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Identification of Epo-independent Human Red Cell Progenitors

The E-cad^{+} Progenitors

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Any living cell carries with it
the experience of a billion years
of experimentation by its ancastors

Max Delbrück

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I ZUSAMMENFASSUNG

Erythrozyten zählen zu den am häufigsten vorkommenden terminal differenzierten Zelltypen des menschlichen Körpers. Durchschnittlich werden täglich ca. 2×10^{11} von ihnen im Körper eines erwachsenen Menschen produziert. Die reifen Erythrozyten entstehen aus multipotenten hämatopoetischen Stammzellen, die über Stadien von erythroiden Vorläuferzellen, erst den sogenannten *burst forming units-erythroid* (BFU-E) und später den *colony forming units-erythroid* (CFU-E), zu kernlosen hämoglobinisierten Zellen differenzieren.

Für die Untersuchung der molekularen Mechanismen der humanen Erythropoese ist die effektive *in vitro* Amplifizierung einer weitgehend homogenen Population der Vorläuferzellen der einzelnen Entwicklungsstadien notwendig. Den Wachstumsfaktoren stem cell factor (SCF) und Erythropoietin (Epo) fällt dabei eine entscheidende Rolle zu. Unter ihrem synergistischen Einfluß lassen sich Epo-abhängige Zellpopulationen, die sich aus BFU-E und CFU-E Typ Zellen zusammensetzen, ausreichend amplifizieren (Panzenböck et al., 1998). Freyssinier et al., 1999 beschrieb erstmals die Isolierung einer Epo-unabhängigen Population von Vorläuferzellen ($CD36^+$ Vorläuferzellen), die ebenfalls erythroide Eigenschaften aufweisen.

Ziel dieser Arbeit war die Isolierung und Charakterisierung von Epo-unabhängigen Vorläuferzellen, die eine frühe erythroide und möglichst homogene Vorläuferzellpopulation darstellen und möglicherweise ein höheres Proliferationspotential aufweisen.

Für die Identifizierung der Epo-unabhängigen Vorläuferzellen, wurden $CD34^+$ Zellen aus Nabelschnurblut aufgereinigt und unter serumfreien Kulturbedingungen und unter Zusatz der Wachstumsfaktoren SCF, Interleukin 3 (IL-3) und eines Fusionsproteins aus IL-6 und löslichem IL-6 Rezeptor (hyper-IL-6) über einen Zeitraum von 8 Tagen kultiviert. Anschließend wurde eine Population von E-cadherin positiven ($E\text{-}cad^+$) Zellen über immunomagnetische Selektion isoliert. Diese neu gewonnenen Epo-unabhängigen $E\text{-}cad^+$ Vorläuferzellen wurden hinsichtlich ihres proliferativen Potentials und ihrer Differenzierungseigenschaften mit SCF/Epo-Vorläuferzellen und $CD36^+$ Vorläuferzellen verglichen. Von allen drei Zelltypen wurden des weiteren

detailierte molekulargenetische Analysen mittels DNA microarray Technologie durchgeführt und die resultierenden Genexpressionsmuster miteinander verglichen.

Die Ergebnisse zeigen, dass die E-cad$^+$ Vorläuferzellen eine frühe, weitgehend homogene Epo-unabhängige Population vom BFU-E Typ darstellen und durch entsprechende Änderungen der Kulturbedingungen zu einer *in vitro* Differenzierung angeregt werden können. Die E-cad$^+$ Vorläuferzellen sind hinsichtlich ihres proliferativen Potentials, ihrer Reaktion auf verschiedene Wachstumsfaktoren, der Expression spezifischer Oberflächenmoleküle und ihrer Genexpressionsmuster mit SCF/Epo-Vorläuferzellen und CD36$^+$ Vorläuferzellen vergleichbar.

Aufgrund der Identifizierung unterschiedlich exprimierter Gene zwischen den Epo-unabhängigen E-cad$^+$ und den Epo-abhängigen SCF/Epo Vorläuferzellen konnten Kanditatengene wie Galectin-3, Cyclin D1, der Anti-Müllerian Hormonrezeptor, Prostata-Differenzierungsfaktor und insulin-like growth factor binding protein 4 identifiziert werden, die als potentielle Regulatoren der Erythropoese in Betracht kommen könnten. Es konnte weiterhin gezeigt werden, dass CD36$^+$ Vorläuferzellen, die aus der selben Zellpopulation wie die E-cad$^+$ Vorläuferzellen immunomagnetisch selektioniert wurden, eine heterogene Population darstellen, die sowohl E-cadherin positive als auch negative Zellen enthält. Die Analyse der Genexpressionsmuster zeigte, dass in den CD36$^+$ Vorläuferzellen zwar auch die Expression erythroid-spezifischen Gene nachgewiesen werden kann, hier aber im Gegensatz zu den E-cad$^+$ Vorläuferzellen auch für Megakaryozyten spezifische Gene stark exprimiert sind.

Die Ergebnisse dieser Arbeit tragen zu einem neuen Modell der *in vivo* Abläufe der Entwicklung roter Blutzellen bei und werden der weiteren Untersuchung der molekularen Mechanismen der Erythropoese dienen.

II ABSTRACT

Red cell development in adult humans results in the mean daily production of $2x10^{11}$ erythrocytes. Mature hemoglobinized and enucleated erythrocytes develop from multipotent hematopoietic stem/progenitor cells through more committed progenitor cell types such as BFU-E and CFU-E. The studies on the molecular mechanisms of erythropoiesis in the human system require a sufficient number of purified erythroid progenitors of the different stages of erythropoiesis. Primary human erythroid progenitors are difficult to obtain as a homogenous population in sufficiently high cell numbers. Various culture conditions for the *in vitro* cell culture of primary human erythroid progenitors have been previously described. Mainly, the culture resulted in the generation of rather mature stages of Epo-dependent erythroid progenitors. In this study our efforts were directed towards the isolation and characterization of more early red cell progenitors that are Epo-independent.

To identify such progenitors, $CD34^+$ cells were purified from cord blood and cultured under serum free conditions in the presence of the growth factors SCF, IL-3 and hyper-IL-6, referred to as SI2 culture conditions. By immunomagnetic bead selection of E-cadherin (E-cad) positive cells, E-cad^+ progenitors were isolated. These Epo-independent E-cad^+ progenitors have been amplified under SI2 conditions to large cell numbers. The E-cad^+ progenitors were characterized for surface antigen expression by flow cytometry, response to growth factors in proliferation assay and for their differentiation potential into mature red cells. Additionally, the properties of E-cad^+ progenitors were compared to those of two other erythroid progenitors: Epo-dependent progenitors described by Panzenböck et al. (referred to as SCF/Epo progenitor), and $CD36^+$ progenitors described by Freyssinier et al. (Panzenböck et al., 1998; Freyssinier et al., 1999). Finally, the gene expression profile of E-cad^+ progenitors was compared to the profiles of SCF/Epo progenitors and $CD36^+$ progenitors using the DNA microarray technique.

Based on our studies we propose that Epo-independent E-cad^+ progenitors are early stage, BFU-E like progenitors. They respond to Epo, despite the fact that they were generated in the absence of Epo, and can completely undergo erythroid differentiation. Furthermore, we demonstrate that the growth properties, the growth factor response and the surface marker expression of E-cad^+ progenitors are similar to those of the SCF/Epo progenitors and the $CD36^+$ progenitors. By the comparison of

gene profiles, we were also able to demonstrate that the Epo-dependent and Epo-independent red cell progenitors are very similar. Analyzing the molecular differences between E-cad$^+$ and SCF/Epo progenitors revealed several candidate genes such as galectin-3, cyclin D1, AMHR, PDF and IGFBP4, which are potential regulators involved in red cell development. We also demonstrate that the CD36$^+$ progenitors, isolated by immunomagentic bead selection, are a heterogeneous progenitor population containing an E-cad$^+$ and an E-cad$^-$ subpopulation. Based on their gene expression profile, CD36$^+$ progenitors seem to exhibit both erythroid and megakaryocytic features.

These studies led to a more updated model of erythroid cell development that should pave the way for further studies on molecular mechanisms of erythropoiesis.

1 INTRODUCTION

1.1 The human hematopoietic system

For epochs in many civilizations blood has been said to be the spirit of life. 4 to 6 liter of blood run through the veins of an adult human being. The essential role of blood is transportation, but also immune reaction, wound healing, pH buffering, temperature regulation in higher vertebrates and skeletal function in erectile organs belong to the functions of blood. With the bloodstream, water, oxygen, carbon dioxide, nutrients, minerals, toxic products of metabolism, antibodies, messenger substances (e.g. hormones) and blood cells are transported.

The blood of vertebrates consists of the liquid component (plasma), leucocytes and red cells. Mature leucocytes are subdivided in a lymphoid compartment producing B- and T-cells and the lymphoid type of dendritic cells (DC) and a myeloid compartment producing the myeloid type of DC, eosinophilic, basophilic and neutrophilic granulocytes, monocytes and megakaryocytes (Fig. 1). Leucocytes belonging to the lymphoid and the myeloid compartment have their function mainly in the immune system and their numbers range daily between 4-8x10^3 cells per mm^3 blood. Leucocytes lifespan is between a few days and 2 weeks. Erythrocytes belong to the myeloid compartment (Fig. 1). About 5x10^6 cells are contained in 1 ml of blood of an adult human being. Red cells owe their name to the respiratory pigment hemoglobin. Their most important function in the blood is transporting oxygen and carbon dioxide between the lung and the places of oxygen consumption or carbon dioxide production, respectively. Erythrocytes have a lifespan of about 120 days.

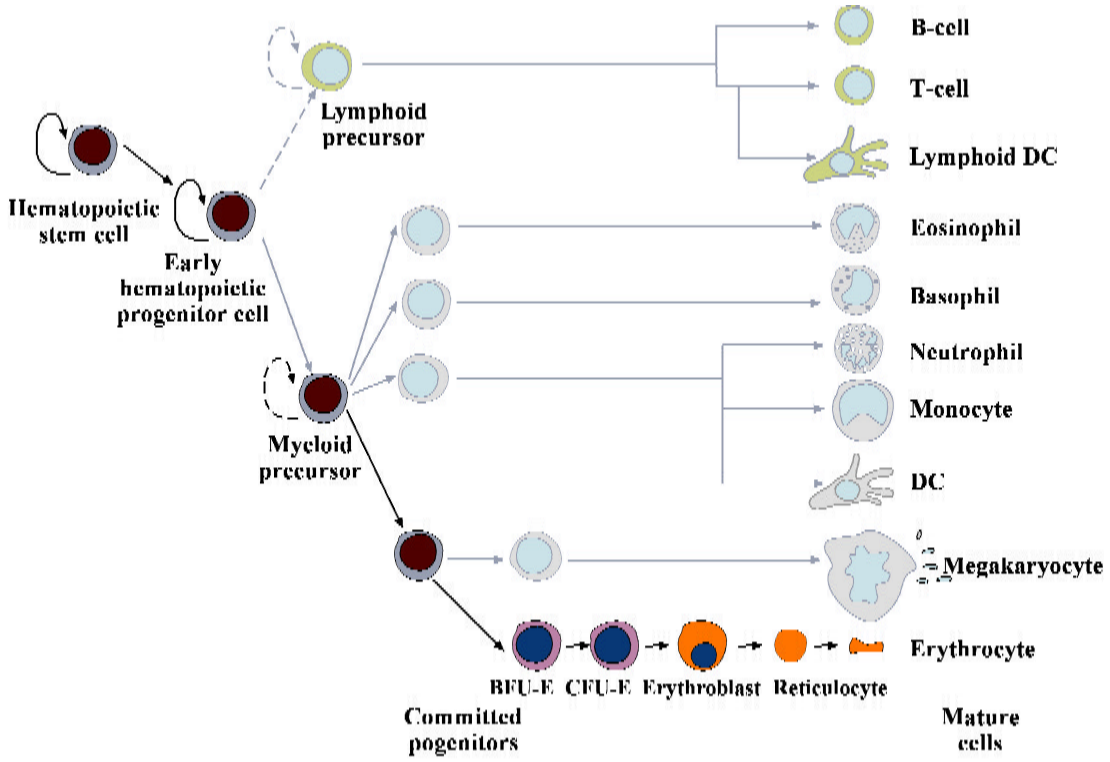

Figure 1.1 : A model of the human hematopoietic system. *All blood cells have their origin in multipotent hematopoietic stem cells. During their development cells undergo various successive steps of differentiation before they finally differentiate into fully functioning mature blood cells.*

Blood cell production of human beings, as in all vertebrates, takes place in specialized tissues. Up to the second month of embryonic development myeloblasts are produced in the connective tissue of the yolk sac. In the second month the intra-embryonic production of blood cells starts in the fetal liver, in the spleen and in lymph nodes. In late gestation and in the early neonatal period a gradual transfer of primitive hematopoietic stem cells to bone marrow takes place (Tavassoli, 1991). During the transition of blood cell production from the embryonic liver to the bone marrow, a large number of primitive blood cells can be found also in peripheral and umbilical cord blood (Broxmeyer, 1991). In adults the majority of blood-forming cells are found in the active bone marrow of spongy bones (epiphysis of long bones and in short, flat bones). Relatively few primitive cells are still contained in the peripheral blood stream.

Blood cells originate from multipotent hematopoietic stem cells (HSC) through successive steps of differentiation. HSC and their immediate progeny, early hematopoietic progenitor cells (HPC), are able to continuously self-renew before they undergo a final differentiation into mature blood cells. An additional common characteristic of HSC and early HPC is the expression of the CD34 antigen, an integral membrane glycoprotein of 90-120 kD that functions as a regulator of cell adhesion to stromal cells within the marrow environment (Greaves et al., 1992; Civin and Gore, 1993; Healy et al., 1995). Approximately 1-3% of the nucleated bone marrow cells have been shown to be positive for CD34 (Greaves et al., 1992; Civin and Gore, 1993). The content of $CD34^+$ cells in umbilical cord blood also has been shown to be around 1% (Kinniburgh and Russell, 1993; Van Epps et al., 1994).

The decision of a HSC or early HPC for a particular differentiation pathway can be controlled either from inside or from outside of the cell by growth factors or by cell-cell interactions. Growth factors can influence the individual hematopoietic stem/progenitor cell population in a selective or instructive manner. In a selective model, hematopoietic stem/progenitor cells commit to a particular lineage independently of the growth factor. The factors act subsequently to control the survival or proliferation of committed progenitor. In the instructive model the growth factors could cause the progenitor to choose a certain lineage (Morrison et al., 1997). Once the decision has been made mature highly specialized blood cells are formed via more committed lineage specific progenitors.

1.2 Erythropoiesis

1.2.1 Characteristics of individual stages

The enormous number and the short lifespan of red erythrocytes make effective mechanisms of red cell production essential. Erythropoiesis in adult humans results in the mean daily production of $2x10^{11}$ red blood cells. The process begins with the commitment of pluripotent hematopoietic stem/progenitor cells into lineage specific progenitors. Committed erythroid progenitors that respond specifically to erythropoietin (Epo) are detected in vitro by formation of discrete erythroid colonies growing in semisolid methycellulose culture medium. The two detectable progenitor types are termed the colony-forming unit-erythroid (CFU-E) and the burst-forming unit-erythroid (BFU-E) (Gregory and Eaves, 1977; Gregory and Eaves, 1978). CFU-

Es are rapid dividing cells that are highly responsive to Epo. After 7 days in the methylcellulose medium CFU-E derived from human mononuclear blood cells form hemoglobinized erythroblast colonies of 8-49 cells. The BFU-Es are more immature cells which divide less frequently. They develop within 2 weeks large colonies (bursts) containing more than 500 hemoglobinized erythroblasts (Gregory and Eaves, 1977; Gregory and Eaves, 1978). Development from the earliest BFU-E to the latest CFU-E is a continuous process.

Morphologically hematopoietic stem/progenitor cells, BFU-E and CFU-E type cells are very similar. It is not possible to distinguish them by light microscopy. The first morphologically recognizable stage within erythropoietic differentiation is the proerythroblast stage (Fig. 1.2 and http://www.med1.de/Experten/Fachrichtungen/Innere/Haematologie/Zyto-kurs). The medium-sized cells have a round nucleus and very fine chromatin. During the subsequent differentiation process cells continuously lose cell size. The nuclear chromatin of macroblasts and basophile erythroblasts is more condensed and more contrasted than in proerythroblasts. The polychromatic erythroblasts show the first stage in erythropoiesis where hemoglobin synthesis is visible. They show a gray cytoplasm in a combined staining with neutral benzindine and histological dye. With further maturation the chromatin condensation is increased and in parallel the accumulation of hemoglobin. The orthochromatic erythroblast has already a reddish cytoplasm. The very condensed nucleus is enucleated in the following maturation step. The resulting cell type is the reticulocyte which still contains ribosomal RNA and therefore still expresses hemoglobin. Reticulocytes and mature erythrocytes were finally released into circulation

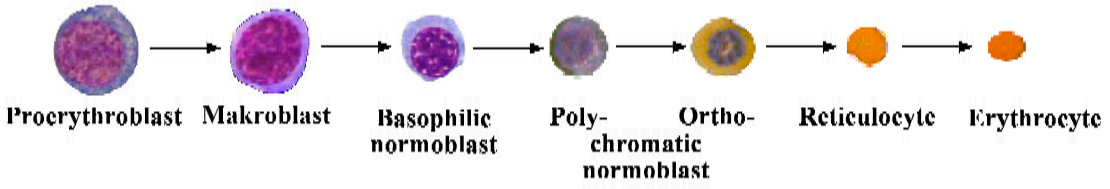

Figure 1.2 : Schematic representation of erythroid cell development.
Erythroid cells of various maturation stages were stained with histological dyes and neutral benzidine. Modified from "http://www.med1.de/Experten/Fachrichtungen/Innere/Haematologie/Zyto-kurs".

During erythropoiesis not only morphological characteristics but also metabolic and molecular characteristics are changed in a specific manner. Among others the differences in surface marker profiles make these changes clear. The expression of CD34, the specific surface marker for hematopoietic stem/progenitor cells, is lost during red cells differentiation. It is already absent on CFU-E (Bühring et al., 1996). Various surface markers, such as SCF receptor (CD117), transferrin receptor (CD71), CD36, E-cad, endoglin (CD105) and Epo receptor (EpoR), are expressed on BFU-Es and CFU-Es. They are subsequently down regulated during differentiation and are no longer detectable on mature red cells (Papayannopoulou et al., 1991; Loken et al., 1987; Kieffer et al., 1989; Bühring et al., 1991; Bühring et al., 1996; Koury and Bondurant, 1988). Reciprocal patterns are reported for red cell specific markers such as KELL, glycophorins A, B and C, Rh associated glycoproteins and band 3 (Gubin et al., 1999; Loken et al., 1987; Lee, S. et al., 1993; Daniels and Green, 2000).

Stages of erythroid cells specifically defined by surface marker profiles can be discriminated by flow cytometry using an appropriate combination of antibodies against corresponding surface markers (Bühring et al., 1996). Changes of surface proteins are also associated with changes in the membrane structure (Vidal et al., 1989; Johnstone, 1992).

1.2.2 Regulation of erythropoiesis by cytokines

Hematopoietic cell growth and differentiation is controlled by a family of polypeptides referred to as cytokines. It is not clear whether these processes are controlled by cytokines (Metcalf, 1989; Metcalf, 1991) or whether the cytokines act subsequently to promote the survival and proliferation of independently committing and differentiating cells (Ogawa, 1989; Suda, J. et al., 1984a; Suda, T. et al., 1984b; Mayani et al., 1993).

These cytokines bind to their cognate receptors, mainly receptor tyrosine kinases (e.g. c-kit, receptor for SCF, epidermal growth factor [EGF] receptor) or receptors signaling through the Janus kinases (JAK)/ signal transducer activator of transcription (STAT) pathway (e.g. erythropoietin receptor [EpoR], most interleukin receptors). The cytokine-receptor binding mediates intracellular signal transduction that results in a modulation of gene expression. Both types of receptors signal through multiple pathways (Beug et al., 1996).

The presence of cytokines is crucial for *in vitro* culture of blood cells. Most of the cytokines show pleiotrophic effects in cell cultures. In human erythropoiesis SCF (also referred to as mast cell growth factor, MGF; Steel factor, SLF; or kit ligand, KL; Krantz, 1991; Broudy, 1997) has an effect on both the pluripotent HSC and the committed lineage restricted erythroid progenitors (Broxmeyer, 1991; McNiece et al., 1991). In primitive stem and progenitor cells SCF promotes the survival (Keller et al., 1995). In red cell progenitors SCF supports proliferation (Nocka et al., 1990; Bartunek et al., 1996). The regulation of the different effects of SCF results from synergistic effects with other factors. In pluripotent stem and progenitor cells, factors like Flt3 ligand (Flt3L), thrombopoietin (Tpo), interleucin 6 (IL-6) and interleucin 3 (IL-3) are reported to have the potential to synergize with SCF (Ikebuchi et al., 1987; Jacobsen et al., 1995; Zeigler et al., 1994; Sui et al., 1995; Nicola, 1994). The combination of SCF and Epo plays a crucial role in the commitment of human erythroid progenitors (McNiece et al., 1991; Muta et al., 1994). IL-3 or IL-6 in combination with SCF were also reported to support the proliferation of early erythroid progenitors (Brugger et al., 1993; Papayannopoulou et al., 1993; Sui et al., 1996). In avian erythroid cell culture systems SCF in combination with transforming growth factors type α and β (TGFα, TGFβ) support the proliferation of committed

erythroid progenitors (Hayman et al., 1993; Steinlein et al., 1995; Gandrillon et al., 1999).

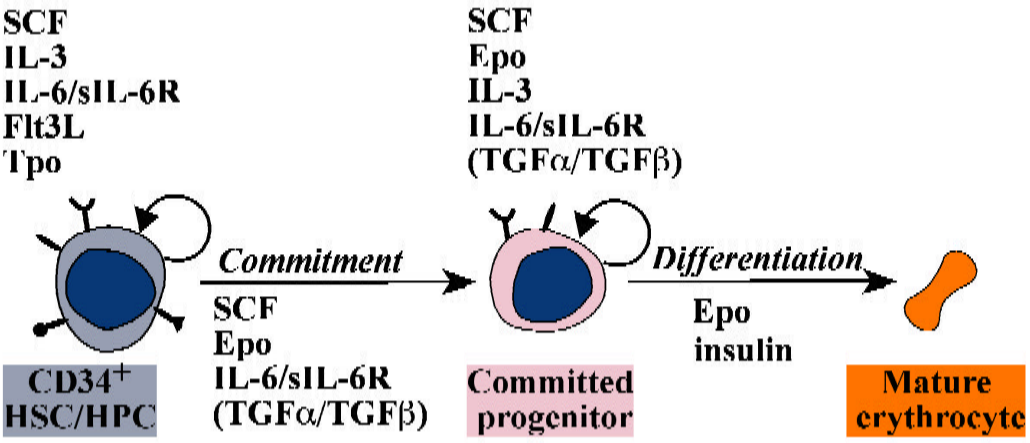

Figure 1.3 : Growth factors play important roles for erythropoiesis in vitro.
Three maturation stages of mammalian erythropoiesis are represented. Growth factors supporting the different stages are listed. Growth factors listed above the cells are reported to support self-renewing and survival of the corresponding cell type. Factors listed below the arrows are reported to be involved in the initiation of red cell commitment and differentiation. The involvement of TGFa and TGFb in erythropoiesis was so far only shown for the chicken system.

C-kit, the receptor for SCF, is expressed on approximately 70% of the $CD34^+$ cells in human bone marrow (Ashman et al., 1991). In the signal transduction pathway of c-kit function via the p85 subunit of phosphatidylinositol 3´kinase (PI3K), phospholipase Cγ (PLC g1), mitogene-activated protein kinase (MAPK)/ERK cascade (Fig 1.4, for review see Linnekin, 1999).

Epo is the primary humoral regulator of erythropoiesis. Epo receptor (EpoR) is its cognate receptor and is involved in both erythroid commitment and, synergizing with insulin, in the induction of red cell differentiation (Muta et al., 1994; Muta et al., 1995; Wu et al., 1995a). Beyond the basophilic erythroblast stage the level of EpoR drops and differentiating cells are no longer Epo-dependent (Koury and Bondurant, 1988). The binding of Epo to the EpoR homodimer on the cell surface induces the phosphorylation and activation of a large signaling complex with proteins belonging

to the JAK/STAT pathway, phosphatidylinositol 3´kinase (PI3K) pathway and ERK/MAPK cascade (Fig 1.4; Ihle et al., 1993; Chin et al., 1998; Lecoq-Lafon et al., 1999; Tilbrook et al., 1997; Witthuhn et al., 1993).

Sui et al. reported that the stimulation of glycoprotein 130 (gp130) by a combination of recombinant human soluble interleukin 6 receptor (sIL-6R) and IL-6 can support the proliferation of erythroid progenitors from human $CD34^+$ cells and can initiate erythropoiesis independent of exogenous Epo but in the presence of SCF. IL-6 itself acts via receptor complexes that belong to the gp130 receptor family (Sui et al., 1995). The receptors of this family share a common signal transducing chain (gp130). IL-6 first binds to the specific IL-6 receptor (IL-6R). This complex then associates with a homodimer of gp130 (for review see Taga and Kishimoto, 1997). It is known that primitive $CD34^+$ progenitors and erythroid progenitors do not express membrane bound IL-6R but gp130 molecules. sIL-6R has been detected in blood at concentrations of 50-80 ng/ml in normal humans (Honda et al., 1992) and so IL-6/sIL-6R complexes can bind to gp130, and thus even cells not expressing the IL-6R can be influenced by IL-6 (Sui et al., 1995). To enhance the effect of IL-6/sIL-6R complexes on gp130 positive cells Fischer et al., 1997 designed a fusion protein by linking IL-6 and sIL-6R covalently. This hyper-IL-6 molecule was shown to be a highly biologically active protein complex and can be used to expand human hematopoietic progenitor cells efficiently (Fischer et al., 1997; Chebath et al., 1997). The intracellular signaling of gp130 was shown to function also via JAK/STAT, PI3K-PKB/akt pathways and ERK/MAPK cascade (Fig 1.4; Kishimoto et al., 1995; Hibi et al., 1996).

In suspension cultures of human early erythroid progenitors IL-3 in synergy with SCF increases the proliferation of cells and also consistently amplifies a population of cells that contain hemoglobin (Papayannopoulou et al., 1993). IL-3 receptor belongs to the gp140 receptor family (Guthridge et al., 1998). The receptors of this family express a unique ligand binding α chain and share a common signal transducing β chain (gp140) (Itoh et al., 1990; Murata et al., 1992; Miyajima et al., 1992). Also the IL-3 induced signal transduction is achieved by recruitment of multiple signal transduction cascades, such as the JAK/STAT signaling pathway, the ERK/MAPK cascade and the PI3K-PKB/akt pathway (Fig 1.4 and for review see Guthridge et al., 1998).

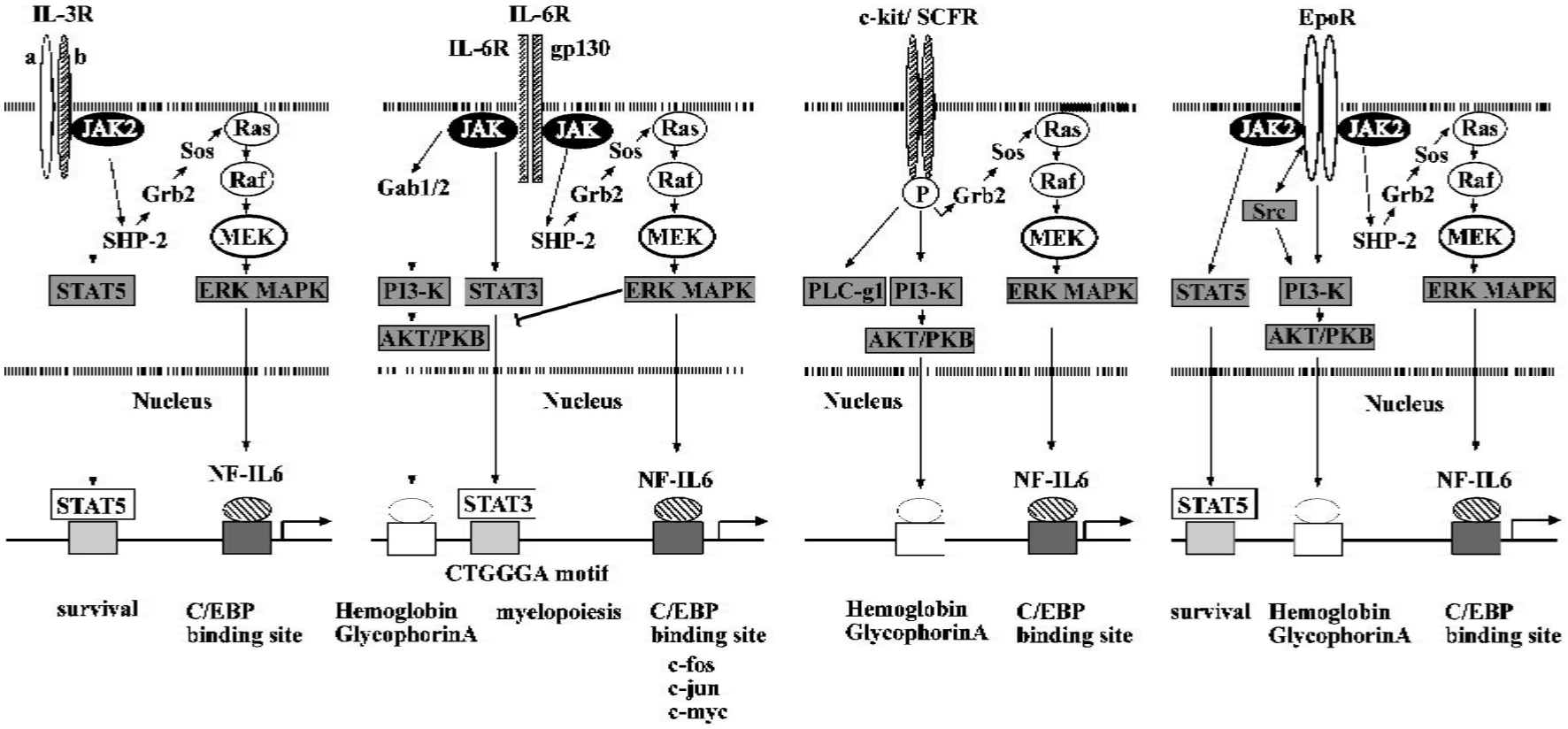

Figure 1.4 : A schematic representation of the signalling pathways stimulated by IL-3, IL-6, SCF and Epo.

Receptor dimerization and activation stimulated by IL-3, IL-6, SCF or Epo result in a number of signalling cascades. The different signalling pathways mediate transcriptional regulations via various transcription factors. (provided by Dr. Ki-Ryang Koh ; sources: EMBO J. 2001. 15: 5666; Blood 2001. 15: 2372; Annu. Rev. Immunol. 1997. 15: 797-819; PNAS 1998. 95: 11107-11112)

1.2.3 Transcriptional regulation of erythropoiesis

The signaling mechanisms of cytokines act through signal transduction cascades which finally mediate transcriptional regulation via transcription factors (Fig. 1.4). Transcription factors are specific DNA binding proteins which are required for the regulation of gene expression. Lineage specific transcription factors play an essential role in the process of lineage specific determination. Numerous transcription factors are thought to translate the information from various signaling pathways into changes in erythroid specific gene expression. These factors participate in critical protein-protein interactions and function as multiprotein complexes. Probably, the composition of these complexes is changed during several stages of human erythropoiesis (Cantor and Orkin, 2002).

The zinc finger–type family of GATA transcription factors consists of 6 members. GATA-1, -2 and -3 are involved in regulation of gene expression in the hematopoietic system whereas GATA-4, -5 and –6 are expressed in non-hematopoietic tissues (for review see Orkin, 1992). GATA-1 is a key regulator in mammalian erythropoiesis and was first identified regulating the expression of globin genes in mice (Tsai et al., 1989). GATA-1 binding motifs later were found in promotor and/or enhancer regions of all erythroid and megakaryocytic-specific genes (Orkin, 1992; Weiss and Orkin, 1995). In proliferating red cell progenitors the GATA-1 proteins are located in the cytoplasm whereas in cells induced to differentiate the protein is translocated into the nucleus (Briegel et al., 1996, Bartunek et al., 2003). GATA-1 is not exclusively expressed in red cells but also in megakaryocytes, mast cells, granulocytes and murine testis tissue (Shivdasani et al., 1997; Tsai et al., 1989; Evans and Felsenfeld, 1989; Ito et al., 1993; Zon et al., 1993). The loss of GATA-1 in mice leads to rapid apoptosis of proerythroblasts, deficirncies in erythroid cell maturation or defects in platelet formation in the murine system (Weiss and Orkin, 1995; Shivdasani et al., 1997). Among others, GATA-2 was reported to be expressed in immature erythroid progenitors and to be down regulated during red cell differentiation (Orkin, 1992; Briegel et al., 1993; Leonard et al., 1993). In immature erythroid progenitors the transcription factor supports the self renewal and maintenance of immaturity of the progenitor cells (Briegel et al., 1993).

The two known friends of GATA proteins (FOG) are also zinc finger type transcription factors which were found to interact with GATA factors (Tsang et al., 1997). FOG-1 is co-expressed with GATA-1 in erythroid and megakaryocytic cells (Tsang et al., 1997). In vitro the interaction of FOG proteins with GATA-1 is essential for the function of GATA-1 in erythroid differentiation (Crispino et al., 1999).

Tal-1/SCL and LMO2 are members of the basic helix-loop-helix (bHLH) transcription factors and also shown to be essential for the early erythropoiesis in mice (Shivdasani et al., 1995; Robb et al., 1995; Wadman et al., 1997). LMO2 and Tal-1/SCL were reported to complex with GATA-1 and E2A to a pentameric transcriptional transactivating complex which may play a role in regulation of erythrocyte specific gene expression (Wadman et al., 1997). The precise functional connections are still not clear (Cantor and Orkin, 2002).

Erythroid krüppel-like factor (EKLF) belongs also to the zinc finger family of transcription factors (Miller and Bieker, 1993). The EKLF-DNA binding motif has been found first to correspond to a motif in the human adult β-globin gene promotor (Orkin et al., 1982). Later experiments suggest that EKLF plays an essential role in the globin switch from embryonic to fetal to adult β-globin expression in humans and mice (Perkins et al., 1996; Wijgerde et al., 1996).

Another transcription factor playing a critical role in regulation of erythropoiesis is the nuclear factor erythroid 2 (NF-E2). NF-E2 binds to AP-1 like motif which is located in enhancer and promotor regions of several erythroid and megakaryocytic specific genes, including human β-globin gene (Kotkow and Orkin, 1995; Ney et al., 1990; Talbot et al., 1990), human porphobilinogen deaminase gene (Mignotte et al., 1989) and human ferrochelatase genes (Taketani et al., 1992). The transcription factor is a heterodimer complex consisting of a 45 kD and an 18 kD subunit. The large subunit has been reported to be expressed specifically in erythroid cells, megakaryocytes and mast cells (Andrews et al., 1993). The loss of the large subunit results in significant reduction in expression of α- and β-globin genes and thus the 45 kD subunit s referred to as a positive regulator of globin gene expression (Kotkow and Orkin, 1995). Additionally, Li and colleagues showed a negative regulation of erythroid proliferation by the 45 kD subunit of NF-E2 (Li et al., 2001).

The signal transducer STAT5 is ubiquitously expressed in hematopoietic cells and is involved in erythroid differentiation and survival (Nosaka et al., 1999; Snow et al., 2002; Socolovsky et al., 1999). The two identified isoforms STAT5A and STAT5B share 90 % homology and are rapidly tyrosine phosphorylated in the transactivation domain upon Epo stimulation (Liu et al., 1995; Azam et al., 1995).

The nuclear transcription factor NF-IL6 (also referred as to C/EBPδ) was originally identified as a DNA-binding protein responsible for IL-1-stimulated IL-6 induction. It belongs to the CCAAT/enhancer binding protein (C/EBP) transcription factor family and is drastically induced by LPS or inflammatory cytokines such as IL-1, TNF, and IL-6 and has a homology with the other members of the C/EBP familiy. NF-IL6 recognize the same nucleotide sequence as other C/EBP, but exhibit distinct patterns of expression. NF-IL6 is phosphorylated and activated by a Ras-dependent MAPK (Akira, 1992). C/EBPα is also known to play a role in the lineage commitment and differentiation of hematopoietic progenitors (Cammenga et al., 2003).

The transcripton factor JunB appears to function as a negative regulator of a number of cell systems, including proliferation in response to negative growth factors (Koo et al., 1992; Chalaux et al., 1998). As with NF-E2, JunB binds to the AP-1 sites. Jacobs-Helbert and colleagues recently reported a role of JunB as a part of the erythroid differentiation program (Jacobs-Helber et al., 2002).

Finally, another important family of transcriptional regulators involved in erythroid development includes nuclear hormone receptors, such as the thyroid hormone receptor TRα, the retinoic acid receptors RXR and RAR, and the steroid hormone receptor and glucocorticoid receptor (Bartunek and Zenke, 1998; Bauer et al., 1998; von Lindern et al., 1999; Wessely et al., 1997; Zenke et al., 1988; Zenke et al., 1990)

The interplay of the different transcription factors during erythropoiesis is not fully understood. It has been reported that transcription factors can act as activators and repressors of specific target genes. Busslinger and colleagues demonstrated that the B-cell specific transcription factor Pax 5 not only supports the differentiation of B-cells but also represses the alternate lineage choices (Nutt et al., 1999). Similar results were reported for the muscle specific transcription factor Pax 7 (Seale et al., 2000).

1.3 *In vitro* system recapitulates the red cell development in cell culture

The studies of the molecular mechanisms of erythropoiesis in the human system require sufficient numbers of purified erythroid progenitors from the different stages of red cell development. Primary human erythroid progenitors are difficult to obtain as a homogenous population in sufficiently high cell numbers. Various cytokine mixtures so far have been described for *in vitro* cell culture of human erythroid progenitors (de Wolf et al., 1994; Mrug et al., 1997; Muta et al., 1994; Panzenböck et al., 1998). These mixtures all included Epo, and resulted in the generation of rather mature stages of Epo-dependent erythroid progenitors.

Wu and colleagues showed that in vivo the early murine erythroid lineage commitment does not require Epo or its receptor (Wu et al., 1995b). Besides Epo several other cytokines or combinations thereof have been reported to stimulate the proliferation of erythroid progenitors including stem cell factor (SCF), interleukin-1 (IL-1), interleukin-3 (IL-3), interleukin-6 (IL-6) in combination with its soluble receptor (sIL-6R) or hyper-IL-6, the fusion protein of both (Dai et al., 1991; Papayannopoulou et al., 1993; Muta et al., 1995; Broudy, 1997; Brugger et al., 1993; Sui et al., 1996; Fischer et al., 1997). Human cord blood derived $CD34^+$ cells were subjected to culture conditions containing combinations of these cytokines, and Epo-independent red cell development was observed (Sui et al., 1996; Baiocchi et al., 2000; Neildez-Nguyen et al., 2002).

In chicken, homogenous populations of erythroid progenitors can be generated *in vitro* from bone marrow in the presence of SCF, SCF+TGFα or TGFα+TGFβ (Hayman et al., 1993; Schroeder et al., 1993; Briegel et al., 1996; Wessely et al., 1997; Gandrillon et al., 1999). The culture conditions containing SCF only cause the growth of more mature progenitors with a reduced ability of self renewal. The properties of the SCF-dependent cells in the chicken system are similar to the properties of the SCF/Epo-dependent progenitor in the human culture system (Bartunek and Zenke, 1998; Panzenböck et al., 1998; von Lindern et al., 1999). Cell culture conditions containing TGFα and TGFα+TGFβ cause the growth of more immature red cell progenitors exhibiting a considerably longer lifespan in vitro (Beug et al., 1995; Steinlein et al., 1995; Hayman et al., 1993; Gandrillon et al., 1999). We suspect the existence of an early Epo-independent human red cell progenitor that is similar to the early TGFα/TGFβ-dependent progenitor in the chicken system.

Furthermore, the studies of the molecular mechanisms of erythroid development require specific markers to enable the purification of distinct cell stages of red cell development.

Stages of erythroid cells specifically defined by surface marker profiles can be discriminated by flow cytometry using appropriate combination of antibodies against corresponding surface markers (Bühring et al., 1996). Freyssinier and colleagues have developed a three step purification method based on immunomagnetic bead selections for CD34 and CD36 positive human cord blood derived cells, grown in the absence of Epo, to obtain a pure red cell progenitor population of BFU-E and CFU-E (Freyssinier et al., 1999). Additionally, E-cad a highly conserved calcium binding transmembrane glycoprotein involved in cell-cell interactions, seems to be more restricted to erythroid progenitors in the hematopoietic system than CD36 (Armeanu et al., 1995). Thus while in initial studies already provided information about some surface markers for early red cell progenitors further surface marker analysis was clearly acquired.

1.4 DNA microarray to investigate cell type specific transcriptional signatures

DNA chip technology is one method of choice for comparison of different cell types. High density oligonucleotide arrays allow the analysis of a large number of genes expressed as mRNA in parallel (Lockhart et al., 1996). Using higher-level data analysis algorithms, expression profiles of different cellular stages can be compared and genes potentially involved in biological processes of interests can be identified by their expression patterns (Eisen et al., 1998; Tamayo et al., 1999). Several groups have recently used the microarray technique to study differential gene expression profiles of various cell types (Hacker et al., 2003, Ju et al., 2003, Park et al., 2002, Steidl et al., 2002, Andrews et al., 1993; Boeuf et al., 2001). We have used gene expression profiling with Affymetrix GeneChip arrays to determing the gene expression repertoire of DC and these studies provided valuable information on the transcription factor Id2 in lineage choice and DC development (Hacker et al., 2003; Ju et al., 2003).

1.5 Objectives

The aim of this study was to identify an early red cell progenitor in the human system which was expected to grow in the absence of Epo.

In particular, the following points had to be addressed:

- Efforts to identify additional growth factor combinations that support growth of Epo-independent early red cell progenitors
- Identification and isolation of an early Epo-independent red cell progenitor out of the hematopoietic stem/progenitor cell population using erythroid progenitor specific early surface markers like CD36 and E-cad
- Characterization of this progenitor type and investigation of its erythroid properties
- Comparison of Epo-dependent and Epo-independent progenitors regarding their gene expression profiles

Gene expression profiles of different progenitor types were compared by DNA chip technology and common and differential expressed genes were determined. Therefore, a further objective of this study was to identify genes of additional potential regulators of growth, commitment and differentiation, like cytokine receptors or transcription factors. New knowledge might lead to more efficient conditions for red cell progenitor amplification *in vitro*. Additionally, an *in vitro* model for erythropoiesis recapitulating the in vivo system as natural as possible would help to answer prospective medical questions.

2 MATERIAL AND METHODS

2.1 Materials

2.1.1 Chemicals

If not specified otherwise, all chemicals were obtained from Merck, Fluka or Sigma in analytical grade.

2.1.2 Media and buffers

2.1.2.1 Media for tissue culture

hu S13 medium	15% (v/v) FCS (Boehringer Mannheim, Germany) 1% (w/v) detoxified, deionised, deliptated BSA 15% (v/v) distilled water 1.9 mM sodium bicarbonate 1.9 mM β-ME 0.128 mg/ml iron saturated human transferrin 1000 U/ml Pen/Strep (GIBCO BRL) in 1x EAGLES medium
StemSpan medium	serum free; StemCell Technologies, Vancouver, Canada

2.1.2.2 Media for bacterial culture

LB medium, pH 7	1% (w/v) NaCl 1% (w/v) bactotrypton (DIFCO) 0.5% (w/v) bacto yeast extract (DIFCO)
LB-Agar	1.5% (w/v) bacto agar (DIFCO) in LB medium
SOC medium, pH 7	0.05% (w/v) NaCl 2% (w/v) bactotrypton (DIFCO) 0.5% (w/v) bacto yeast extract (DIFCO) 2.5 mM KCl 10 mM $MgCl_2$ 20 mM glucose

2.1.2.3 Buffers and solutions

TBE buffer, pH 8.0	89 mM Tris/HCl 89 mM boric acid 2 mM EDTA;
MOPS buffer, pH 7.0	200 mM 8-Morholino-propane-sulfonic acid 50 mM NaAcetate 10 mM EDTA
SSC, pH 7.0	150 mM NaCl 15 mM NaCitrate
Church buffer, pH 7.5	0.5 M NaP_i 7% (w/v) SDS 1 mM EDTA
PBS	137 mM NaCl 2.7 M KCl 8.2 mM Na_2HPO_4 1.5 mM KH_2PO_4

2.1.3 Growth factors

Factor	**F.c.**	**Source**
amphiregulin (AR)	10 ng/ml	R&D systems
betacellulin (BTC)	0.3 ng/ml	R&D systems
dexamethasone (Dex)	10-6 M	Sigma
rhu epidermal growth factor (EGF)	10 ng/ml	Roche Diagnostics, Mannheim,Germany
rhu erythropoietin (Epo)	1 U/ml	Roche Diagnostics, Mannheim,Germany
Factor	**F.c.**	**Source**
acid fibroblast growth factor (aFGF)	10 ng/ml	Promega
basic FGF (bFGF)	50 ng/ml	Promega
rhu Flt3 ligand (Flt3L)	50 ng/ml	PeproTech, London, UK
rhu granulocyte macrophage-	500 U/ml	Leukomax, Novartis, Nürnberg, Germany

colony stimulating factor (GM-CSF)		
rhu growth hormone (GH)	100 ng/ml	Norditropin, Novo Nordisk Pharma
heparin binding EGF (HB-EGF)	5 ng/ml	R&D systems
heregulin β1 (HRGβ1)	10 ng/ml	Promega
rhu insulin	0.9 µg/ml	Actrapid HM40, Novo Nordisc Pharma
rhu insulin like growth factor 1 (IGF-1)	40 ng/ml	Sigma
rhu interleukin 3 (IL-3)	20 ng/ml	Sandoz, Novartis, Vienna, Austria
hyper-interleukin 6 (hyper-IL-6, IL-6/soluble IL-6 receptor fusion protein; Fischer et al., 1987)	5 ng/ml	a gift from S. Rose-John, Kiel, Germany
platelet derived growth factor (PDGF)	10 ng/ml	a gift from C. H. Heldin, Uppsala, Sweden
rhu stem cell factor (SCF)	100 ng/ml	Amgen Inc., Thousand Oaks, USA
rhu thrombopoietin (Tpo)	20 ng/ml	Amgen Inc., Thousand Oaks, USA
rhu transforming growth factor β1 (TGF-β1)	4 ng/ml	R&D systems
rhu transforming growth factor alpha (TGF-α)	10 ng/ml	Promega

2.1.4 Antibodies

2.1.4.1 Primary antibodies

Antigen	**Host**	**type**	**Clone**	**Source**
CD1a	mouse	monoclonal	NA1/34	DAKO
CD11a, LFA-1, α-chain	mouse	monoclonal	HI111	BD Biosciences
CD11b, Mac-1	rat	monoclonal	M1/70	BD Biosciences
CD11c	mouse	monoclonal	B-Ly6	BD Biosciences
CD14	mouse	monoclonal	RM052	Immunotech
CD18, LFA-1, β_2 chain	mouse	monoclonal	6,7	BD Biosciences
CD19-FITC	mouse	monoclonal	HD37	DAKO

CD33, siglec-3	mouse	monoclonal		Cymbus Biotech CBL
CD36, thrombospondin receptor	mouse	monoclonal	FA6-152	Immunotech
CD36-PE	mouse	monoclonal	CB38	BD Biosciences
CD71, transferrin receptor	mouse	monoclonal	Ber-T9	DAKO
CD117, c-kit, SCF receptor	mouse	monoclonal	YB5.B8	BD Biosciences
CD233, band 3	mouse	monoclonal	BIII-136	Sigma
CD235a/b, glycophorin A/B	mouse	monoclonal	E3	Sigma
CD238, KELL	mouse	monoclonal	BS 45	Biotest AG, Germany
E-cadherin	mouse	monoclonal	HECD-1	R&D systems
EpoR, erythropoietin receptor	rabbit	polyclonal		Upstate
HLA-DR, MHC II	mouse	monoclonal	CR3/43	DAKO

2.1.4.2 Secondary antibodies

Conjugate	**Antigen**	**Host**	**Source**
FITC	mouse IgG	goat	Jackson Immuno Research
FITC	rabbit IgG	goat	Sigma
PE	mouse IgG	goat	Sigma
Conjugate	**Antigen**	**Host**	**Source**
HRP	mouse IgG	sheep	Amersham
HRP	rabbit IgG	donkey	Amersham

2.1.5 PCR primers

Name	**Target**	**Sequence (5´=>3´)**
huEpo sense	human Epo	ATC ACG ACG GGC TGT GCT GAA CAC
huEpo antisense	human Epo	GGG AGA TGG CTT CCT TCT GGG CTC
huGAPDH forward	human GAPDH	AAG TGT AAG GTC GGA GTC AA
huGAPDH reverse	human GAPDH	GCC AGT GGA CTC CAC GAC GT

2.1.6 Bacteria and cell lines

XL-1 blue: recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F^proAB lac1^{q}ZVM15Tn10(Tet^{r})]^{c}

HepG2 Human hepatoma cell line; ATCC ref. no: HB-8065

2.1.7 Vectors

pCR™2.1 (Invitrogen)

2.1.8 DNA chips

GeneChip Test3 Array and HG_U95Aver2 Array, Affymetrix

2.2 Cells and tissue culture

2.2.1 Human cord blood cells

2.2.1.1 Preparation of mononuclear cells and culture of SCF/Epo progenitors

Cord blood cells, scheduled for discard and collected according to institutional guidelines, were obtained after normal full-term pregnancies. After placental delivery the umbilical veins were cannulated and aspirated. Approximately 30-50 ml cord blood were routinely recovered and collected in syringes containing 100 U sodium heparin (Heparin Natrium, Braun 5000 I.E./0.5 ml) and 100 U/ml Pen/Strep (GIBCO-BRL). Residual blood clots were removed by passage through a 70 µm cell strainer (Becton Dickinson), mononuclear cells (MNC) were isolated using Ficoll density separation (Biocoll separation solution, density 1.077 g/cm^3; Biochrom KG, Berlin, Germany). MNC were cultured first at 3x10^6 cells/ml for 1-3 days in human S13 medium at 37°C in 5% CO_2 atmosphere and high humidity (95%) essentially as described (Panzenböck et al., 1998). Later cells were cultured at 2x10^6 cells/ml. Culture medium was supplemented with 1 U/ml recombinant human Epo, 100 ng/ml recombinant human SCF, 10^{-6} M dexamethasone. In some experiments 1% of culture supernatant of SCF producing CHO-SCF cells (Neben et al., 1994) was used as a

source of SCF. Partial medium changes were done every second day. To monitor cell proliferation, cells were counted every second day with an electronic cell counter device (CASY1, Schaerfe Systems, Reutlingen, Germany) and cumulative cell numbers were determined.

2.2.1.2 Isolation and *in vitro* culture of hematopoietic stem/progenitor cells

$CD34^+$ hematopoietic stem/progenitor cells were obtained from MNC (2.2.1.1) by immunomagnetic bead selection with the CD34 Progenitor Cell Isolation kit (Miltenyi Biotec, Bergisch Galdbach, Germany). After Ficoll centrifugation MNC were labeled with MACS MicroBeads according to the protocol of the isolation kit. In brief, MNC were incubated with FcR blocking reagent and hapten-conjugated anti-CD34 antibody (20 min, 4°C). Cells were washed and reacted with microbeads conjugated to anti-hapten antibody (20 min, 4°C). $CD34^+$ cells were obtained by two cycles of immunomagnetic bead selection and selected cells were counted with an electronic cell counter device (CASY1). $CD34^+$ cells were plated first at $0.5x10^6$ cells/ml, later at $1.5x10^6$ cells/ml in StemSpan Medium (serum free culture medium; StemCell Technologies Inc., Vancouver, Canada) supplemented with 100 ng/ml SCF, 10 ng/ml IL-3 and 5 ng/ml hyper-IL-6, referred to as SI2 conditions, or supplemented with 100 ng/ml SCF, 5 ng/ml hyper-IL-6, 20 ng/ml Tpo and 50 ng/ml Flt3L, referred to as STIF conditions. New culture medium containing the corresponding cytokines was added every second day.

2.2.1.3 Selection of E-cad$^+$ and CD36$^+$ cells

E-cad^+ and $CD36^+$ cells were obtained by immunomagnetic bead selection similar to the isolation of $CD34^+$ cells (2.2.1.2). Briefly, on day 7 or 8 of cell culture amplified hematopoietic stem/progenitor cells were collected by centrifugation, counted, washed and monoclonal CD36 or E-cad IgG1 antibody was added at a final concentration of 1 µg/10^6 cells and incubated for 20 min at 4°C. After washing with separation buffer cells were incubated 20 min at 4°C with rat anti-mouse IgG1 antibody coupled to magnetic beads (Miltenyi Biotec). Immunomagnetic separation and subsequent cell culture were performed as described previously (2.2.1.2).

2.2.1.4 *In vitro* commitment and differentiation of progenitor cells

For erythroid commitment SI2 cells, purified E-cad$^+$ and CD36$^+$ progenitor cells at day 8 of culture were washed twice with 1x EAGLES medium and than cultured for additional 2–4 days in human S13 medium supplemented with 1 U/ml Epo, 100 ng/ml SCF, 10^{-6} M dexamethasone (Panzenböck et al., 1998).

To induce differentiation, SCF/Epo progenitors at day 10 or committed cells at day 2–4 of culture were recovered, washed twice with 1x EAGLES medium and seeded at $4x10^6$ cells/ml in culture medium containing 1 U/ml Epo and 1 µg/ml insulin Panzenböck et al., 1998). Medium was partially replaced daily by new culture medium plus factors. Erythroid commitment and differentiation was followed by measuring cell size (CASY1), by flow cytometry and by staining cytospin preparation for hemoglobin (see 2.2.3).

2.2.2 Freezing and thawing of cells

Cells were counted and harvested. $5-10x10^6$ cells were resuspended in 1 ml of FCS and DMSO was added dropwise to a final concentration of 10%. Cells were frozen in a polystyrene box at -80°C to allow a slow cooling. After 5-7 days cells were transferred to liquid nitrogen for longterm storage.

For thawing, cells were immersed in a 37°C water bath, transferred in a 50 ml Falcon tube and DMSO concentration was gently reduced by the dropwise addition of 10 ml cold culture medium. Cells were centrifuged and resuspended in new culture medium to remove DMSO. Cells were seeded twice as densely as usual and adjusted to maintenance density the following day.

2.2.3 Cytospins

For analysis of cell morphology, cells were cytocentrifuged onto glass slides (700 rpm, 7 min; Cytospin 2, Shandon Inc., Pittsburgh, USA) and stained with neutral benzidine and histological dyes as previously described (Beug et al., 1982, Panzenböck et al., 1998). Photographs were taken with Axiophot II microscope

(Zeiss) and a Kontron ProgRes 3012 CCD camera and processed with Adobe Photoshop software.

2.2.4 Cell proliferation assays

2.2.4.1 ^{3}H-thymidine incorporation

Cell proliferation was assessed quantitatively by measuring the rate of ^{3}H-thymidine incorporation (Kowenz et al., 1987, Panzenböck et al., 1998). $4x10^{4}$ cells per well were incubated in microtiter plates for 48 hrs at 37°C in 100 µl culture medium containing various growth factors or combinations thereof. ^{3}H-thymidine (0.75 µCi per well; specific activity 30 Ci/mmol; Amersham, Little Chalfont, UK) was added and cells were incubated for 2 hrs. Then cells were lysed by one cycle of freezing and thawing, and harvested onto filter mates. ^{3}H-thymidine incorporation was measured in a Microbeta counter (Wallac, Turku, Finnland).

2.2.4.2 CFSE labeling

$2.5x10^{5}$ cells were washed in PBS and incubated with 5,6-carboxylfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Inc. Eugene, USA; f.c. 2.5 µM in PBS) for 10 min at 37°C. After washing, cells were seeded in medium containing various growth factors or combinations thereof. The loss of CFSE was analyzed after 2 days by flow cytometry with FACScalibur device and CellQuest software (BD Bioscience, SanJose, CA). When required cells were exposed to specific antibodies and PE labeled secondary antibodies as described (2.2.6.1).

2.2.5 Colony assay

$5x10^{4}$ and $1x10^{5}$ cells were plated in 0.75 ml aliquots in 12 well plates containing methylcellulose medium. Methylcellulose medium contained 0.9% methylcellulose in Iscove's Modified Dulbecco's Medium (IMDM; MethoCult H4100, Stemcell Technologies Inc., Vancouver, Canada), supplemented with 30% heat-inactivated FCS, 1% detoxified BSA, 2 mM L-glutamine, 0.1 mM β-ME, 0.128 mg/ml iron-saturated human transferrin (Sigma), 3 U/ml Epo, 50 ng/ml SCF, 10 ng/ml IL-3, 10

ng/ml GM-CSF and 2 µg/ml insulin. Cultures were incubated for 14 days in 5% CO_2 and high humidity at 37°C. Triplicate plates containing 30 and more colonies were analyzed for colonies using a stereo microscope. Burst-forming units erythroid and colony-forming units erythroid type colonies (BFU-E and CFU-E, respectively) were evaluated at day 10 and 14. Similarly, colony-forming units granulocyte, erythrocyte, monocyte, macrophage (CFU-GEMM) colonies, colony-forming units granulocyte, macrophage (CFU-GM), macrophages and dendritic cell colonies were identified morphologically and evaluated.

2.2.6 Flow cytometry

2.2.6.1 Single fluorescence staining

Surface antigen expression of cells was analyzed by flow cytometry. Fluorescence staining was done according to Diebold et al., 1999. Cells were pre-incubated with 1% BSA and 1% human IgG in PBS for 1 hr and than reacted with specific antibodies for 1 hr (Venimmun; Behringwerke, Marburg, Germany). This incubation was followed by 1 hr reaction with FITC or PE-conjugated anti-mouse, anti-rabbit or anti-goat IgG (Fc specific; Sigma). Cells were washed once and resuspended in PBS containing 1% BSA and propidium iodide (PI, 2 µg/ml; Sigma) for gating on viable cells. For flow cytometry a FACScalibur device with CELLQuest software (BD Bioscience, SanJose, CA) was used.

2.2.6.2 Double fluorescence staining

In addition to the previously described staining procedure a second specific antibody was incubated for 1hr. An antibody directly labeled with a different fluorescence molecule than the secondary antibody of the first staining was used.

2.3 Molecular biology methods

2.3.1 RNA isolation

Cells were counted and washed with ice cold PBS. For isolation of RNA, Qiagen RNeasy Mini Kits including a DNase digestion (Qiagen, Hilden, Germany) were

used. RNA was resuspended in 100 µl RNAse free water (Qiagen) and the concentration was measured (2.3.2). RNA preparations were stored at –40°C.

2.3.2 Measurement of RNA and DNA

RNA and DNA concentrations were determined using UV transparent 96 well plates (Costar, Corning Inc. Life Science, Acton, USA) and a SpectraMax Spectrometer (Molecular Devices, Sunnyvale, USA). Concentrations and OD 260/280 nm ratios were calculated by Softmax software (Molecular Devices).

2.3.3 Reverse transcriptase polymerase chain reaction (RT-PCR)

2.3.3.1 cDNA synthesis

A standard random primed first-strand cDNA synthesis reaction consisted of 5 µg total heat-denatured RNA as template, 25 pmol/µl pd(N6) random hexamers (Amersham Biosciences, Uppsala, Sweden), 25 U RNAsin (Promega GmbH, Mannheim Germany), 125 U MuMLV reverse transcriptase (Promega), 10 µM dNTPs in a final volume of 25 µl MuMLV buffer supplied by the manufacturer. The reaction was performed at 37°C for 1 hr and stopped by heat inactivation (5 min at 95°C). 2 µl of the resulting cDNA preparation were subjected to standard PCR.

2.3.3.2 Polymerase chain reaction (PCR)

PCR was performed in a PTC-200 thermal cycler (MJ Research, Inc. Waltham, USA).

Briefly, 3 µl reverse transcribed cDNA was added to 47 µl PCR reaction mix containing 1xPCR buffer (-$MgCl_2$), 2 mM $MgCl_2$, 4 µM dCTP, dGTP, dTTP and dATP each, forward and reverse primers for human Epo and GAPDH (see 2.1.5) in a final concentration of 2 pmol/µl and 1U Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany).

After an initial denaturation for 120 sec at 95°C, 35 PCR cycles were performed as follows: denaturation for 30 sec at 95°C, annealing for 30 sec at 58 or 68°C, elongation for 30 sec at 72°C. The 35 cycles were followed by a 4 min elongation period at 72°C. PCR products were analyzed by agarose gel electrophoresis and stored at 4°C.

2.3.4 Agarose gel electrophoresis

Solutions of agarose (Gibco BRL, Invitrogen life technologies, Carlsbad, USA) in TBE were melted in a microwave oven and cooled down to 70°C before addition of 50 µg EtBr per 100 ml agarose solution. DNA samples in DNA- and RNA in RNA-loading buffer were loaded and electrophoresis was performed in TBE. The BioDoc Analyze system (Biometra, Göttingen, Germany) was used for visualization and documentation of the results.

2.3.5 Cloning of DNA fragments

2.3.5.1 Gel purification of DNA fragments

Agarose gel electrophoresis using 1.5% SeaPlaque agarose (FMC Bioproducts, Rockland, USA) in TBE buffer was performed. Bands of correct size were excised and DNA fragments were purified using the QIAquick kit (Qiagen). DNA was resuspended in elution buffer supplied by the manufacturer.

2.3.5.2 Ligation

Gel purified DNA fragments were cloned using the TA Cloning Kit (Invitrogen, Leek, Netherlands) ligating 20 ng of PCR fragments into 50 ng pCR2.1 vector in a final volume of 10 µl. Ligation was performed overnight at 16°C, according to the manufacturers instructions.

2.3.5.3 Heat shock transformation

For the transformation, Ca^{2+} heat shock competent E. coli cells were thawed on ice. 5 µl ligation mixture were added to 50 µl cell suspension. After 20 min incubation on

ice, a heat shock was performed at 42°C for 45 sec, and the cells were placed on ice. 950 µl of SOC medium was added, and the mixture was incubated 30 min at 37°C, vigorously shaken. Bacteria were plated on LB agar plates containing 150 µg ampicillin per ml.

2.3.5.4 Detection of recombinant plasmids using b-glactosidase

For blue/white selection 75 µl of X-Gal solution (25 mg/ml EtOH) and 25 µl 200 mM IPTG were plated on the agar plates 30 min prior to plating the bacteria. Only white colonies were picked for further analysis.

2.3.6 Plasmid preparation

2.3.6.1 Plasmid mini preparation

Bacteria of 1.5 ml overnight culture were harvested by centrifugation and lysed in 150 µl TELT buffer (50 mM Tris-HCl pH 7.5, 62.5 mM EDTA, 2.5 M $LiCl_2$, 0.4 % Triton X-100) containing 15 µl lysozyme (10 mg/ml). After an incubation of 5 min at RT, bacterial lysates were boiled for 2 min and incubated 5 min on ice. Supernatant was cleared from proteins and genomic DNA by centrifugation and the pellet was removed. DNA was precipitated using 115 µl isopropanol and the pellet was washed in 70% EtOH. The dried DNA pellet was resuspended in 50 µl sterile water and subjected to an analytical restriction digest.

2.3.6.2 Plasmid midi preparation

Plasmid DNA was prepared using the plasmid midi kit (Qiagen) according to the manufacturer's protocol. DNA was resuspended in an appropriate volume of water and stored at –20°C.

2.3.7 Sequencing

Nucleotide sequences were determined using the BigDye™ Terminator Cycle Sequencing Kit (PerkinElmer Life and Analytical Sciences, Inc., Boston, USA) according to the manufacturer's protocol. The Sequences were analyzed on an

ABIPrism 310 Genetic Analyser (Perkin Elmer) and identified on the basis of homology by BLAST alignment tools (www.ncbi.nlm.nih.gov/blast/blast.cgi).

2.3.8 Radioactive labeling of probes

^{32}P labeled probes were prepared with the "High Prime Labeling Kit" (Roche Diagnostics) and α-^{32}P dCTP (3000 Ci/mmol; Easytides, NEN Research Products, Boston, USA) according to the manufacturer's protocol. The probe was precipitated with 1x volume isopropanol and 5 µg glycogen as a carrier. Afterwards, the probe was washed with 70% EtOH in order to remove non-incorporated dCTP, resuspended in water, denatured 5 min at 95°C and chilled on ice prior to hybridization.

2.3.9 Southern blot hybridization

Southern transfer of PCR fragments from 1.5% agarose gels onto Gene-Screen membrane (NEF-972, NEN Research Products) was performed as described (Asubel, F.M. et al. [Eds.], Current Protocols in Molecular biology, section 2.9.1-2.9.6). 10xSSC buffer was used as transfer buffer. After crosslinking the blotted DNA to the membrane using an UV crosslinker (Biorad, Hercules, USA), the blot was pre-hybridized for 1 hr in Church buffer containing 0.1 mg/ml salmon sperm DNA (Roche Diagnostics) as blocking reagent. Hybridization was performed overnight at 65°C in Church buffer containing ^{32}P-labeled fragments of human Epo cDNA. Membranes were washed with washing buffer (40 mM NaP_i, 1% (w/v) SDS) and exposed to X-ray film for various time periods.

2.3.10 DNA Microarray analysis and bioinformatics

Gene expression analysis was performed using the Affymetrix GeneChip System. Human HG_U95Aver2 gene chip arrays (Affymetrix, Inc., Santa Clara, USA) were used. In brief, 7µg of total RNA were used for cDNA synthesis according to the Expression Analysis Technical Manual (Affymetrix). cRNA was generated with the BioArray High Yield Transcription Labeling Kit (Enzo, Farmingdale, USA) and fragmentated. The quality of *in vitro* transcription and fragmentation was controlled by agarose gel electrophoresis. Subsequently, 15 µg cRNA were hybridized to arrays

(GeneChip Hybridization Oven 640, Affymetrix; 16h, 45°C). DNA microarrays were stained using the GeneChip fluidics station 400 (Affymetrix), washed and scanned by HP Gene Array Scanner (Hewlett Packard). Scanned GeneChip.DAT files were initially analyzed by the GeneChip Analysis Suit Software (MAS version 5, Affymetrix). Data output by MAS version 5 were further analyzed in GeneSpring™ Software (Silicon Genetics, Redwood City, CA).

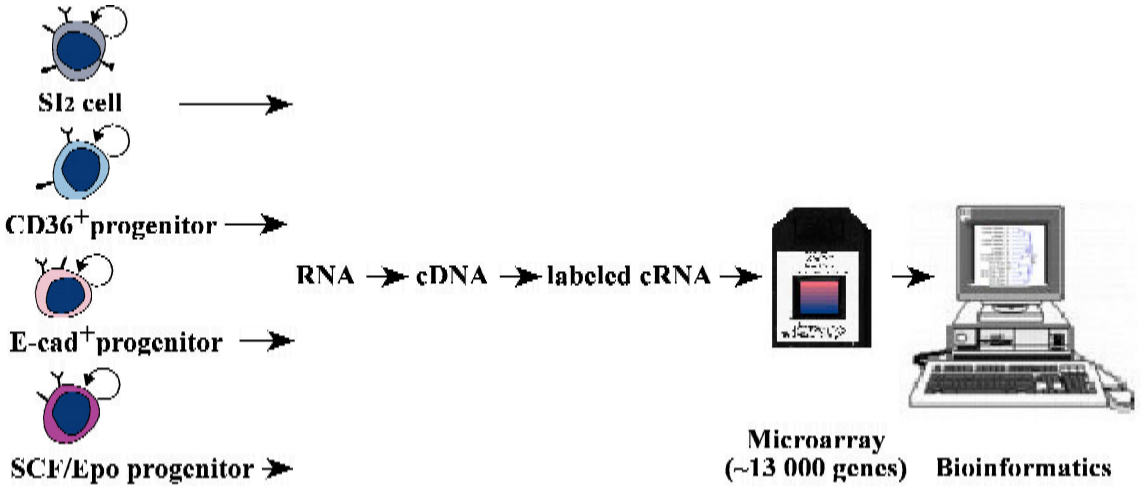

Figure 2.1 : Experimental design.

Different cell types were obtained from tissue culture, RNA was isolated and processed. Following the probe preparation, labeled cRNA was hybridized on DNA arrays. After scanning of arrays data analysis was performed.

Before analysis in GeneSpring™ Software data were normalized per sample and gene using the median value of the genes' expression values. Transcripts called "absent" by Affymetrix software in more than 2 samples of all samples analyzed were considered as being not expressed. The expressed transcripts were compiled into a data base of 7,370 transcripts. Classification of genes into functional groups was done according to Hacker et al., 2003.

Hierarchical clustering (Eisen et al., 1998) of all analyzed samples was done with GeneSpring™ Software using the Pearson correlation with a separation ratio of 0.5 and a minimum distance of 0.001 for the 7,030 expressed genes. To enhance the reliability GeneSpring function "Add data file restriction" was used to filter for genes "present" across replicates in the sample groups. Respective lists of transcripts that

were present in 3 out of 4 SI2 cell samples, 2 out of 3 $CD36^+$ progenitor samples, both E-cad^+ progenitor samples and in 5 of the 6 SCF/Epo progenitor samples were established.

As another means of testing the variation of signals the coefficient of variation (CV=stdev/mean) in % was used. CV of reference transcripts encoding for housekeeping genes like GAPDH, cyclophillins, β-actin and ribosomal genes of all samples was calculated by Microsoft Excel software. The distribution of CV ranged from 40-60%. All transcripts showing a CV higher than 60% within replicates were excluded from the gene lists. Of 12,625 transcripts tested, 4,899 passed the restriction for $CD36^+$ progenitors, 4,545 transcripts for E-cad^+ progenitors and 4,455 transcripts for SCF/Epo progenitors. Respective gene lists of $CD36^+$ progenitors, E-cad^+ progenitors and SCF/Epo progenitors were intersected via Venn-diagrams. Means of expression levels of transcripts expressed in two intersected cell types, referred to as commonly expressed genes, were filtered for Max/Min ≥2 to obtain differentially expressed genes.

3 RESULTS

3.1 Purification and characterization of different progenitor cells

3.1.1 Purification of hematopoietic stem/progenitor cells from cord blood

Human umbilical cord blood samples were depleted from mature erythrocytes by Ficoll purification (see 2.2.1.1). $2.9 \pm 1.4 \times 10^6$ MNC were routinely purified from 1 ml cord blood. These cells were either cultivated in SCF, Epo and Dex (see 2.2.1.1) or subjected to $CD34^+$ immunomagnetic bead selection (see 2.2.1.2). The total cell recovery after $CD34^+$ selection was reproducibly 1.25 ± 0.53 % of MNC.

$CD34^+$ cells were cultured in StemSpan serum free culture medium containing SCF, IL-3 and hyper-IL-6 referred to as SI2 conditions or containing SCF, Tpo, hyper-IL-6 and Flt3L referred to as STIF conditions (see 2.2.1.2). After 8 days of culture $CD36^+$ and E-cad $^+$ progenitors were selected by magnetic bead selection from amplified hematopoietic stem/progenitor cells. The total cell recovery after the corresponding selections was reproducibly 20-30%.

3.1.2 Efforts to identify early red cell progenitors by growth factor response

3.1.2.1 Proliferative response to multiple growth factor combinations

These experiments were performed in order to identify a growth factor combination supporting the proliferation of early red cell progenitors, which might be Epo-independent. Therefore, growth factors were chosen which are known to act as mitogens in various tissues. The rate of DNA synthesis of cells in response to these factors was measured.

SCF/Epo progenitors, previously amplified with the corresponding growth conditions for 10 days, were starved for 8 hrs without growth factors. Subsequently cells were incubated with IGF-1, insulin, GH, bFGF, PDGF, EGF, HRGβ1 and Dex in combination with Epo, SCF or both factors. After 48 hrs incubation the proliferative response of cells was assessed by ^{3}H-thymidine incorporation.

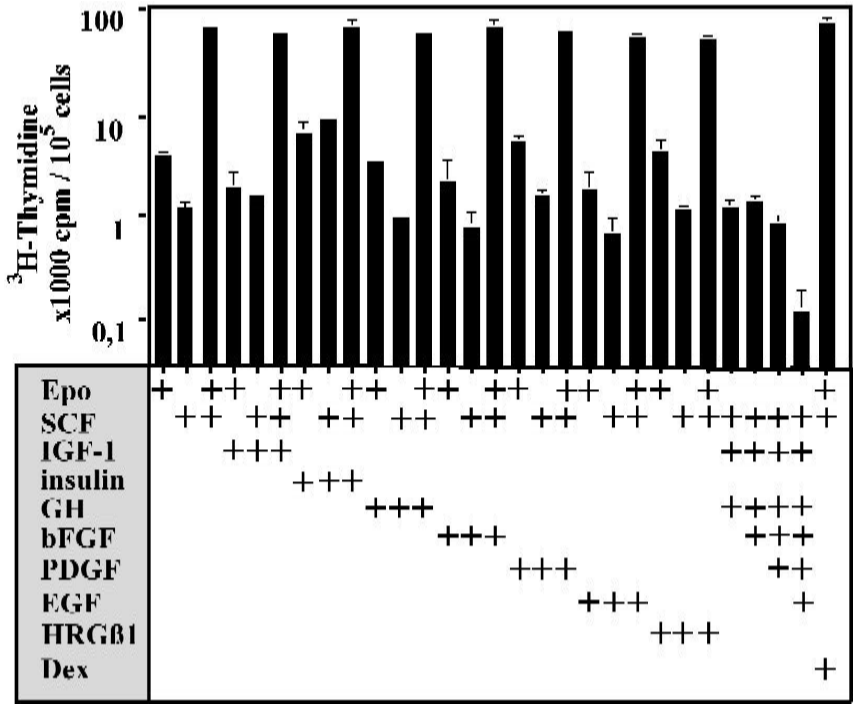

Figure 3.1 : Response of SCF/Epo cells to multiple growth factors.

^{3}H-thymidine incorporation in response to Epo, SCF, IGF-1, insulin, GH, bFGF, PDGF, EGF, HRGb1 and Dex is shown. Factors were applied in various combinations. ^{3}H-thymidine incorporation was determined 48 hrs after addition of factors. Results are means of triplicate values. For details see 2.2.4.1.

Epo and SCF applied simultaneously was the most potent factor combination (Fig. 3.1). As described before (Panzenböck et al., 1998) both factors synergize in inducing DNA synthesis. There was no increase of DNA synthesis when additional factors were combined with SCF and Epo. Insulin enhanced the effects of either Epo or SCF on ^{3}H-thymidine incorporation to some extend but not when all three factors were combined. No factor combination without Epo showed a proliferative potential on starved SCF/Epo cells which would support growth of an Epo-independent early progenitor.

3.1.2.2 Proliferative response of SCF/Epo cells to HER ligands and hyper-IL-6

TGFα plays an important role in growth of early chicken red cell progenitors (Hayman et al., 1993; Steinlein et al., 1995; Beug et al., 1995). TGFα belongs to the

group of human EGF receptor (HER) ligands. These results led to the investigation of the effect of TGFα and other HER ligands, like epidermal growth factor (EGF), amphiregulin (AR), heparin binding (HB)-EGF, betacellulin (BTC), heregulin β1 (HRGβ1), on human red cell progenitors. Additionally, a stimulation of Epo-independent growth of primitive human red cells by a complex of IL-6/soluble IL-6 receptor has been reported (Sui et al., 1995). Thus, the IL-6/ soluble IL-6 receptor fusion protein hyper-IL-6 designed by Fischer et al., 1997 was used to investigate this effect on starved SCF/Epo progenitors.

Fig. 3.2 shows the proliferative potential of SCF, Epo, TGFα, EGF, AR, HB-EGF, BTC, HRGβ1 and hyper-IL-6 in combination with SCF or, SCF and Epo. Factors were added after 8 hrs of starvation SCF/Epo progenitors at day 10 of cell culture. As controls, the effects of standard conditions with SCF, Epo, Dex and of starving conditions without factors are shown. The effect was measured after 48 hrs in a ^{3}H-thymidine incorporation assay. All factors were tested in normal and low serum conditions to measure the influence of FCS on the growth factor response.

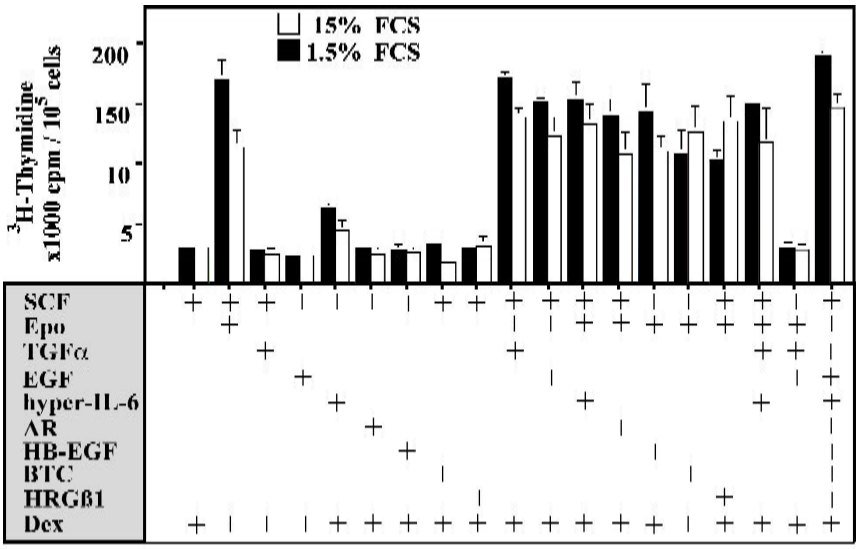

Figure 3.2 : Response of SCF/Epo cells to HER ligands and hyper-IL-6.

^{3}H-thymidine incorporation in response to SCF, Epo, hyper-IL-6 and the HER ligands: TGFa, EGF, AR, HB-EGF, BTC, HRGb1 is shown. Cells were seeded in respective culture media containing either 1.5% or 15% FCS. Factors were applied individually or in various combinations. ^{3}H-thymidine incorporation was determined 48 hrs after addition of factors. Results are means of triplicate values.

Again, the most effective combination of factors was Epo and SCF applied simultaneously. There was no significant increase of DNA synthesis when other factors were combined. HER ligands added together with SCF did not show any additional influence to the effect of SCF itself. There was a slight effect of hyper-IL-6 in combination with SCF in comparison to HER ligand combinations with SCF. TGFα and EGF in combination seemed to inhibit the effect of SCF and Epo.

In most of the experiments normal FCS content seemed to decrease the effect of growth factors. This might be due to the fact that FCS contains substances, which have inhibiting effects on the activity of growth factors due to unspecific binding.

A titration of AR and HRGβ1 was performed in order to investigate whether HER ligands are effective in different concentrations as the ones that have been previously used. Various concentrations of both ligands and in combination with SCF were added to 10 day old SCF/Epo progenitors after 8 hrs of starvation, and increase of DNA synthesis was measured.

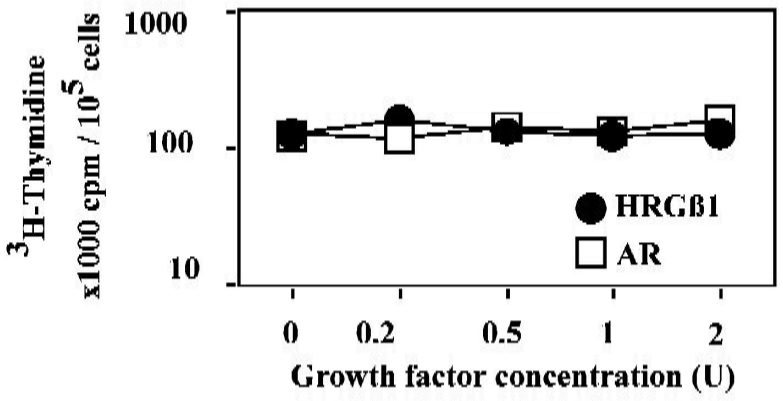

Figure 3.3 : Titration of HER ligands.

^{3}H-thymidine incorporation in response to different concentrations of AR and HRGb1 is shown. Factors with increasing concentration of factors were applied. ^{3}H-thymidine incorporation was determined 48 hrs after addition of factors. Results are means of triplicate values.

Even for different concentrations, no effect in ^{3}H-thymidine incorporation was observed for AR and HRGβ1.

In conclusion there was no evidence found that HER ligands support the proliferation of SCF/Epo progenitors, in particular not the Epo-independent growth.

3.1.3 Hematopoietic stem/progenitor cells under different growth conditions

3.1.3.1 Growth properties under SI2 and STIF conditions.

For *in vitro* amplification of early hematopoietic stem/progenitor cells various cytokines and cytokine combinations were reported to have a stimulating effect. Previous work of our research group has shown that liquid culture of stem/progenitor cells in serum free StemSpan medium supplemented with SCF, Tpo, hyper-IL-6 and Flt3L (STIF condition) supports the proliferation of these cells (Hacker et al., 2003; Ju et al., 2003). Sui and colleagues suggest the amplification of cord blood derived stem/progenitor cells in serum free medium complemented with SCF, IL-6/sIL-6R and IL-3 (Sui et al., 1996). A similar growth factor combination, where IL-6/sIL-6R was replaced by hyper-IL-6, is referred to here as SI2 conditions. To compare both growth conditions, growth properties were tested by counting cells every day and determining cumulative cell numbers.

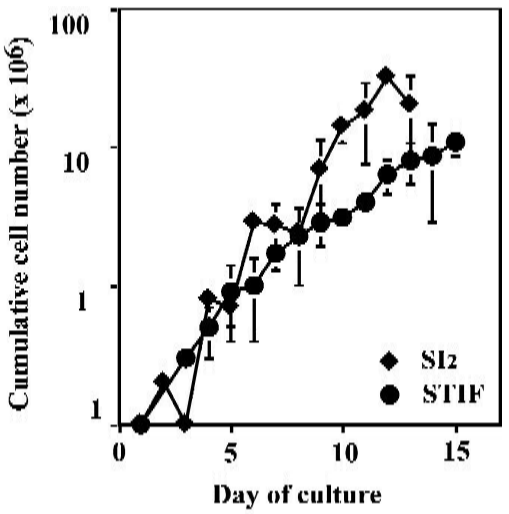

Figure 3.4 : Growth of hematopoietic stem/progenitor cells under different growth conditions.

Hematopoietic stem/progenitor cells were grown either under SI2 or STIF conditions. Cell density was determined every second day and cumulative cell numbers were calculated. Average values of 5 experiments are shown.

The growth curves in Fig. 3.4 show that both STIF and SI2 conditions supported the logarithmic cell growth within the first 15 days of cell culture. After 2 weeks the total cell number had increased about 100-fold for STIF conditions and about 200-fold for

SI2 conditions. Conclusively, SI2 conditions seemed to provide the hematopoietic stem/progenitor cells more effectively compared to STIF conditions.

3.1.3.2 Growth factor dependence

To assess the individual contribution of growth factors used under STIF and SI2 conditions for growth of hematopoietic stem/progenitor cells, the rate of DNA synthesis induced by these factors was measured. Therefore, cells were generated separately under both growth conditions. At day 10 of culture cells were starved for 8 hrs in StemSpan medium without factors. SCF, Tpo, hyper-IL-6, Flt3L, IL-3 were added individually or in combination, and after 48 hrs of incubation ^{3}H-thymidine incorporation was measured. Additionally, the effect of Epo on SI2 CELL grown in different conditions was determined.

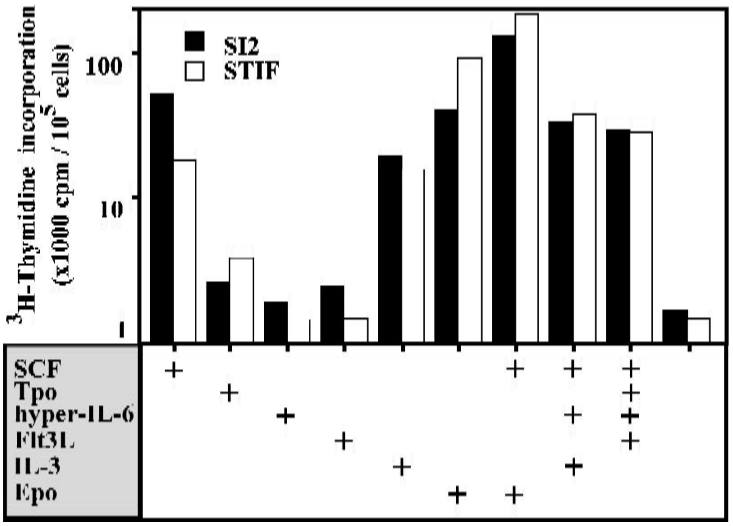

Figure 3.5 : Growth factor response of hematopoietic stem/progenitor cells grown in STIF or SI2 conditions.

^{3}H-thymidine incorporation in response to SCF, Tpo, hyper-IL-6, Flt3L, IL-3 and Epo is shown. Factors were applied individually or in various combinations as indicated for hematopoietic stem/progenitor cells grown in STIF or SI2 conditions. ^{3}H-thymidine incorporation was determined 48 hrs after addition of factors. Results are means of triplicate values.

Experiments showed that hematopoietic stem/progenitor cells grown in STIF or SI2 conditions showed similar response to different growth factors and their combinations.

It was not surprising to see an effect of SCF in both experiments. Also, IL-3 supported the proliferation of both hematopoietic stem/progenitor cells groups very effectively. In contrast, hyper-IL-6 seemed not to support the proliferation in either case. Tpo and Flt3L showed a very mild effect on cells grown in SI2 conditions. Tpo showed also a mild effect in STIF derived cells and Flt3L was without an effect.

Surprisingly, hematopoietic stem/progenitor cells grown either in STIF or SI2 conditions showed a clear response to Epo and to Epo in combination with SCF, despite the absence of Epo in the culture medium when cells were generated. Epo and SCF supplemented simultaneously stimulated most effectively the proliferation as shown for SCF/Epo progenitors (see 3.1.2.2).

3.1.3.3 Cell surface marker expression

The differences and common features of different growth conditions were also monitored by assessing the expression of specific cell surface markers by flow cytometry.

To determine the nature of cells grown in STIF (following referred to as STIF cells) or SI2 conditions (following referred to as SI2 cells) several lineage specific surface markers were analyzed (Table 3.1).

Lineage	Surface marker	STIF conditions	SI2 conditions
early progenitors	CD117/SCF receptor	+	+
	CD71/transferrin receptor	++	++
myeloid	CD33/siglec-3	+++	+++
	CD14	-	-
	MHC class II	(+)	(+)
	CD11a/αL integrin	+++	+++
	CD18/β2 integrin	+++	+++
	CD1a	-	-

Lineage	Surface marker	STIF conditions	SI2 conditions
erythroid	EpoR	+	+
	CD235ab/glycophorin A/B	-	-
	CD238/KELL	-	(+)
	CD233/band 3	-	-
others	CD36	+	++
	CD44	+++	+++
	CD105/endoglin	-	-
	E-cad	+	+

Table 3.1 : Cell surface marker expression of hematopoietic stem/progenitor cells in different growth conditions.

Hematopoietic stem/progenitor cells at day 8-10 of culture were reacted with specific antibodies. Cells were than analyzed by flow cytometry. Expression levels are indicated: +++ high expression; ++ expression; + weak expression; (+) expression not in all samples; - no expression

Cells grown either in STIF or SI2 conditions showed a similar pattern of surface marker expression. Cells expressed several specific myeloid markers (CD33, CD11a and CD18) but not every myeloid marker. EpoR was the only erythroid specific marker which was clearly expressed. KELL, glycophorin A/B and band 3 are known to be expressed at late stages of erythropoiesis. In both hematopoietic stem/progenitor cell groups these molecules were not expressed. This was not surprising since the cells were expected to be early hematopoietic progenitors. This fact was also supported by the expression of surface molecules (transferrin and SCF receptor) which have been reported to be markers for early hematopoietic progenitors (Broxmeyer, 1991; McNiece et al., 1991; Bühring et al., 1996). CD44 and endoglin are not specific marker for certain hematopoietic lineages. CD44 was highly expressed in cells under both growth conditions whereas endoglin was not expressed.

CD36 and E-cad are also not specific marker proteins for hematopoietic cells but they were reported to be also present on cell surfaces of early erythroid progenitors (see 1.3). Both molecules were expressed on hematopoietic stem/progenitor cells, no matter in which condition they were grown. Fig. 3.6 shows the simultaneous

expression of E-cad and CD36 on hematopoietic stem/progenitor cells grown in SI2 conditions as analyzed by flow cytometry.

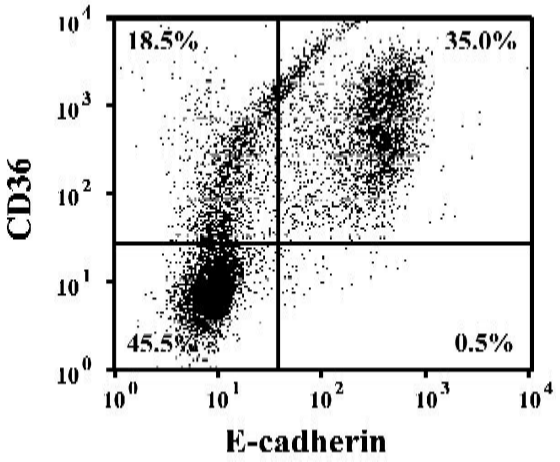

Figure 3.6 : Simultaneous expression of E-cadherin and CD36 on hematopoietic stem/progenitor cells.

Hematopoietic stem/progenitor cells grown under SI2 conditions for 8 days were double labeled with CD36-(PE) and E-cad specific antibodies. Numbers show the percentages of individual populations. Gating was for living cells. A representative experiment is shown.

The staining of cells with anti-E-cad and anti-CD36 antibodies revealed at least three distinct cell populations: a CD36 positive population ($CD36^+/E\text{-}cad^-$), an E-cad and CD36 double positive population ($E\text{-}cad^+/CD36^+$) and a population which was negative for both molecules ($E\text{-}cad^-/CD36^-$). It was obvious that all E-cad positive cells also expressed CD36, but there was a subpopulation of CD36 positive cells which did not express E-cad. Percentages of these populations are shown in Table 3.2.

Growth conditions	**$CD36^+/E\text{-}cad^-$**	**$E\text{-}cad^+/CD36^+$**	**$E\text{-}cad^-/CD36^-$**
STIF	14.6 ± 9%	20.9 ± 0.4%	63.1 ± 7.8%
SI2	21.1 ± 6.3%	32.3 ± 11.5%	44.3 ± 13.8%

Table 3.2 : Simultaneous expression of E-cadherin and CD36 on hematopoietic stem/progenitor cells amplified in STIF or SI2 conditions.

Hematopoietic stem/progenitor cells after 7-10 days of culture were reacted simultaneously with antibodies against E-cad and CD36. Expression was analyzed by flow cytometry. Numbers show the percentage of E-cad$^-$/CD36$^+$, E-cad$^+$/CD36$^+$ and E-cad$^-$/CD36$^-$ cell populations and the respective standard deviation.

The percentage of E-cad and CD36 double positive cells was higher under SI2 conditions than under STIF conditions. This was also observed for the E-cad$^-$/CD36$^+$ population. The double negative population was more abundant under STIF culture conditions. In SI2 derived cells CD36 expression seemed to be higher and in few samples a weak expression of KELL was found, which was absent in STIF derived cells.

3.1.4 Comparison of different progenitor cell types

3.1.4.1 Growth properties

Our efforts to identify immature red cell progenitors led to the purification of both the CD36 positive (referred to as CD36$^+$ progenitors) and E-cad positive cells (referred to as E-cad$^+$ progenitors) from SI2 cells by immunomagnetic bead selection. To compare the growth properties of SI2 cells, E-cad$^+$, CD36$^+$ and SCF/Epo progenitors, cells were cultivated in the corresponding growth conditions, counted every second day and cumulative cell numbers were determined.

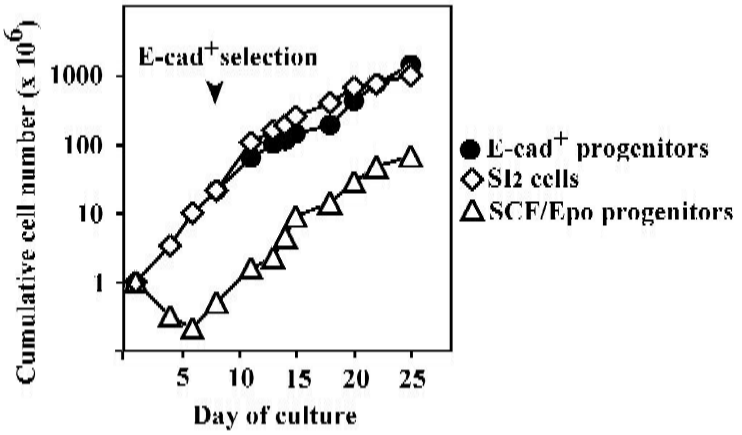

Figure 3.7 : Growth kinetics of human hematopoietic progenitors.
SI2 cells and E-cad$^+$ progenitors were grown in the presence of SCF, IL-3 and hyper-IL-6 under serum free conditions. Growth of SCF/Epo progenitors was performed according to Panzenböck et al., 1998. Cell density was determined every second day and cumulative cell numbers were calculated. Representative experiments are shown.

Both SI2 and SCF/Epo cells grown under the corresponding conditions showed similar growth properties from day 5-25 of culture. During the first week of culture the cell number of SCF/Epo progenitors decreased, but cells recovered after a few days and showed as well logarithmic growth properties from day 5 onward. At day 8 of culture SI2 cells were subjected to immunomagnetic bead selection for either E-cad or CD36. After selection progenitors were cultivated again under SI2 conditions. During the following days of culture both E-cad$^+$ (Fig. 3.7) and CD36$^+$ progenitors (data not shown) showed the same behavior in growth as the SI2 cells.

3.1.4.2 Growth factor dependence of SI2 cells

3.1.4.2.1 Growth factor dependence determined by ^{3}H-thymidine incorporation

To show the individual contribution of growth factors used for proliferation of different hematopoietic progenitors, the growth factor response of the respective cells was determined. At day 10 of cell culture or alternatively 2 days after immunomagnetic bead selection for E-cad$^+$ or CD36$^+$ progenitors, cells were starved for 8 hrs under serum free conditions without growth factors. Next, SCF, IL-3, hyper-

IL-6 and Epo were added individually or in combinations. After 48 hrs of incubation ^{3}H-thymidine incorporation was measured (see 2.2.4.1).

All 4 different cell types showed similar responses to the analyzed growth factors (Fig. 3.8). There was no effect, or only low effect, of IL-3 and hyper-IL-6. It was not surprising to see an effect of SCF on all cell types since all cells were grown in the presence of SCF. All analyzed cell types showed a response to Epo, despite the fact that SI2 cells, E-cad^{+} and CD36^{+} progenitors were previously grown in the absence of Epo. The SCF/Epo factor combination, the SI2 factor combination and SI2 factors plus Epo induced as well a high response in all 4 cell types. All 4 cell types analyzed seemed to be very similar in their growth factor response.

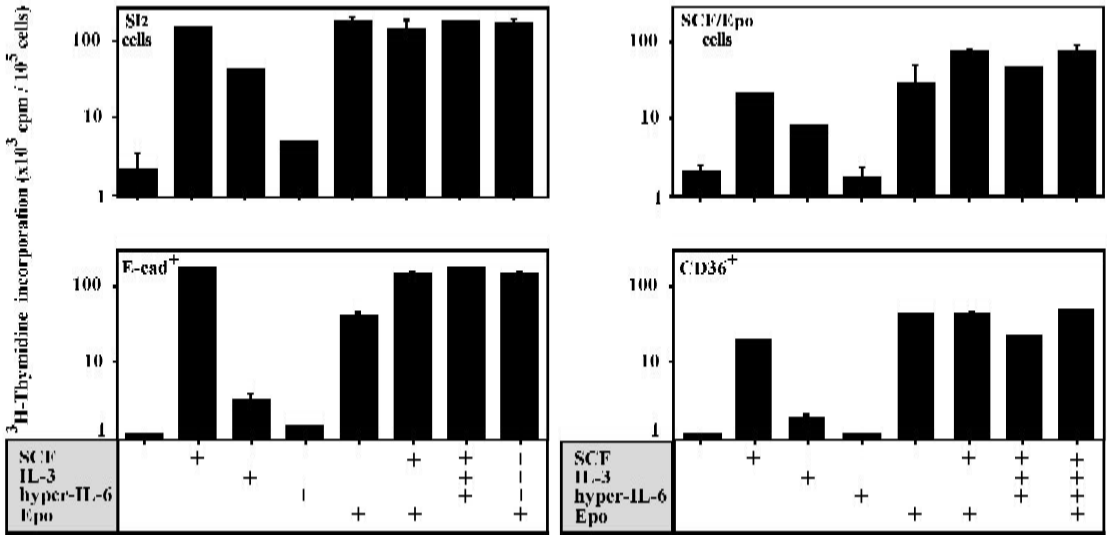

Figure 3.8 : Growth factor response of different hematopoietic progenitors.
^{3}H-thymidine incorporation in response to SCF, hyper-IL-6, IL-3 and Epo is shown. Factors were applied individually or in various combinations to SI2 cell, E-cad^{+}, CD36^{+} and SCF/Epo progenitor cells. ^{3}H-thymidine incorporation was determined 48 hrs after addition of factors. Results are means of triplicate values.

3.1.4.2.2 Growth factor response determined by CFSE labeling

The method of CFSE labeling was chosen to determine on individual cells the growth factor response of E-cad^{+} cells. Therefore, SI2 or SCF/Epo cells at day 10 of culture were labeled with the fluorescence dye CFSE and incubated with SCF, IL-3, hyper-

IL-6, Epo or combinations thereof (for details see 2.2.4.2). Cells proliferating in response to the respective growth factors lost the fluorescent signal of CFSE during cell divisions (Fig. 3.9.A). Loss of CFSE was followed by flow cytometry analysis. In addition CFSE labeled SI2 cells were stained with an E-cad specific antibody to observe the behavior of E-cad^{+} positive cells within the SI2 cell population. Cells were analyzed for 3 days after subjection to the respective growth factors.

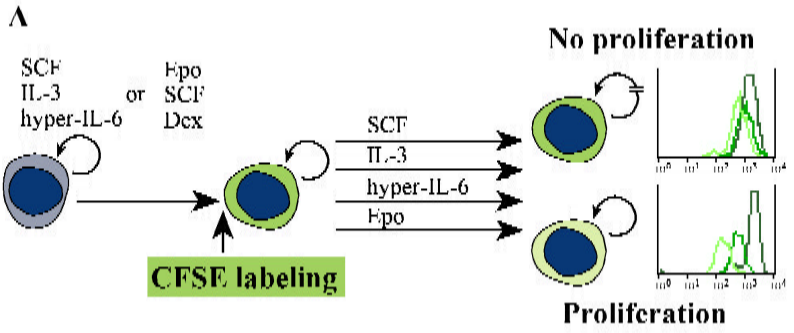

Figure 3.9 : Growth factor response of SI2 cells determined by CFSE labeling.
A: *Method of CFSE labeling. Progenitors were grown under SI2 or SCF/Epo conditions. Cells were labeled with the fluorescent dye CFSE at day 10 of culture and then grown in the presence of single growth factors or growth factor combinations for an additional 3 days. Proliferation of cells in response to the growth factors was analyzed by flow cytometry. Note the decrease of the fluorescent signal in proliferating cells.*

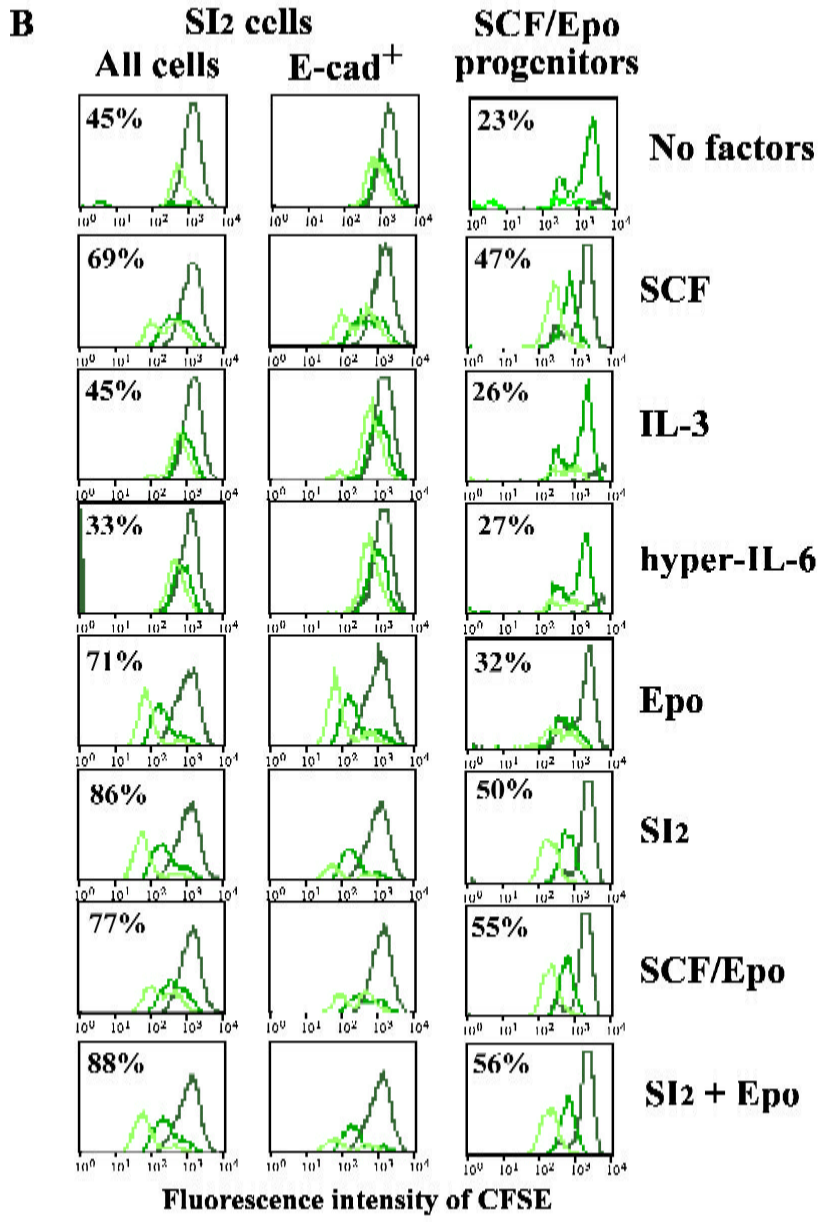

Figure 3.9 : Growth factor response of SI2 cells determined by CFSE labeling.

B: *Response of SI2 cells, E-cad+ cells within SI2 cell population and SCF/Epo progenitors to different growth factors. Cells were labeled with CFSE and fluorescence was measured after 1, 2 and 3 days of proliferation in SCF, IL-3, hyper-IL-6, Epo or SI2, SCF/Epo and SI2+Epo conditions. For further analysis cells were stained with an E-cad specific antibody. "All cells" refers to the entire cell population. "E-cad+" refers to E-cad positive cells. Gating was for living cells. The percentage of viable cells is shown in the upper left corner.(**d1**, d2 ,**d3** of CFSE labeling)*

The growth factor response analyzed by CFSE labeling showed a similar pattern in all three cell types. A daily loss of CFSE staining was observed when cells were treated with SCF, Epo, or the combination of both, in SI2 and SI2+Epo conditions. There was no effect of IL-3 and hyper-IL-6 detectable neither on SI2 cells nor on E-cad$^+$ progenitors, both factors showed a weak effect in SCF/Epo progenitors at day 2 of measurement. Surprisingly, SCF/Epo progenitors show just a weak response to Epo as a single factor.

In all experiments the viability of SI2 cells was higher than the viability of SCF/Epo progenitors. The highest differences in viability were found in experiments with Epo, SI2 and the combination of SI2+Epo growth factor combinations.

3.1.4.3 Phenotypes and cell surface marker expression under different growth conditions

To characterize the progenitor populations, cell surface antigen expression was monitored. The co-expression of E-cad and CD36, and different lineage specific markers was analyzed (Fig. 3.10). After immunomagnetic bead selection for CD36 the co-expression analysis showed clearly that there were still two subpopulations within the CD36$^+$ progenitors, an E-cad negative and an E-cad positive. On the contrary, the E-cad$^+$ progenitors showed just one main double positive population with co-expression of E-cad and CD36. SCF/Epo progenitors contained also just one population, namely the E-cad positive cells.

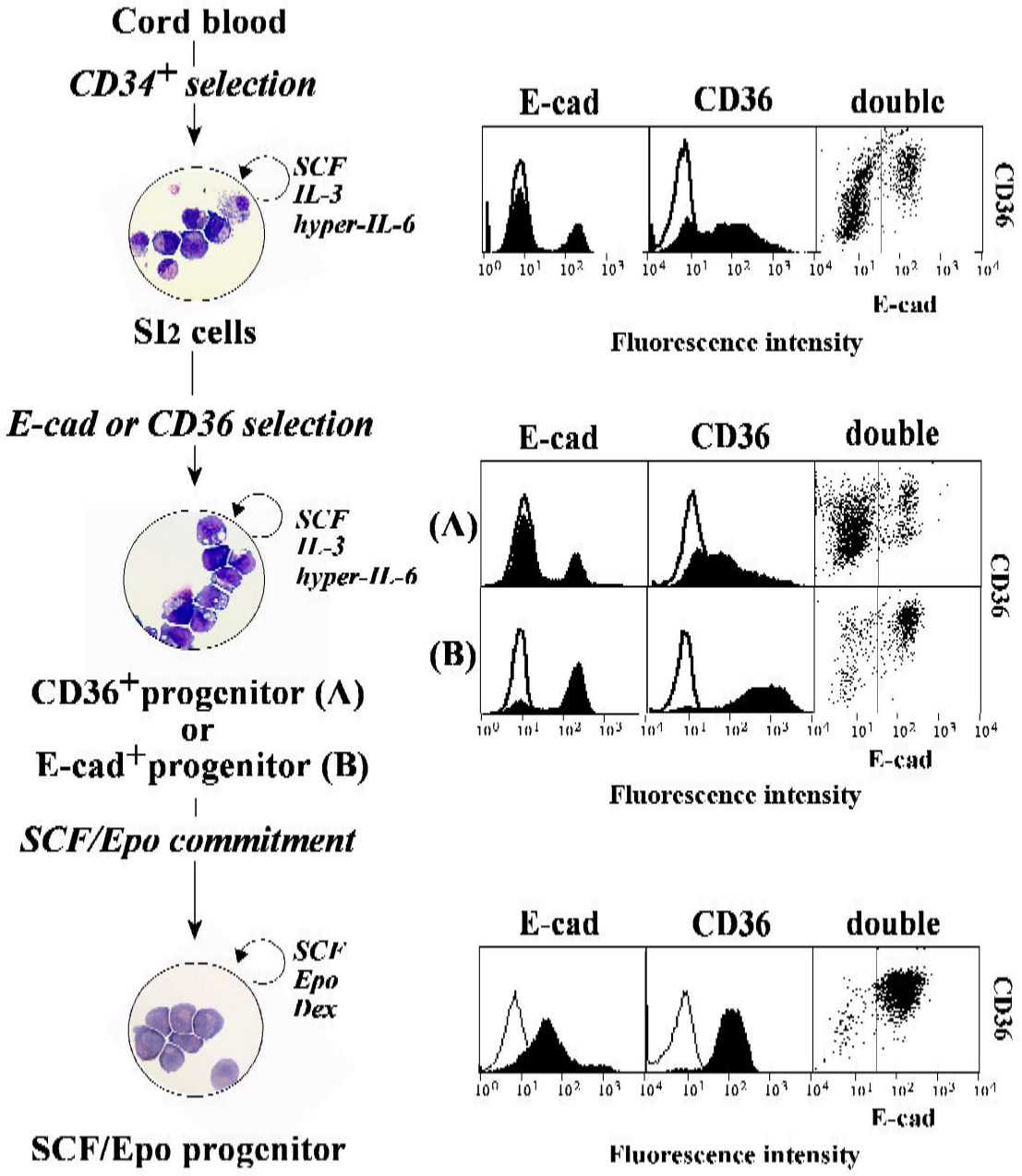

Figure 3.10 : E-cadherin and CD36 expression on different progenitors.

Photographs of SI2 cells, E-cad$^+$ and SCF/Epo progenitors are shown. Cells were subjected to cytocentrifugation and stained with neutral benzindine and histological dyes. Flow cytometry profiles, corresponding to the different progenitors and to CD36$^+$ progenitors, show the expression of the surface markers E-cad and CD36.

(A: CD36$^+$ progenitors after immunomagnetic bead selection and B: E-cad$^+$ progenitors after immunomagnetic bead selection)

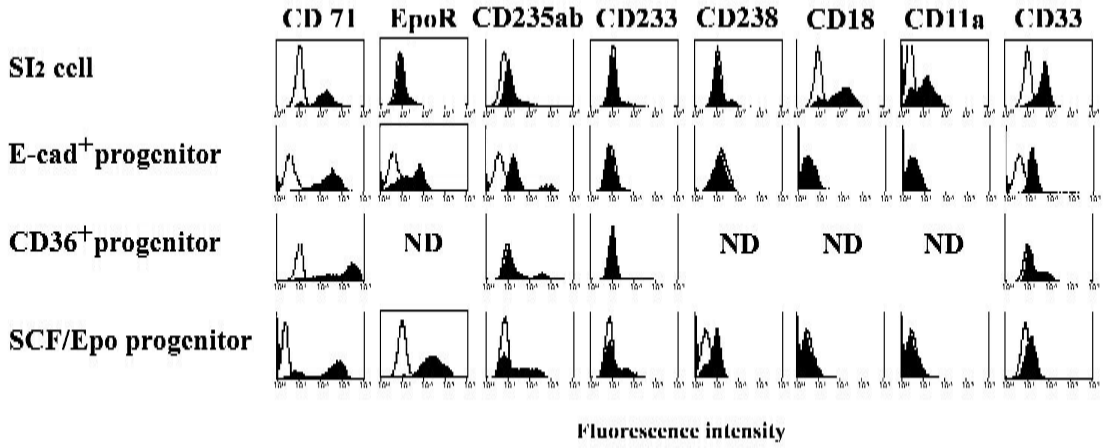

Figure 3.11 : Cell surface expression profiles of different progenitor populations. *Different progenitors were analyzed by flow cytometry for expression of erythroid specific markers: erythropoietin receptor (EpoR), glycophorin a/b (CD235a/b), band 3 (CD233) and KELL (CD238), myeloid specific markers: b2 integrin (CD18), a integrin (CD11a), CD33, CD14 and CD1a. Open areas represent staining of the respective cells with the corresponding secondary antibody as control.*

After the analysis of co-expression of E-cad and CD36, the expression of different lineage specific surface marker molecules was also analyzed on SI2 cells, E-cad$^+$, CD36$^+$ and SCF/Epo progenitors. The transferrin receptor (CD71) was highly expressed on all four progenitor cell types. Red cell specific markers such as erythropoietin receptor (EpoR), glycophorin A/B (CD235ab), band 3 (CD233) and KELL (CD238) were not expressed in SI2 cells. EpoR was effectively expressed on E-cad$^+$ and, as expected, on SCF/Epo progenitors. Low levels of glycophorin A/B as an early marker for erythropoiesis were detectable on SCF/Epo as well as on E-cad$^+$ and CD36$^+$ progenitors. Low levels of band 3 expression could be observed only on SCF/Epo progenitors. This progenitor type was also the only one which expressed KELL. SCF/Epo progenitors were clearly negative for all analyzed myeloid markers (CD18, CD11a, CD33, CD14 and CD1a). A proportion of SI2 cells expressed myeloid markers. Cells were negative for the macrophage specific CD14 and the DC specific CD1a but positive for the integrin LFA-1 (CD18, CD11a). E-cad$^+$ progenitors did not express LFA-1. CD33 expression on SI2 and E-cad$^+$ cells was always positive but the expression levels were variable in different preparations.

In conclusions, SI2 cells showed more myeloid properties concerning the surface marker profile whereas the SCF/Epo progenitors showed an erythroid specific surface marker profile. $CD36^+$ and E-cad^+ progenitors seemed to be more similar to the SCF/Epo progenitors than to SI2 cells in respect to the investigated surface markers. Only in the expression of CD33 are they similar to SI2 cells.

In addition, the morphology of SI2 cells, E-cad^+ and SCF/Epo progenitors was analyzed (Fig.3.10). Aliquots of the respective progenitor populations were taken, subjected to cytospin centrifugation and stained with neutral benzindine and histological dyes.

All three types of progenitor populations showed the typical morphological character of hematopoietic progenitors. The picture of SI2 cells showed a more heterogeneous population, whereas E-cad^+ and SCF/Epo progenitors looked very homogeneous. The vacuolization in SCF/Epo progenitors was lower than in E-cad^+ progenitors. Vacuoles present in E-cad^+ progenitors might be due to serum free growth conditions.

3.1.4.4 Potential of erythroid commitment and differentiation.

3.1.4.4.1 SCF/Epo commitment

After 8 days of culture in SI2 conditions SI2 cells were selected for either E-cad or CD36 positive cells by immunomagnetic bead selection. Selected cells were cultured for additional 2–4 days under culture conditions containing SCF, Epo and Dex according to Panzenböck et al., 1998. This commitment phase was followed by analyzing the expression of CD36, E-cad, CD71, erythroid lineage specific antigens and CD33 by flow cytometry.

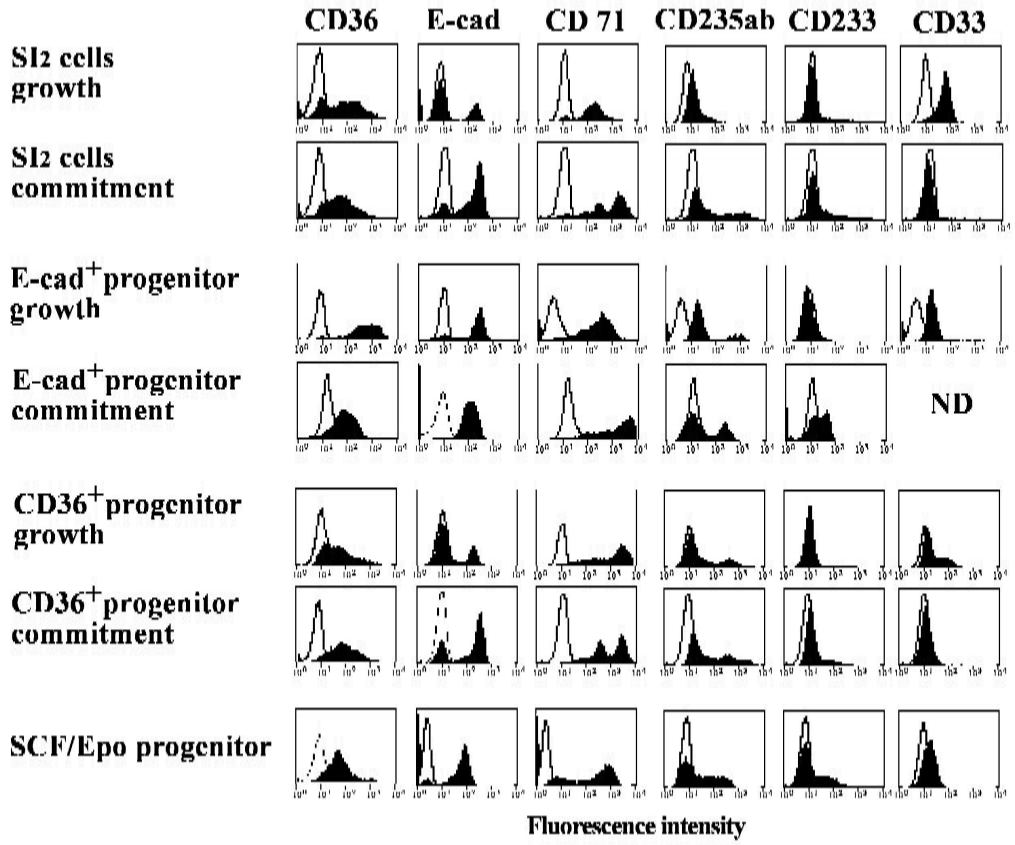

Figure 3.12 : Expression of various surface markers on different progenitor cells during the commitment in SCF/Epo conditions.

SI2 cells, E-cad$^+$ and CD36$^+$ progenitors growing under SI2 conditions were subjected to SCF/Epo commitment conditions (material and methods: 2.2.1.4). The commitment was followed by flow cytometry. Different lineage specific marker proteins were analyzed as indicated. For comparisons, SCF/Epo progenitors, not selected for CD34, were analyzed for the same antigens. Open areas represent staining of the respective cells with the corresponding secondary antibody as negative control.

All progenitor populations, SI2 cells, CD36$^+$ and E-cad$^+$ progenitors showed changes in their surface marker expression under the influence of SCF/Epo commitment conditions (material and methods: 2.2.1.4). In SI2 cells the ratio of cells negative for CD36 to cells positive for this marker was shifted in favor of the CD36$^+$ population. A similar shift in the favor of E-cad positive cells was detectable not only in SI2 cells but also in the CD36$^+$ progenitor population. The expression of the transferrin receptor (CD71) was enhanced in SI2 cells and E-cad$^+$ progenitors during the

SCF/Epo treatment. All three analyzed cell populations showed a slightly increased expression of the early erythroid surface marker glycophorin A/B (CD235ab) whereas no clear differences were detectable in the expression of band 3 (CD233). SI2 cells and $CD36^+$ progenitors were negative for CD33 after commitment. The analysis of SCF/Epo progenitors (not selected for CD34) for the same surface markers offered the possibility of comparing the committed SI2 cells, $E\text{-}cad^+$ and $CD36^+$ progenitors. It was clearly shown that all committed progenitor populations derived from $CD34^+$ cells generated surface marker profiles similar to the profile of SCF/Epo red cell progenitors.

In addition, growth properties of $E\text{-}cad^+$ progenitors during SCF/Epo commitment was analyzed. After magnetic bead selection at day 8 of cell culture $E\text{-}cad^+$ progenitor cells were cultivated either again under SI2 conditions or under SCF/Epo conditions. As a control the growth of the original SI2 cell population was measured during the same period.

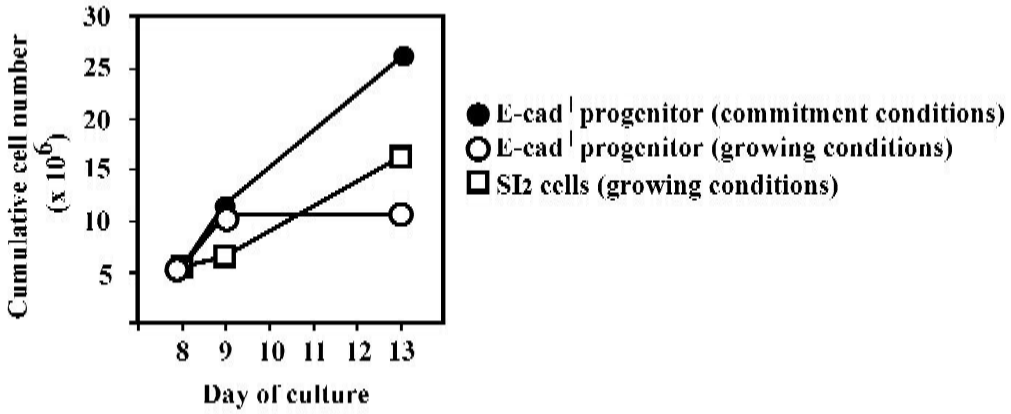

Figure 3.13 : Growth kinetics of $E\text{-}cad^+$ progenitors during the commitment under SCF/Epo conditions.

At day 8 of culture after magnetic bead selection $E\text{-}cad^+$ progenitors were grown either in SI2 conditions (referred to as growing conditions) or according to Panzenböck et al. (referred to as commitment conditions; Panzenböck et al., 1998, see 2.2.1.4). As a control SI2 cells were cultivated in SI2 conditions. Cell density was determined and cumulative cell number was calculated. Representative experiments are shown.

E-cad$^+$ progenitors in SCF/Epo commitment conditions showed the best growth properties but it was not clear whether this was due to a response to SCF and Epo or to more optimal growth conditions because of the serum containing culture medium.

3.1.4.4.2 Differentiation properties

The clonogenic potential of different progenitor populations was assessed in colony assay. Therefore cells were seeded after 10 days of liquid culture or 2 days after immunomagnetic bead selection in semi solid methylcellulose medium supplemented with Epo, SCF, IL-3, GM-CSF and insulin (see 2.2.5) that support the commitment and differentiation of both erythroid and myeloid blood cell lineages. About two weeks later the number of colonies, colony type and frequencies were evaluated.

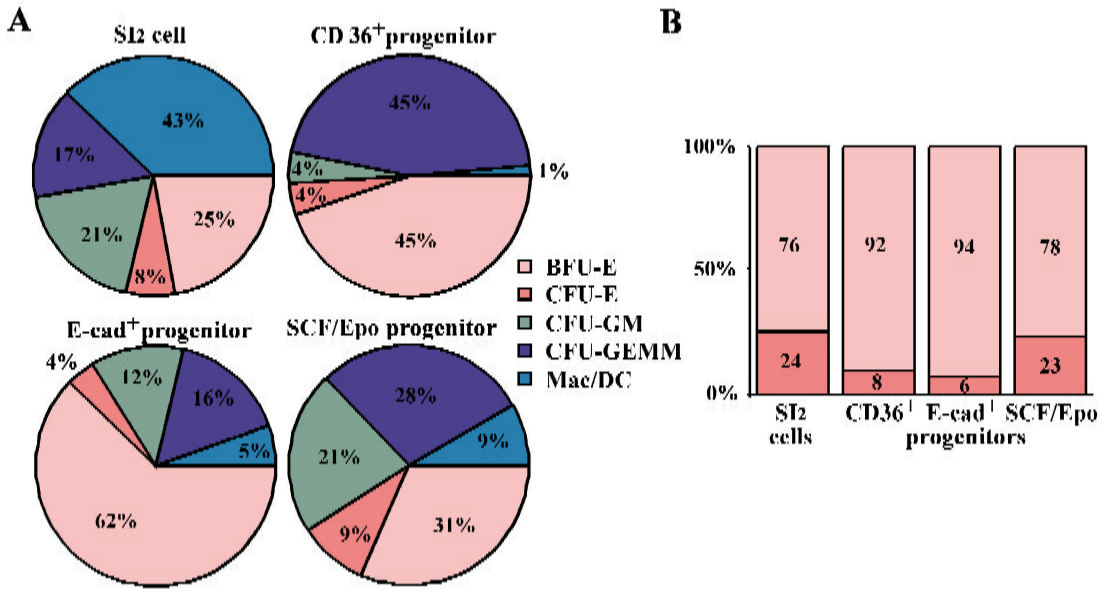

Figure 3.14 : Colony assay of different progenitors.

A: *SI2 cells, CD36$^+$, E-cad$^+$ and SCF/Epo progenitors were seeded in semisolid methyl cellulose medium with SCF, Epo, IL-3, GM- CSF and insulin. After two weeks BFU-E, CFU-E, CFU-GM, CFU-GEMM and colonies of macrophages/dendritic cells (Mac/DC) were determined. Numbers show the percentage of the corresponding colonies. Results are means of 4 different experiments.*

B: *incidence of erythroid colonies from the same cells as in (A) was observed after 10 or 14 days for CFU-E and BFU-E type colonies, respectively. The percentage of CFU-E and BFU-E type colonies of the total number of erythroid colonies is shown.*

All investigated cell populations showed a potential for both erythroid and myeloid lineage commitment. The myeloid component of SI2 cells seemed to account for more than three-quarters of the population. Remarkable was the high percentage of CFU-GEMM in $CD36^+$ progenitor cells.

E-cad^+ cells showed the highest potential for erythroid differentiation in colony assays. The sum of erythroid colonies represented nearly three-quarters of all colonies. The erythroid component of $CD36^+$ progenitors was less than 50%, followed by SCF/Epo progenitors with 40% and SI2 cells with 33%. In all four populations the percentage of CFU-E colonies was much lower than the percentage of BFU-E colonies (Fig. 3.14B).

In E-cad^+ and $CD36^+$ progenitors the frequency of BFU-E type colonies was particularly high and significantly higher than that obtained for SI2 cells and SCF/Epo progenitors. This finding indicated that E-cad^+ and $CD36^+$ progenitors exhibit features of early erythroid progenitors with properties similar to BFU-Es.

Fig. 3.15 shows representative photographs of BFU-E colonies derived from different progenitor types. In all BFU-E colonies a hemoglobin accumulation was observed but colonies of SI2 cells and E-cad^+ progenitors seemed to produce less hemoglobin than the BFU-E colonies derived from SCF/Epo progenitors. Additionally the portion of BFU-E colonies derived from SI2 cells and E-cad^+ progenitors was slightly smaller than that derived from SCF/Epo progenitors.

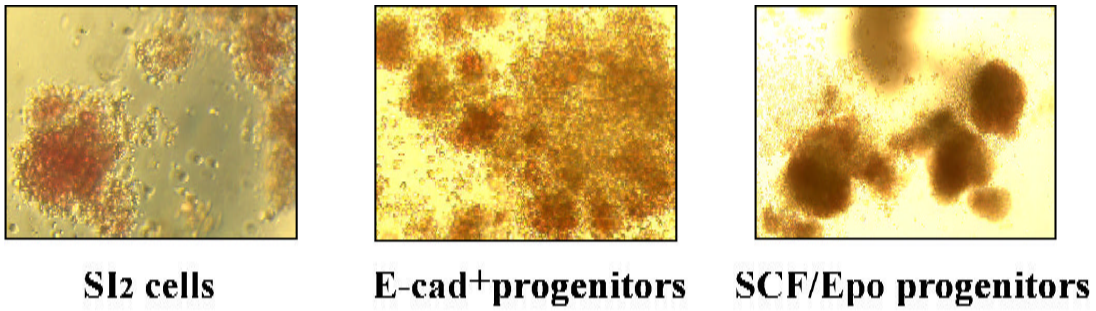

Figure 3.15 : BFU-E from different progenitors.

After 10 days of culture SI2 cells, E-cad$^+$ and SCF/Epo progenitors were seeded in semisolid methyl cellulose medium with SCF, Epo, IL-3, GM- CSF and insulin. After about two weeks photographs of BFU-E colonies were taken. Representative colonies are shown.

Panzenböck et al., 1998 described an *in vitro* differentiation system for SCF/Epo progenitors that supports erythroid cell differentiation in liquid tissue culture. Following induction of differentiation in the presence of Epo and insulin, cells begin to accumulate hemoglobin and gradually acquire the morphology of normal red cells within 5 days. This system was used to investigate the differentiation potential of E-cad$^+$ progenitors since this population was observed to be the more potent in erythroid lineage commitment colony assays.

First cells were withdrawn from the growth factors of SI2 conditions and subjected to the differentiating conditions containing Epo and insulin (see 2.2.1.4). Surprisingly the cells did not undergo a differentiation but died within 2 days (data not shown). Consequently E-cad$^+$ progenitors were first subjected to a commitment phase in SCF/Epo commitment conditions (see 2.2.1.4) for 2-4 days and than transferred to the corresponding differentiation conditions.

Following culture in Epo and insulin, cell size decreased from 12 µm to 6-7 µm at 4 days of differentiation (Figs. 3.16A and B). During this time cells accumulated hemoglobin as revealed by benzindine staining of cytospins preparations (Figs. 3.16B).

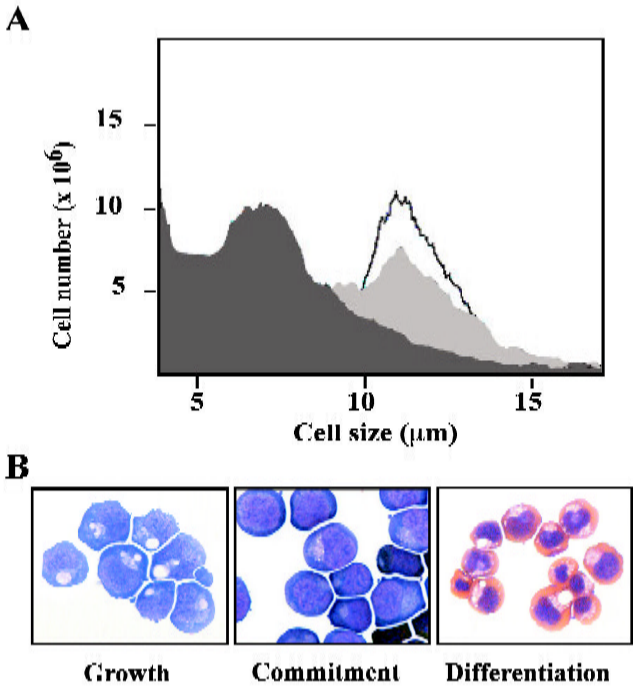

Figure 3.16 : Morphology of E-cad$^+$ cells during commitment and differentiation.
A: *Cell size profiles demonstrate the reduction in cell size during differentiation: undifferentiated cells at day 8 of culture under SI2 conditions, white; committed cells after 2 days of SCF/Epo growing conditions, gray; differentiated cells at day 4 in the presence of Epo and insulin, dark.*
B: *Aliquots of cell cultures were subjected to cytocentrifugation and stained with neutral benzindine and histological dyes. Photographs of E-cad$^+$ progenitors are shown.*

In addition, commitment and differentiation of E-cad$^+$ progenitors was followed by flow cytometry analysis of surface markers. During the differentiation of E-cad$^+$ progenitors E-cad and CD36 expression was downregulated. As expected, erythropoiesis specific markers, like glycophorin A/B (CD235ab), band 3 (CD233) and KELL (CD238), were not expressed in the starting cell population but upregulated during SCF/Epo commitment and differentiation. There was no detectable regulation of EpoR. CD33 was completely absent in differentiating cells.

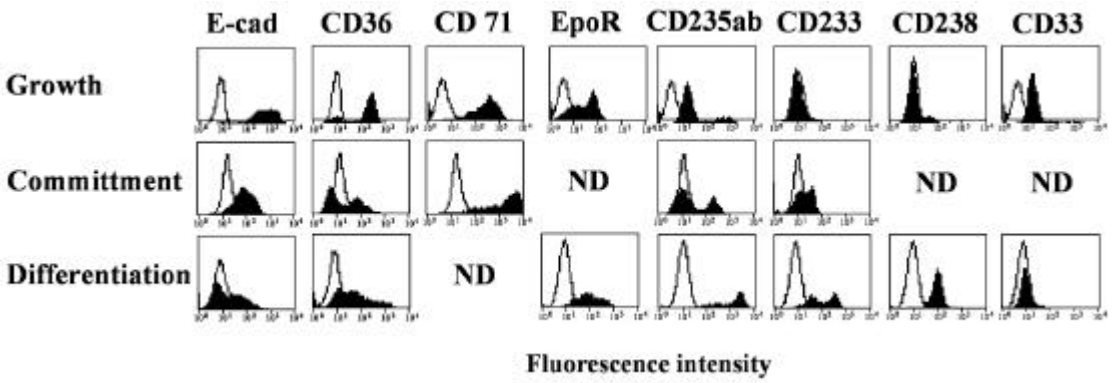

Figure 3.17 : Expression of various surface markers on E-cad$^+$ progenitors during commitment and differentiation.

E-cad$^+$ progenitors growing in SI2 conditions were subjected to SCF/Epo commitment conditions and Epo/insulin differentiation conditions. Commitment and differentiation was followed by flow cytometry. Different lineage specific marker proteins were analyzed as indicated. Open areas represent staining of the respective cells with the corresponding secondary antibody as negative control.

3.1.5 Epo-independence of different progenitor cells types

Previously it was shown that erythroid cells can themselves produce endogenous Epo and therefore have the potential to proliferate and differentiate in the absence of exogenous Epo (Sato et al., 2000). It was thus important to determine whether SI2 cells, E-cad$^+$ and CD36$^+$ progenitors, when grown under SI2 conditions in the absence of exogenous Epo, can indeed produce Epo. SI2 cells and SCF/Epo progenitors at day 10 of culture, and E-cad$^+$ and CD36$^+$ progenitors at day 2 after immunomagnetic bead selection were lysed and total RNA was extracted. Expression of human Epo was evaluated by RT-PCR. Total RNA of the human hepatoma cell line HepG2 served as a positive control.

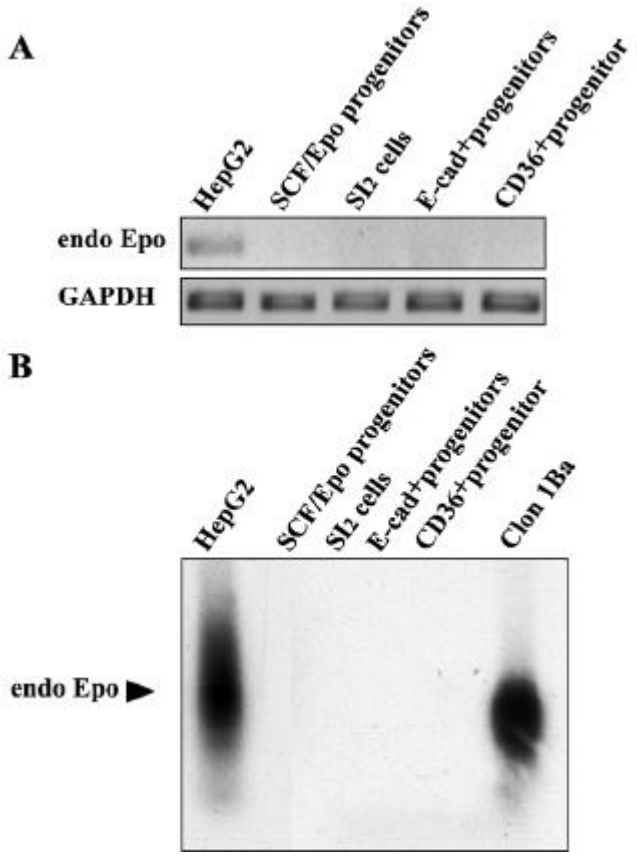

Figure 3.18 : Expression of endogenous Epo in different progenitor types.

A: *SCF/Epo progenitors, SI2 cells, E-cad$^+$ progenitors and CD36$^+$ progenitors were analyzed for expression of human Epo by RT-PCR. HepG2 cells served as a positive control. GAPDH specific signals are shown in the lower panel as a control.*

B: *PCR products were resolved by agarose electrophoresis and subjected to Southern blot hybridization using a human Epo specific probe. Blots were exposed to X-ray film.*

No detectable endogenous human Epo expression was shown in the investigated progenitor cell types by normal RT-PCR (Fig. 3.18A). The amplification product of HepG2 RNA was excised, DNA was extracted and the fragment was cloned into pCR2.1 vector. Two clones were chosen and subjected to sequence analysis. The cloned fragments were sequenced from either end using T7 an M13rev sequence primers, respectively. Both clone 1Ba and clone 1Bb were identified as the expected fragments of human Epo. Alignment with published human Epo sequence (Accession no: NM000799) revealed 100% identity.

To confirm the result of RT-PCR and to enhance signals, PCR fragments were subjected to Southern blot hybridization using the fragment of clone 1Ba as a human Epo specific probe. (Fig. 3.18B). Again no expression of human Epo was detectable in SI2 cells, SCF/Epo, E-cad$^+$ and CD36$^+$ progenitors. As expected, both of the positive controls showed a strong signal.

3.2 Gene expression analysis by DNA microarray

3.2.1 RNA isolation and cRNA synthesis

To determine differences between various progenitor cell types on a genome wide scale, cells were subjected to transcriptional profiling by DNA microarray analysis using Affymetrix GeneChip arrays that contain probe sets of about 13,000 human genes. Mononuclear cells were isolated by density gradient separation from individual cord blood and either subjected to CD34$^+$ selection or cultured in SCF/Epo growth conditions. After selection CD34$^+$ hematopoietic stem/progenitor cells were cultured in SI2 growth conditions for 8 days and than selected for E-cad or CD36. After selection CD36$^+$ and E-cad$^+$ progenitors were let to proliferate for 2 additional days (see 2.2.1). After a total of 10 days of culture the RNA from different cell populations was isolated. Cord blood samples of 8 individuals were analyzed by DNA chip technology.

Table 3.3 : mRNA samples hybridized to DNA microarrays.
The experiment number is indicated with the respective date of cell preparation and the isolated cell types. 1-4 different progenitor cells types were obtained per cord blood preparation. Sets KK_1 and KK_2 are provided by Dr. Koh.

Experiment number	Date	SI2 cells	CD36$^+$ progenitors	E-cad$^+$ progenitors	SCF/Epo progenitors
01	21.10.99				+
02	19.05.00				+
03	09.06.00				+
1	11.10.00	+	+		
2	31.10.00	+			

Experiment number	Date	SI2 cells	CD36$^+$ progenitors	E-cad$^+$ progenitors	SCF/Epo progenitors
3	08.12.00	+	+	+	
4	13.07.01	+	+	+	+
5	09.07.01			+	+
KK_1					+
KK_2					+

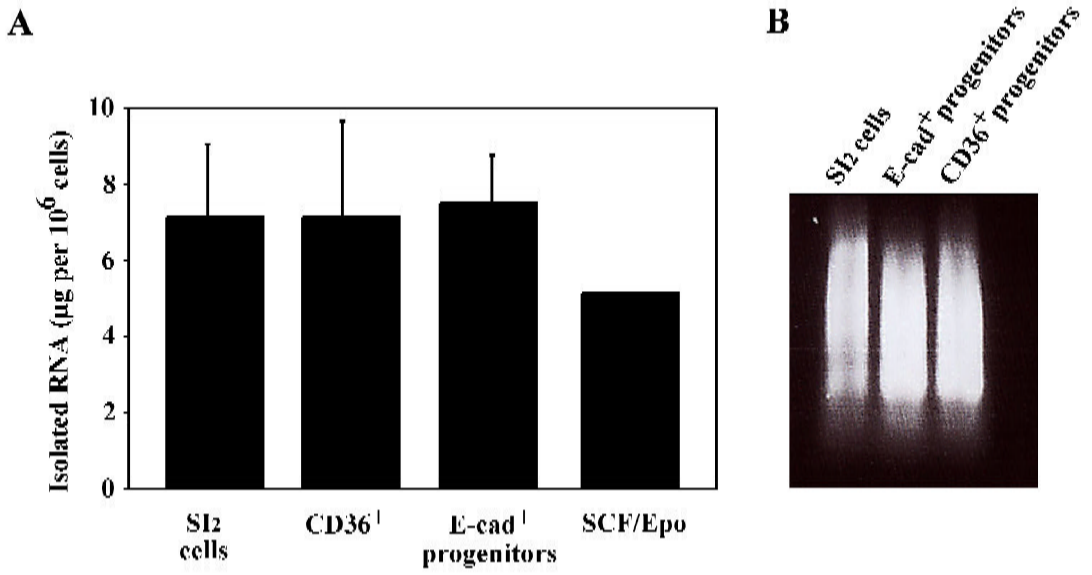

Figure 3.19 : RNA isolation from different progenitor types and fragmentation control of cRNA.

A: *Average amounts of RNA isolated from 10^6 cells of the corresponding cell type are shown. Results are means of all performed experiments of the corresponding cell type (Table 3.3).*

B: *Biotinylated cRNA of SI2 cells, E-cad$^+$ and CD36$^+$ progenitors were analyzed for the success of fragmentation. cRNA samples were resolved by 1% agarose gels electrophoresis and stained with EtBr. A representative experiment is shown*

In total, RNA from 17 different samples was isolated. On average 5-10 µg RNA were isolated from 10^6 cells. The ratio of isolated RNA per cell did not show significant differences for the individual progenitor types (Figure 3.19). After isolation all RNA were subjected to the procedure described by Affymetrix, Inc. Fragmented biotin-labeled cRNA (Fig. 3.19B) were hybridized on human HG_U95Aver2 Affymetrix chips.

3.2.2 Data analysis

Scanned GeneChip.DAT files were initially analyzed by the GeneChip Analysis Suit Software (MAS version 5, Affymetrix, Inc.). The average intensity of each probe was calculated and the results were reported in tabular format as a GeneChip.CHIP file. The analysis determined also "present" or "absent" calls for transcripts. The investigated transcripts were annotated according to Hacker et al., 2003.

3.2.2.1 Number of expressed genes

About 13,000 genes are represented on an HG_U95Aver2 Affymetrix chip. As expected all analyzed genes were found to be expressed in one cell type. Figure 3.20 shows the percentage of expressed genes for individual samples. Transcripts called "absent" by GeneChip® software in more than 2 of all analyzed samples were considered as being expressed.

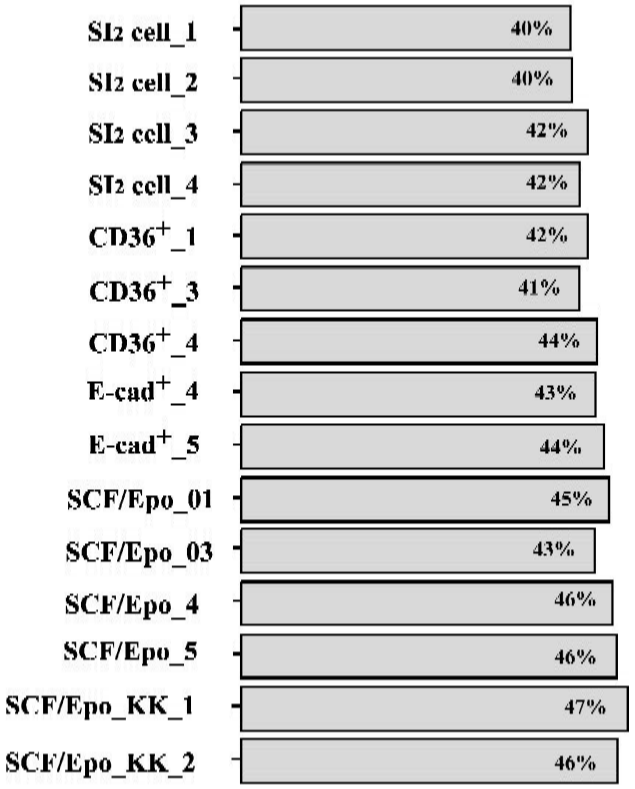

Figure 3.20 : Number of expressed genes for individual experiments.
The percentage of expressed genes out of 12,558 *tested genes for individual samples is shown.*

For every sample 40-47% of the 12,558 analyzed transcripts were consistantly found to be defined as expressed. The samples SCF/Epo_02 and E-cad$^+$_3 showed low numbers of expressed genes (25-35%) and were not further analyzed. All transcripts found to be expressed were compiled into a data base of 7,370 transcripts, referred to as 7k database.

3.2.2.2 Comparison of individual experiments

To investigate the relationship of all four progenitor types, hierarchical clustering was performed using multiple data sets from 2-6 independent experiments. The hierarchical cluster was prepared by GeneSpring® software. To determine the similarity between individual samples the average linkage was chosen as cluster method. The Pearson correlation with a minimum distance of 0.001 was chosen to define the proximities. The analysis was based on the 7k database (see 2.3.10).

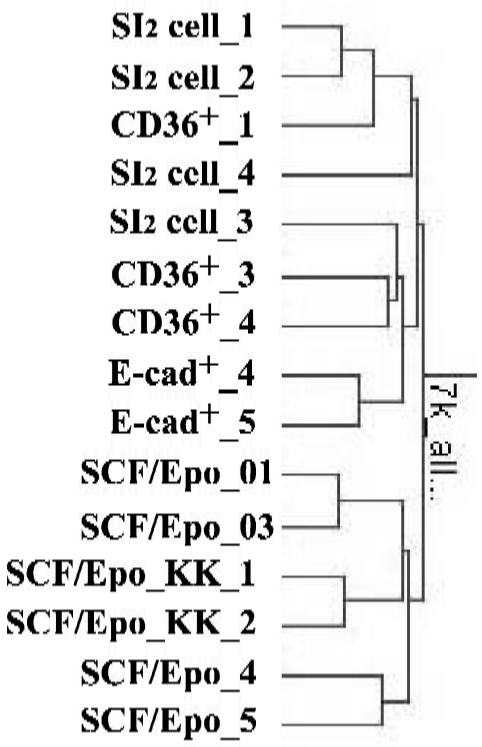

Figure 3.21 : Similarity of individual samples.

Similarity of individual samples is shown in a dendrogram. Results are obtained by hierarchical clustering using Genespring® software. *Proximities were defined by Pearson Correlation.*

The dendrogram shows that nearly all samples of the same progenitor cell type clustered together. All SCF/Epo progenitors were found in a single cluster. Also both samples of E-cad$^+$ progenitors showed a high similarity and clustered together. The samples of CD36$^+$ progenitors and SI2 cells were found in one cluster. Furthermore, E-cad$^+$ progenitors were found to be more closely related to CD36$^+$ progenitors and the SI2 cells than to SCF/Epo progenitors, which most probably reflects the fact that the E-cad$^+$ progenitors are a component of these cell populations (see Fig. 3.10).

3.2.2.3 Comparison of different progenitor types

3.2.2.3.1 Comparison of E-cad$^+$ and SCF/Epo progenitors

Because it is known that SI2 cells are a mixed cell population and the main focus was on the definition of the erythroid potential of E-cad$^+$ progenitors, the following analyses were applied for E-cad$^+$ and SCF/Epo progenitors. To show the commonly and differentially expressed genes between these two cell types, lists of expressed genes were compared in a Venn-diagram using GeneSpring™ Software. Therefore, respective lists of transcripts that were present in both E-cad$^+$ progenitor samples and in 5 out of the 6 SCF/Epo progenitor samples were established.

4,545 transcripts passed the criteria for E-cad$^+$ progenitors and 4,455 transcripts for SCF/Epo progenitors, and the respective gene lists were intersected in Venn-diagrams. Means of expression levels of transcripts expressed in two of intersected cell types, referred to as commonly expressed genes, were filtered for Max/Min ≥2 to obtain differentially expressed genes.

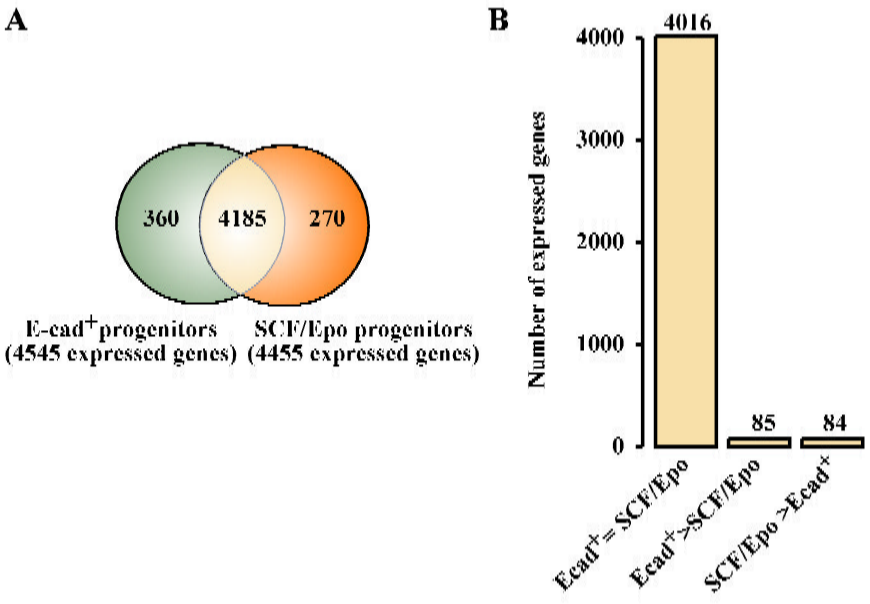

Figure 3.22 : Commonly and differentially expressed genes in E-cad$^+$ progenitors and SCF/Epo progenitors

(A) *Depicted is a Venn-diagram showing the number of commonly and differentially expressed genes between E-cad$^+$ progenitors and SCF/Epo progenitors. The*

overlapping region of two circles refers to commonly expressed genes. Indicated are numbers of expressed transcripts.

(B) *Comparison of average values of commonly expressed genes leads to transcripts showing equal expression levels (Max/Min<2; E-cad$^+$ = SCF/Epo) and to differentially expressed transcripts (Max/Min>2). Transcripts with higher expression levels in E-cad$^+$ progenitors than in SCF/Epo progenitors referred to as E-cad$^+$ >SCF/Epo and transcripts with lower expression levels in E-cad$^+$ progenitors than in SCF/Epo progenitors referred to as SCF/Epo >E-cad$^+$. Numbers indicate the respective number of transcripts.*

Figure 3.22A shows that the majority of genes (4,185) were expressed in both E-cad$^+$ and SCF/Epo progenitors; 360 were unique for E-cad$^+$ progenitors and 270 were unique for SCF/Epo progenitors. Additionally, of the 4,185 genes expressed in both E-cad$^+$ and SCF/Epo progenitors the majority (4,016) did not show significant differences in expression levels between both progenitor cell types (Fig. 3.22B). Within this group of genes with similar expression levels the majority of red cell specific genes were found, such as erythroid specific globins, enzymes for porphyrin biosynthesis, ferritin, transferrin receptor, EpoR, glycophorin B, C and E, rhesus antigens CD241 and CD240 CE, band 3 and KELL. The erythroid specific transcription factors EKLF, NF-E2 and GATA-1 also showed similar expression levels (data not shown).

A minority of genes was found to be differentially expressed: 85 genes were >2fold higher expressed in E-cad$^+$ progenitors than in SCF/Epo progenitors and 84 genes were >2fold higher in SCF/Epo progenitors (Fig. 3.22B). From this analysis we conclude: (1) 96% of the genes analyzed are expressed in both E-cad$^+$ and SCF/Epo progenitors indicating that these progenitors are very much related. This is also reflected in their biological properties, e. g. their ability to generate mature red blood cells. (2) There are gene sets of differentially expressed genes that form transcriptional signatures of E-cad$^+$ progenitors (445 genes) and SCF/Epo progenitors (354 genes), respectively (see appendix).

To investigate these signature genes in detail expression values were subjected to more stringent restrictions (Fig. 3.23). For cell type specific genes averages of

normalized expression values had to be >1.5. For the differentially expressed genes the restrictions were enhanced for both the average of normalized expression values and the ratio between averages of both cell types. For E-cad$^+$ progenitors average values had to be >3, for SCF/Epo progenitors >1.5. All selected genes should have shown additionally a change in expression by at least 2.5 fold. This resulted in a group of about 15 genes for both groups of unique expressed genes and for both groups of differentially expressed genes. Finally, every gene was then manually evaluated for consistency of individual values in every sample and the expression values were compared to the expression values in other cell types using data sets of DC, B cells, CD34$^+$ cells and differentiating erythroid cells. This analysis led to two final sets of 4 genes for each progenitor type (Fig. 3.23).

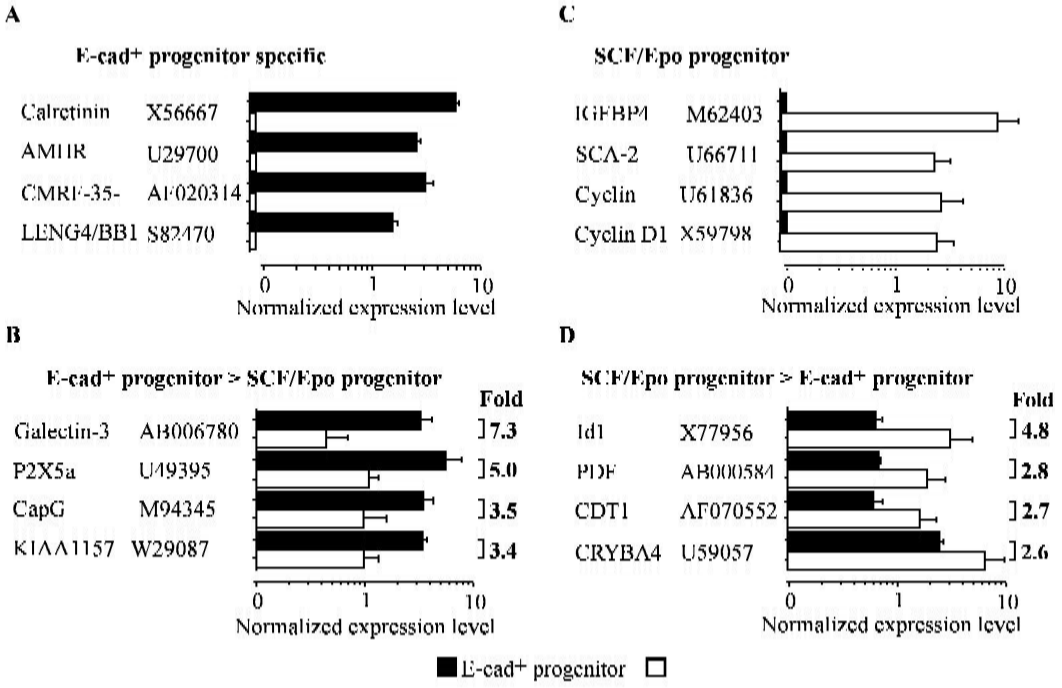

Figure 3.23 : Expression levels of selected candidate genes.

Expression levels determined by microarray analysis in E-cad$^+$ progenitors (means of 2 experiments; filled bars) and in SCF/Epo progenitors (means of 6 experiments, open bars) are shown with the standard deviation for selected genes. All diagrams are shown with log scale. Accession numbers are indicated next to the gene names.

(A and C) *Genes expressed specifically in E-cad$^+$ progenitors (A) or SCF/Epo progenitors (C), as shown in Fig 3.22A, were selected. (AMHR refers to Anti-*

Müllerian Hormone Receptor, LENG4/BB1 to Leukocyte receptor cluster 4, IGFBP4 to Insulin Like Growth Factor Binding Protein 4, Sca-2 to Stem Cell Antigen 2)
(B and D) *Genes expressed higher in E-cad$^+$ progenitors (B) and higher in SCF/Epo progenitors (D), as shown in Fig 3.22B, were selected. Numbers are fold changes of means of normalized expression levels between both cell types. P2X5a refers to purinergic receptor P2X, CapG to gelsolin-like capping protein, Id1 to Inhibitor of DNA binding 1, PDF to Prostate Differentiation Factor, CRYBA4 to Beta-A4 Crystalline*

Most of the genes that were exclusively or differentially expressed in E-cad$^+$ progenitors and SCF/Epo progenitors (Fig 3.23) have not been described in the context of red cell development before and in the following their identity will be commented on briefly.

The gene set that is unique for E-cad$^+$ progenitors contains calretinin, anti-müllerian hormone receptor II (AMHR II), CMRF-35H9 and leukocyte receptor cluster 4 (LENG4; Fig. 3.23A). Calretinin showed the highest expression within this group of E-cad$^+$ progenitor affiliated genes. Calretinin (also referred to as calbindin 2) is a Ca^{2+} binding protein containing an EF-hand as calcium binding motif (Schwaller et al., 2002). AMHR II represents a serine/threonine kinase receptor that binds Anti-müllerian hormone, a member of the TGFβ family that is involved in the regression of Müllerian ducts in male fetuses (Josso et al., 2001). The CMRF-35H9 has been suggested to encode a cell surface glycoprotein with so far unknown function (Green et al., 1998). Leukocyte receptor cluster 4 (LENG4/BB1) has been implicated in formation of bladder and breast carcinomas (Fukunaga-Johnson et al., 1996).

Four genes that were highly expressed in E-cad$^+$ progenitors comprise galectin-3, ATP receptor P2X5a, gelsolin-like capping protein (CapG) and KIA1157 (Fig. 3.23B). Galectin-3 is a soluble galactose-specific lectin that was also reported to be weakly expressed in CD34$^+$ progenitor cells (Le Marer, 2000). P2X5a belongs to the ionotropic ATP receptor family P2X (North, 2003). These receptors act as ligand gated ion channels and are frequently expressed at high levels in brain and immune system. P2Y family members have also been shown to interact with extracellular nucleotides. For P2Y, a role in proliferation and maturation of blood cells has been

proposed (Sak et al., 2003). CapG interacts with actin in a Ca^{2+} dependent manner by blocking the barbed ends of actin filaments. KIA1157 is a sequence of unknown function but shows sequence similarity to a protein phosphatase in Drosophila melanogaster (http://bioinformatics.weizmann.ac.il/cards).

Genes which were expressed only in SCF/Epo progenitors include insulin-like growth factor binding protein 4 (IGFBP4), stem cells antigen 2 (SCA-2), cyclin G1 interacting protein and cyclin D1 (Fig. 3.23C). IGFBP4 can interact with both molecules IGF-1 or IGF-2 and influences the activity of these molecules on the affected cells. SCA-2, also referred to as lymphocyte antigen 6 (Ly-6E) or retinoic acid induced gene E (Rig-E), belongs to the Ly-6 family of cell surface proteins and is found on a number of hematopoietic cell lineages. The role of the putative cyclin G1 interacting protein is unknown. Cyclin D1 (also referred to as parathyroid adenomatosis 1 [PRAD 1], B cell lymphoma 1 [Bcl-1] or CCND1) is a G1 cyclin, which acts as an important regulator of the G1/S transition of the cell cycle.

Figure 3.23D shows the four transcripts differentially expressed with a higher expression level in SCF/Epo progenitors: inhibitor of DNA binding 1 (Id1), prostate differentiation factor (PDF), Cdt1 and beta crystalline–A4 (CRYBA4). Id1 belongs to the family of basic helix-loop-helix (HLH) proteins and acts as a transcription factor antagonizing HLH transcriptional activators (Yokota, 2001). It is required for the G1 progression and consequently involved in the controlling of cell growth and/or differentiation. PDF also referred to as growth differentiation factor 15 (GDF15) or macrophage inhibitory cytokine (MIC-1), is a cytokine and belongs to the TGFβ superfamily (Bootcov et al., 1997; Bottner et al., 1999a). It is widely expressed and its function is not clear yet (Bottner et al., 1999b). The cell cycle dependent replication initiation factor Cdt1 plays an essential role in DNA replication. CRYBA4 was reported to be a structural component of the vertebrate eye lenses.

The results of the DNA microarray analysis imply that E-cad$^+$ and SCF/Epo progenitors are very similar in their molecular expression profile but also exhibit distinct differences. Additionally, while for some of the molecules a putative function in E-cad$^+$ and SCF/Epo progenitors can be inferred (see 4.2.2), the function of other molecules in these cells remains elusive.

3.2.2.3.2 Comparison of E-cad$^+$ and CD36$^+$ progenitors

CD36$^+$ cells derived from SI2 cells have the potential to differentiate into mature red cells (Freyssinier et al., 1999). In this thesis work it was shown that E-cad$^+$ progenitor cells were also able to recapitulate red blood cell differentiation *in vitro* (see 3.1.4.4). Furthermore, it was demonstrated that CD36$^+$ progenitors, obtained by immunomagnetic bead selection, contained at least two main cell populations: E-cad positive and E-cad negative cells (Fig. 3.10). To investigate the expression profile of CD36$^+$ progenitors and their relationship to E-cad$^+$ progenitors, DNA microarray data of both progenitor types were analyzed and compared. Lists of expressed genes of these two cell types were compared in a Venn-diagram using GeneSpring™ Software. Therefore, lists of transcripts that were present in all three CD36$^+$ progenitor samples and in both E-cad$^+$ progenitor samples, were established.

4,721transcripts passed the criteria for CD36$^+$ progenitors and 4,545 transcripts for E-cad$^+$ progenitors, and the respective gene lists were intersected in Venn-diagrams. The 4,326 commonly expressed genes, were filtered for Max/Min ≥2 to obtain differentially expressed genes.

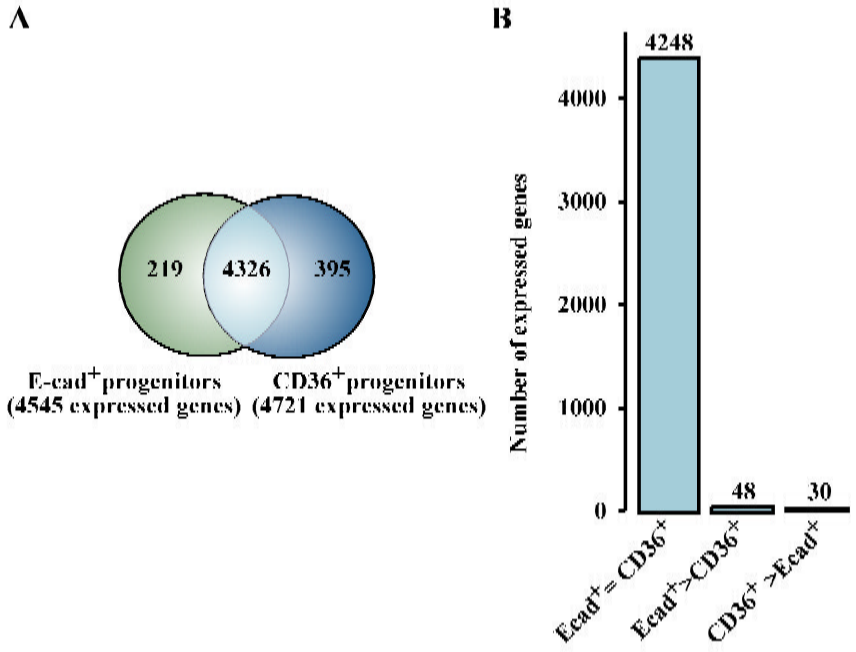

Figure 3.24 : Commonly and differentially expressed genes in CD36$^+$ progenitors and E-cad$^+$ progenitors

(A) *Depicted is a Venn-diagram showing the number of commonly and differentially expressed genes between E-cad$^+$ progenitors and CD36$^+$ progenitors. The overlapping region of two circles refers to commonly expressed genes between corresponding cell types. Indicated are numbers of expressed transcripts.*

(B) *Comparison of average values of commonly expressed genes leads to transcripts showing equal expression levels (Max/Min<2; E-cad$^+$ = CD36$^+$) and to differentially expressed transcripts (Max/Min>2). Transcripts with higher expression levels in E-cad$^+$ progenitors than in CD36$^+$ progenitors are referred to as E-cad$^+$ > CD36$^+$ and transcripts with lower expression levels in E-cad$^+$ progenitors than in CD36$^+$ progenitors are referred to as CD36$^+$>E-cad$^+$. Numbers indicate the respective number of transcripts.*

Figure 3.24A shows that the majority of genes (4,326) were expressed in both E-cad$^+$ and CD36$^+$ progenitors; 219 were unique for E-cad$^+$ progenitors and 395 were unique for CD36$^+$ progenitors. Additionally, the 4,326 commonly expressed genes were analyzed for differentially expressed transcripts. In both E-cad$^+$ and CD36$^+$

progenitors the majority of transcripts (4,248) did not show significant differences in expression levels between both progenitor cell types (Fig. 3.24B).

As expected, within the group of genes with similar expression levels (Fig. 3.24B: E-cad$^+$ = CD36$^+$) the majority of red cell specific genes were found, like globin α, β and γ, rhesus and blood group antigens, the erythroid 5-aminolevulinate synthase, ferritin, transferrin receptor, EpoR, glycophorin B, C and E and band 3. The erythroid specific transcription factors EKLF, NF-E2 and GATA-1 as well as GATA-2 showed also similar expression levels (data not shown).

A minority of genes was found to be differentially expressed: 48 genes were >2fold higher expressed in E-cad$^+$ progenitors than in CD36$^+$ progenitors and 30 genes were >2fold higher in CD36$^+$ progenitors (Fig. 3.24B). Not surprisingly, the majority (4,248) of transcripts were not only commonly expressed but exhibited also similar expression levels.

E-cad$^+$ progenitors represent a subpopulation of CD36$^+$ progenitor cells (Fig. 3.10) and for this reason we will consider only the transcriptional signature of CD36$^+$ progenitors. The transcriptional signature of CD36$^+$ progenitors comprised 425 genes. To investigate these signature genes in detail, expression values were subjected to more stringent restrictions, corresponding to the restrictions used for SCF/Epo progenitors in 3.2.2.3.1. This resulted in a group of about 15 genes. Finally, every gene was then manually evaluated for the consistency of individual expression values. The analysis led to a final set of 8 genes (Fig. 3.25).

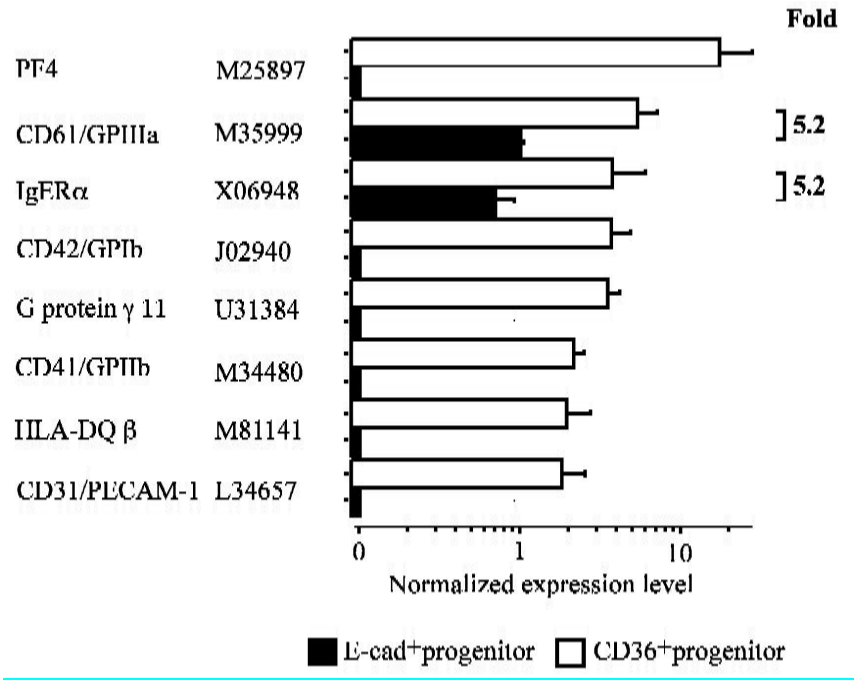

Fig 3.25 : Expression levels of selected candidate genes.

Expression levels determined by microarray analysis in CD36$^+$ progenitors (means of 3 experiments; open bars) and in E-cad$^+$ progenitors (means of 2 experiments, filled bars) are shown with the standard deviation for selected genes. The diagram is shown with log scale. Accession numbers are indicated next to the gene names.
Genes expressed specifically or higher in CD36$^+$ progenitors were selected. Numbers are fold changes of means of normalized expression levels between both cell types. (PF4 refers to platelet factor 4, CD61/GPIIIa refers to glycoprotein IIIa, IgERa refers to high affinity IgE receptor alpha-subunit, CD42/GPIb refers to glycoprotein Ib, CD41/GPIIb refers to glycoprotein IIb, HLA-DQ b refers to MHC class II molecule, CD31/PECAM-1 refers to platelet/epithelial cell adhesion molecule 1)

Surprisingly, 5 out of the 8 transcripts encode genes playing an essential role in platelets, a cell type known to be closely related to the erythroid lineage in the hematopoietic system (see Fig. 1.1). Platelet factor 4 (PF4) showed the highest expression within the group of CD36$^+$ progenitor affiliated genes and is a member of the CXC chemokine family (Sulpice et al., 2002). PF4 is produced by megakaryocytes and participates in inflammatory responses by attracting monocytes and neutrophils (Boehlen and Clemetson, 2001; Xia and Kao, 2003). Glycoprotein IIIa (GPIIIa,

CD61) and glycoprotein IIb (GPIIb, CD41) were found to be highly expressed in $CD36^+$ progenitors, but in contrast to GPIIIa, GPIIb was not found to be expressed in E-cad^+ progenitors. Both molecules form the platelet specific fibrinogen receptor (also referred to as glycoprotein IIb/IIIa [GPIIb/IIIa]), a heterodimeric membrane glycoprotein. Fibrinogen receptor links the extracellular fibrin matrix to the platelet contractile apparatus and is required for the platelet aggregation and clot retraction (Phillips et al., 1980; Hynes, 1992; Ruoslahti, 1991). Glycoprotein Ib (GPIb, CD42) was also exclusively expressed in $CD36^+$ progenitors. It is known as a subunit of the von-Willebrandt-factor receptor (vWF-R). vWF-R binds to its ligand at high blood shear stress mediated by blood flow and initiates the adhesion and thus contributes to formation of a thrombus (Uff et al., 2002).

Platelet endothelial cell adhesion molecule 1 (PECAM-1, CD31) is a 130-kd transmembrane glycoprotein and a member of a receptor family with immunoreceptor tyrosine-based inhibitory motifs. PECAM-1 is expressed on platelets, certain T-cells, monocytes, neutrophils, and vascular endothelial cells and is involved in a variety of cellular processes (Cicmil et al., 2002). It serves as a physiological negative regulator of platelet-collagen interactions that may function to negatively limit growth of platelet thrombi on collagen surfaces (Jones et al., 2001). High affinity IgE receptor alpha-subunit (IgERα) was found not only on the surface of platelet but also on mast cells, basophils, eosinophils and DC. IgER plays a key role in IgE-mediated allergic reaction (Takahashi et al., 2002).

Only 2 of the candidate genes are not known to be related to platelets: the G protein γ 11 and the MHC class II molecule HLA-DQβ1. G protein γ 11 is a subunit of heteromeric G proteins, is required for the GTPase activity and is involved in the G-proteine mediated signal transduction. G protein γ 11 is abundantly expressed in many tissues (http://bioinformatics.weizmann.ac.il/cards). HLA-DQβ1 belongs to the HLA class II beta chain paralogues. It plays a central role in the immune system by presenting peptides derived from extracellular proteins. Class II molecules are expressed in antigen presenting cells, such as B lymphocytes, DC and macrophages (http://harvester.embl.de/harvester/O197/O19712.htm).

These results implicate that $CD36^+$ progenitors, obtained by immunomagnetic bead selection, have megakaryocyte/platelet and other myeloid components besides their red cell properties.

4 DISCUSSION

4.1 Growth factor combinations supporting the Epo-independent growth of human red cell progenitors

4.1.1 Epo-independent growth in SCF/Epo progenitors

Previous studies with human hematopoietic progenitors establish that SCF and Epo are the most prominent growth factors required for *in vitro* growth of erythroid progenitors (Panzenböck et al., 1998; Muta et al., 1994; Muta et al., 1995; McNiece et al., 1991). It has been shown in colony assays that erythroid progenitors grown in SCF/Epo conditions contain both early BFU-E and late CFU-E type progenitors (Panzenböck et al., 1998). It has also been reported that several combinations of SCF with growth factors other than Epo, such as IL-3 and hyper-IL-6 in the human system (see 1.2.2) and TGFα/TGFβ in the chicken system (Beug et al., 1995; Steinlein et al., 1995; Hayman et al., 1993; Gandrillon et al., 1999) support an Epo-independent growth of red cell progenitors.

We thus suspected the existence of an early human red cell progenitor able to grow Epo-independently. To amplify such progenitors our efforts were directed towards the investigation of the optimal growth factor combination. First, growth factors were chosen which are known to act as mitogens in various tissues. Progenitors, amplified in SCF/Epo growth conditions for 10 days (2.2.1.1), were subjected to various cytokines and combinations thereof (Figs. 3.1 and 3.2, 3.1.2.1 and 3.1.2.2). The most potent factor combination in these experiments was Epo and SCF applied simultaneously. Not surprisingly, it was found that progenitors, amplified in SCF/Epo growth conditions, are SCF and Epo-dependent. Of the tested factor combinations, only hyper-IL-6 combined with SCF showed a slight effect on the tested cells.

In conclusion, there was no evidence found that other factors or combinations thereof support an Epo-independent growth of SCF/Epo progenitors.

4.1.2 Amplification of hematopoietic stem/progenitor cells in different growth conditions

The CD34 antigen is a defining hallmark of hematopoietic stem/progenitor cells (Civin and Gore, 1993). $CD34^+$ cells present in human umbilical cord blood constitute a very heterogeneous cell population (Mayani and Lansdorp, 1998). Several groups have described the isolation of $CD34^+$ cells, their amplification and the generation of different cell lineages such as erythroid, myeloid, B lymphoid, DC and megakaryocytic cells (Mayani and Lansdorp, 1998; Ju et al., 2003). The *in vitro* amplification of $CD34^+$ cells depends on cytokines present in culture. Several combinations of early acting factors (including SCF, Tpo, IL-3, Flt3L, IL-6, IL-1, GM-CSF) can be used (Piacibello et al., 1997). Previous work of our research group has shown that liquid culture of hematopoietic stem/progenitor cells in serum free StemSpan medium supplemented with SCF, Tpo, hyper-IL-6 and Flt3L (referred to as STIF condition) supports the proliferation of amplified human $CD34^+$ cells (Hacker et al., 2003; Ju et al., 2003) which subsequently can differentiate into various cell types (e.g. DC (Hacker et al., 2003; Ju et al., 2003) and erythroid cells [unpublished data]). Sui and colleagues suggest for the first time the amplification of cord blood derived stem/progenitor cells in serum free medium complemented with SCF, IL-6/sIL-6R and IL-3. For our experiments IL-6/sIL-6R was replaced by hyper-IL-6 and conditions were referred to as SI2 conditions. For these SI2 culture conditions an Epo-independent development of human erythroid cells was reported (Sui et al., 1996; Baiocchi et al., 2000; Neildez-Nguyen et al., 2002).

We isolated $CD34^+$ cells from umbilical cord blood by immunomagnetic bead selection and grew the cells in either STIF or SI2 conditions to compare both culture conditions for their suitability to support the *in vitro* culture of Epo-independent red cell progenitors. Cells grown in the respective culture conditions showed similar properties for their growth rate and their growth factor response (3.1.3.1 to 3.1.3.3). Hematopoietic stem/progenitor cells grown either in STIF or SI2 conditions surprisingly showed a clear response to Epo and to Epo in combination with SCF, despite the absence of Epo in the culture medium before. Epo and SCF supplemented simultaneously stimulate most effectively the proliferation as shown for SCF/Epo progenitors (3.1.2.2). Furthermore, EpoR, SCF-R and transferrin receptor were expressed in cells of both culture conditions, but with weak or medium expression

levels (Tab.3.1). The expression of these molecules is a characteristic of early red cell progenitors (Panzenböck et al., 1998; Broxmeyer, 1991; McNiece et al., 1991; Bühring et al., 1996), which proves that the stem/progenitor cells contain a population of red cell progenitors. On the other hand, the expression of several specific myeloid markers (CD33, CD11a and CD18) suggests that amplified stem/progenitor cells are a heterogeneous cell population. Additionally, SI2 conditions seemed to support the growth of cells more effectively than STIF conditions (Fig 3.4).

CD36 and E-cad were expressed on stem/progenitor cells, grown under SI2 and STIF conditions, but the percentage of E-cad$^+$/CD36$^+$ and E-cad$^-$/CD36$^+$ cell populations were higher under SI2 conditions than under STIF conditions (Tab. 3.2). The double negative populations had a higher percentage in STIF cells.

These results led us to the assumption that hematopoietic stem/progenitor cells cultured in SI2 conditions have more erythroid properties compared to the cells grown in STIF conditions. For this reason and because the proliferation was more effective, it was decided to further use SI2 conditions for amplification of CD34$^+$ cells.

4.2 Identification and isolation of a human early Epo-independent red cell progenitor present in SI2 cells population

Four different cord blood derived progenitor types, SI2 cells, CD36$^+$, E-cad$^+$ and SCF/Epo progenitor cells, were investigated in this study. CD36$^+$ and E-cad$^+$ progenitors, derived from SI2 cells, and SCF/Epo progenitors, are known to be red cell progenitors. Therefore, SI2 cells and SCF/Epo progenitors were used as control for the comparison of cell populations. Amplified human hematopoietic stem/progenitor cells are a heterogeneous cell population. To obtain pure erythroid progenitors a second selection step was necessary. For this second purification step, Freyssinier and colleagues have created an immunomagnetic bead selection using anti-CD36 antibodies (Freyssinier et al., 1999). We observed that these CD36$^+$ cells contain at least two subpopulations concerning their simultaneous expression of E-cad and CD36 (Fig. 3.10A). Since the E-cad molecule seemed to be more restricted to the erythroid lineage in hematopoietic cells (Armeanu et al., 1995) and E-cad$^+$ progenitors were a homogeneous population concerning the simultaneous expression of E-cad and CD36 (Fig. 3.10B), we established a three step isolation method to enrich the cell population for E-cad positive cells. First the properties of these cells will be discussed.

4.2.1 E-cad$^+$ progenitors: Early Epo independent BFU-E type progenitors

In this study we described the Epo-independent E-cad$^+$ progenitors which were efficiently obtained from cord blood derived SI2 cells. These progenitor cells were grown effectively under serum-free SI2 conditions. We characterized the progenitor cells by their cell surface marker expression and observed the expression of marker molecules for early multilineage hematopoietic progenitors (c-kit, CD71, CD33). Interestingly, we also detected the erythroid specific EpoR, despite the fact that the cells were previously grown in the absence of Epo. Lee and colleagues already observed that the expression of EpoR does not require the presence of Epo in murine yolk sac blood islands during the primitive Epo-independent erythropoiesis (Lee, R. et al., 2001). Erythroid differentiation markers (glycophorin A/B [CD235ab], Kell [238] and band 3 [CD233]) and markers for myeloid lineage specific molecules, like CD18, CD11a (Fig. 3.11), CD19, CD14 and CD1a (data not shown), were not expressed on E-cad$^+$ progenitors. In SI2 cells CD18, CD11a were also expressed but no EpoR could be detected. Thus, the surface marker profile of the cells was very similar to the profiles of SI2 cells and Epo-dependent SCF/Epo progenitors (Panzenböck et al., 1998; C.H. unpublished data). The differences to SCF/Epo progenitors, the absence of glycophorin A/B and band 3, the bimodal expression of EpoR and the reduced production of hemoglobin in colony assays, support the idea that E-cad$^+$ progenitors are less mature than Epo-dependent SCF/Epo progenitors. The observation of Epo-independent growth was also supported by the fact that no endogenous Epo was expressed as has been reported for erythroid precursors (Stopka et al., 1998; Sato et al., 2000).

Although the growth factor Epo seems not to be crucial for proliferation, the E-cad$^+$ progenitors responded effectively to this growth factor, as we expected, since they express EpoR on their surface (Fig 3.11). This fact supports the hypothesis that lineage specific growth factors like Epo act subsequently to promote the survival and proliferation of independently committing cells (Ogawa et al., 1989; Suda, J. et al., 1984a; Mayani et al., 1993).

The second remarkable single factor response we found was the response to SCF. SCF seems to be the main player in the proliferation of E-cad$^+$ progenitors. The

combination of SCF with IL-3 and hyper-IL-6 did not significantly improve the cell proliferation (Fig. 3.8). These results suggest a more assisting role for both multilineage competent factors in early progenitor survival than has been reported before (Kinoshita et al., 1995; Zha et al., 1996; Fukada et al., 1996). The growth factor responses of the other investigated cell types were similar. This fact was additionally confirmed by the CFSE labeling assay (Fig 3.9B).

The differentiation of E-cad$^+$ progenitors was shown to require an additional commitment phase under SCF/Epo growing conditions because cells were unable to differentiate directly as it was shown for SCF/Epo progenitors (Panzenböck et al., 1998; C.H. unpublished data). The commitment of SI2 cells, CD36$^+$ and E-cad$^+$ progenitors was followed by analyzing their surface marker profile. The characteristic surface antigen pattern of all 3 progenitor types were shifted in favor of erythroid properties. Erythroid markers, such as glycophorin A/B (CD235ab) and band 3 (CD233) were up regulated and CD33 was down regulated (Fig. 3.12). In committed SI2 cells and CD36$^+$ progenitors 2 subpopulations were still detectable, an E-cad negative and an E-cad positive subpopulation, and concerning the transferrin receptor (CD71) a subpopulation expressing the molecule at lower or at higher level. The profile of committed E-cad$^+$ progenitors was most similar to the profile of SCF/Epo cells. Additionally, the E-cad$^+$ progenitors, subjected to SCF/Epo growing conditions, showed a more effective amplification rate compared to SI2 cells and CD36$^+$ progenitors (Fig. 3.13).

The clonogenic potential of E-cad$^+$ progenitors also reflected their features of early erythroid progenitors. An almost pure BFU-E population was observed for E-cad$^+$ progenitors in colony assays, whereas the distribution of early BFU-E and late CFU-E type progenitors in the amplified SI2 cells and SCF/Epo progenitors was more mixed (Fig. 3.14B). The percentage of myeloid type colonies was significantly decreased in E-cad$^+$ progenitors compared to the other analyzed progenitor populations, although the assay conditions support a multilineage differentiation (Fig. 3.14A). These results indicate a high purity of E-cad$^+$ progenitors.

There was no lack of evidence to reflect the erythroid origin of E-cad$^+$ progenitors. In addition to the clonogenic potential and the surface marker profile of red cell progenitors, we were also able to show a change in the surface marker profile of

committing and differentiating cells in an erythroid manner (Fig 3.17), a decrease in cell size and hemoglobinization in Epo/insulin differentiation conditions (Fig 3.16).

Thus, our method of isolation of E-cad$^+$ cells out of the human cord blood derived SI2 cells leads to an almost pure population of Epo-independent early red cell progenitors. The purity of our progenitors is an advantage for studying the Epo-independent phase of erythroid development. The three-step stimulation protocol described by Neildez-Nguyen et al., where purified CD34$^+$ stem/progenitor cells undergo an Epo-independent phase of 8 days before a pure red cell population is generated using Epo as stimulus, does not allow this particular investigation (Neildez-Nguyen et al., 2002).

The advantage is also evident in relation to the method of Freyssenier and colleagues who isolated the CD36 positive population out of CD34$^+$ stem/progenitor cells (Freyssinier et al., 1999). We showed that in CD36$^+$ progenitors at least two subpopulations exist and that they show a lower erythroid potential in colony assay than E-cad$^+$ progenitor. Additionally, the CD36$^+$ cells were reported to be a mixed population of BFU-E and CFU-E type progenitors (Freyssinier et al., 1999). We, on the other hand, observed an almost pure BFU-E type population for E-cad$^+$ progenitor cells in our experiments (Fig 3.14B).

Bühring and colleagues reported that E-cad was exclusively expressed in CFU-E type progenitors and normoblasts but not in BFU-E type progenitors (Bühring et al., 1996). In contrast to our methods, in this study fresh bone marrow cells were analyzed in colony assays and the cells were not amplified *in vitro* in Epo free culture conditions. These methodical differences might be the reason why no BFU-E type colonies were observed. Possibly the amount of Epo-independent BFU-E type E-cad$^+$ progenitors is so minute in the initial bone marrow tissue that no BFU-E colonies can be observed without *in vitro* amplification.

The expression of E-cad seems to be more restricted to the red cell lineage in the hematopoietic system, even to distinct developmental stages of erythrocytes (Armeanu et al., 1995; Armeanu et al., 2000; Bühring et al., 1996). Thus, the use of E-cad as selection marker is a clear advantage.

4.2.2 The transcriptional profiles of Epo-independent E-cad$^+$ and Epo-dependent SCF/Epo progenitor cells

In many cases, microarray analysis was used to compare the gene expression patterns of different cell types or of specific cellular stages of development (Hacker et al., 2003, Ju et al., 2003, Park et al., 2002, Steidl et al., 2002, Andrews et al., 1993; Boeuf et al., 2001). Starting from the hypothesis that E-cad$^+$ and SCF/Epo progenitor cells represent different cell types, we chose the DNA chip technology to investigate common features and differences between both on the transcriptional level.

The fact that 4,016 genes out of 7,030 defined as expressed show a similar expression level in both cell types, pleaded for a strong similarity of E-cad$^+$ and SCF/Epo progenitor cells. Genes that have been described as red cell specific, e.g. EpoR, glyc B, GATA-1, or genes known to be regulated during red cell development, e.g. c-kit, CD71, GATA-2 and CD33 showed similar expression levels in both cell types (data not shown). Furthermore, gp130 as an important common co-receptor of the IL-6 pathway, and E-cad and CD36 were found in this group (data not shown). These facts partially reflected the results from flow cytometry analysis.

Although both cell populations seemed to be very similar in their transcriptional pattern, they showed important differences, which support the hypothesis that E-cad$^+$ and SCF/Epo progenitor cells represent different cell types. First, in the hierachical cluster, E-cad$^+$ progenitors and SCF/Epo progenitors did not cluster together (Fig. 3.21). On the contrary, the E-cad$^+$ progenitors cluster was closer to the CD34$^+$ and CD36$^+$ cells. Secondly, transcripts differentially expressed in E-cad$^+$ progenitors and SCF/Epo progenitors formed cell type specific gene profiles.

Interestingly, some of the erythroid specific genes, EKLF, NF-E2, glycophorin A and band 3 showed higher expression levels in SCF/Epo progenitors (data not shown), which might also indicate a more mature developmental stage for SCF/Epo progenitors.

Out of the cell type specific gene profiles we finally defined single signature genes for each progenitor type. Roughly, the signature genes with known function can be subdivided into 3 groups. Functionally, AMHR, IGFB4 and PDF are involved in growth factor mediated signaling; Galectin-3, cyclin D1 and cdt1 are involved in the cell cycle regulation, calretinin and CapG in the Ca^{++} metabolism.

Calretinin is abundant in neuronal cells and closely related to calbindin D28K, which is involved in Vitamin D dependent calcium absorption (Parmentier and Lefort, 1991). For red cell development it has also been reported that Ca^{++} metabolism plays a role. Ca^{++} was shown to induce the down regulation of c-myb expression during erythropoiesis (Magocsi et al., 1999).

Both AMH and PDF belong to the TGF-β family and signal through heteromeric serine/threonine kinase receptor complexes. Members of this family have been reported to have important functions in cell growth, differentiation, apoptosis and embryonic development (for review see Kingsley, 1994). AMHR II, which we showed to be specifically expressed in E-cad$^+$ progenitors (Fig 3.23A), belongs to the type II receptors of the TGF-β receptor family and can interact with various TGFβ type I receptors *in vitro*. Furthermore, it was reported that AMH signaling results in Smad1 activation through AMHR II (Josso et al., 2001). PDF belongs to the group of four transcripts differentially expressed with a higher expression level in SCF/Epo progenitors (Fig 3.23D). In microarray analysis of differentiating red cells, it was shown to be up regulated during differentiation (C.H. unpublished data). Its function is still not clear but Krieglstein and colleagues suggest a promoting role in the neuronal cell survival (Krieglstein et al., 2002). Previously TGFβ itself was reported to play a major role in the growth of chicken erythrocytic progenitors (Gandrillon et al., 1999). In the chicken system SCF-dependent erythroid progenitors seem to be more mature than the TGFα/TGFβ-dependent erythroid progenitors, which exhibit a considerably longer lifespan *in vitro* (Steinlein et al., 1995; Beug et al., 1995; Hayman et al., 1993; Gandrillon et al., 1999). AMH and PDF might influence the maturation stage of the respective red cell progenitor type in a cell specific manner, whereas the up-regulation of PDF during apoptosis suggests functional involvement in cell maturation.

IGFBP4 belongs to the group of genes specifically expressed in SCF/Epo progenitors (Fig. 3.23C). It is known to modulate the IGF-1 concentration by acting as a functional antagonist of IGF-1 (Aron, 1992). IGF-1 plays a stimulating role in erythropoiesis (Aron, 1992). Previously we described galectin-3 as a transcript highly expressed in E-cad$^+$ progenitors (Fig 3.23B). Among others, galectin-3 has been already reported to be weakly expressed in early CD34$^+$ cells of bone marrow (Woo et al., 1991, Le Marer, 2000). Functionally, members of the galectin family have been

shown to be involved not only in cell adhesion regulation but also in the control of cell proliferation and apoptosis (Hughes, 1994; Akahani et al., 1997; Krugluger et al., 1997). Recently, Tao and colleagues investigated apoptosis-related genes in human cord blood $CD34^+$ progenitors by gene expression profiling. Among others, galectin-3 was shown to be up regulated during induction of apoptosis by etoposide treatment (Tao et al., 2003). Galectin-3 seems to prevent apoptosis by inducing cell cycle arrest at different stages, depending on the apoptotic stimuli (Lin et al., 2000).

Cyclin D1 belongs to the set of genes which were expressed only in SCF/Epo progenitors (Fig 3.23C) and it is a molecule which is essential for the cell cycle in both G1 phase and G1/S transition. Cyclin D1 was reported to be regulated by STAT5A (Matsumura et al., 1999). The transcription factor STAT5A is known to be activated by a variety of cytokines, including Epo. Early murine erythroblast ($CD71^{high}$ $Ter119^{high}$) might be the target of EpoR/STAT5 signaling (Socolovsky et al., 2001). This indicates an involvement of cyclin D1 in cytokine-dependent growth of erythroid progenitor cells. Galectin-3 was reported to be expressed in $CD34^+$ cells, and cyclin D1 was suggested to play a role in later erythroid differentiation. This supports our hypothesis that E-cad^+ progenitors are less mature than SCF/Epo progenitors.

4.2.3 E-cad^+ and SCF/Epo progenitor cells - two maturation stages in red cell development

Finally, we were able to demonstrate that E-cad^+ and SCF/Epo progenitor cells had very similar properties. The cell growth in the respective growing conditions followed a similar growth kinetic (Fig 3.7). As mentioned before, both progenitor types had similar surface marker profiles, including the simultaneous expression of E-cad and CD36 (Fig. 3.10). They were responsive to Epo and effectively differentiated into erythrocytes. The majority of expressed genes showed similar expression levels in microarray analysis, including several erythroid specific genes.

But we also observed distinct differences. The most important difference is the Epo-independence of E-cad^+ progenitors and the Epo-dependence of SCF/Epo progenitors. Differences in the expression of the erythroid surface markers EpoR, glycophorin A/B, Kell and band 3 were also observed (as mentioned before). The differentiation of

E-cad$^+$ progenitors was shown to require an additional commitment phase in SCF/Epo growing conditions because cells were unable to differentiate directly as was shown for SCF/Epo progenitors (Panzenböck et al., 1998; C.H. unpublished data). All differences indicate that E-cad$^+$ progenitor cells are less mature than the SCF/Epo progenitors. Additionally, differences between both progenitor types were shown in the cell type specific gene profiles generated. In the groups of single signature genes, we found clues for different maturation stages of the analyzed cell types.

SCF/Epo progenitors are very similar to the SCF-dependent erythroid progenitor obtained from the bone marrow of chicken (Panzenböck et al., 1998; Wessely et al., 1997; Bartunek and Zenke, 1998). In the chicken system, SCF-dependent erythroid progenitors seem to be more mature than the TGFα/TGFβ-dependent erythroid progenitors, which exhibit a considerably longer lifespan *in vitro* (Steinlein et al., 1995; Beug et al., 1995; Hayman et al., 1993; Gandrillon et al., 1999). Therefore our studies suggest that the human Epo-independent E-cad$^+$ progenitor cells described here represent a potential functional equivalent to the TGFα/TGFβ--dependent progenitor in the chicken system.

4.3 CD36$^+$ progenitors – a heterogeneous cell population

4.3.1 Different and common features of CD36$^+$ and E-cad$^+$ progenitors

CD36 is considered to be a surface marker of red cell precursors (van Schravendijk et al., 1992) but also a marker of late megakaryocytic and monocytic cells (de Wolf et al., 1994; Nakahata and Okumura, 1994). To obtain pure populations of red cell precursors, the separation of the CD36$^+$ subpopulation using magnetic anti-CD36 beads has been reported previously (Freyssinier et al., 1999; Scicchitano et al., 2003). Recently, these techniques have been simplified even more by the commercial availability of purified human CD36 cord blood progenitors (Poetics™, Cambrex Bio Science Verviers S.P.R.L., Verviers, Belgium).

We used a separation and amplification protocol similar to the protocol of Freyssinier et al. (Freyssinier et al., 1999) and investigated the CD36$^+$ progenitors in parallel to the E-cad$^+$ progenitors. First, we observed that these CD36$^+$ cells contained at least two subpopulations concerning the simultaneous expression of E-cad and CD36, and that the E-cad$^+$ progenitor is one of these subpopulations. Both progenitors have thus

similar characteristics. They showed the same behavior in their growth kinetics (Fig. 3.7; data not shown for CD36) and had comparable patterns in their growth factor response, tested by ^{3}H-thymidine incorporation (Fig. 3.8). As was discussed for E-cad^{+} progenitors, SCF seems to be the main player in the proliferation of CD36^{+} progenitors too. CD36^{+} progenitors also responded effectively to Epo although the growth factor seems not to be crucial for their proliferation. The fact that they did not express endogenous Epo also pleads for the Epo-independence of the progenitors (Fig. 3.18).

Both progenitor types expressed CD71 in high levels on their surface and did not express band 3. Additionally, the expression of CD71 that is abundant on proliferating cells, in the whole cell populations supports the assumption that both CD36^{+} and E-cad^{+} cells are proliferating populations.

In CD36^{+} progenitors two subpopulations were found not only for the simultaneous expression of E-cad and CD36, but also for the expression of glycophorin A/B and CD33. In each case, one subpopulation was negative and one positive (Fig. 3.11). This fact also confirms the observation that CD36^{+} progenitors are a heterogeneous cell population.

In growing CD36^{+} progenitors the majority of cells is negative and the minority is positive for E-cad. During a commitment phase in SCF/Epo commitment conditions, this proportion is shifted in favor of the E-cad positive cells (Figs. 3.10A and 3.12). It is not clear whether the population of E-cad negative cells acquire E-cad during the commitment phase or whether few positive cells are particularly supported in their proliferation.

The heterogeneity of CD36^{+} progenitors was also shown in their clonogenic potential. Only half of the colonies were of erythroid origin (Fig 3.14A). In contrast the distribution of BFU-E and CFU-E type colonies was very similar to the results shown for E-cad^{+} progenitors, where an almost pure BFU-E population was observed (Fig. 3.14B).

In this study the differentiation properties of CD36^{+} progenitors in liquid differentiation conditions were not further investigated. The fact that CD36^{+} cells derived from SI2 have the potential to differentiate into mature red cells has been shown by Freyssinier et al. (Freyssinier et al., 1999). The differentiation potential of

commercially available CD36$^+$ progenitors in culture conditions, which were similar to our SI2 conditions, and in the addition of Epo, was previously analyzed by Scicchitano et al. (Scicchitano et al., 2003). It has been shown that CD36$^+$ progenitors can differentiate into fully mature red cells, but it is not clear whether all or only a subpopulation of CD36$^+$ progenitors can do so.

4.3.2 The gene expression profile of CD36$^+$ progenitors

Three samples of human CD36$^+$ progenitors were analyzed by microarray technique and resulting data were compared to the data of 2 samples from E-cad$^+$ progenitors. Similar to the results of E-cad$^+$ progenitors, CD36$^+$ progenitors express 42-44% of the analyzed genes.

In the hierarchical cluster CD36$^+$ progenitors were found to cluster near to E-cad$^+$ progenitor samples, but they did not cluster together (Fig. 3.21). In contrast, CD36$^+$ progenitors clustered also near to SI2 cells and did not form a separate branch. The relatedness of all three progenitor types might reflect the fact that both CD36$^+$ and E-cad$^+$ progenitors are components of the SI2 cell population.

It was not surprising that the majority of genes (4,248) showed similar expression levels in both cell types (Fig. 3.24), since E-cad$^+$ progenitors were a subpopulation of CD36$^+$ progenitors. In this study we demonstrated that E-cad$^+$ progenitors are fully competent red cell progenitors. Thus, it is clear that at least the E-cad$^+$ progenitor subset of the CD36$^+$ cells expresses red cell specific transcripts, such as globin α, β and γ, rhesus and blood group antigens, erythroid 5-aminolevulinate synthase, ferritin, transferrin receptor, EpoR, glycophorin A, B, C and E, and band 3. Also the transcription factors EKLF, NF-E2, GATA-1 and GATA-2, known to play a role in the erythroid development, showed similar expression levels (data not shown). Additionally, Scicchitano et al. reported the regulation of some of these transcripts during the differentiation of CD36$^+$ progenitors (Scicchitano et al., 2003).

As has been done previously, we defined a transcriptional signature for the CD36$^+$ progenitors, including all genes which were shown to be expressed exclusively or at higher level in this progenitor type. The transcriptional signature of CD36$^+$ progenitors comprises 425 genes. We finally focused on 8 genes out of this cell type specific gene profile yielding the highest normalized expression levels (Fig. 3.25). 6

out of these 8 signature transcripts encode for genes playing a role in megakaryocytes and platelets (PF4, CD61,CD42, CD41, CD31 and IgER α). These results implicate that $CD36^+$ progenitors, obtained by immunomagnetic bead selection, have megakaryocyte/platelet and other myeloids components besides their red cell properties.

Previously, numerous experiments showed a close relationship between erythropoiesis and megakaryocytopoiesis (Higuchi et al., 1997). The glycoprotein CD36, here used as a selection marker to obtain Epo-independent red cell progenitors, is also known to be a major protein on the surfaces of megakaryocytes and platelets (Ikeda, 1999). Recent advances have shown the particular importance of three transcription factors playing central roles not only in erythroid differentiation but also in megakaryocytic and platelet differentiation. The functions of GATA-1, NF-E2 and FOG-1 in erythropoiesis were previously described (see 1.2.3). In thrombopoiesis, GATA was found to play crucial roles e.g. in the transcriptional regulation of rat PF4, human CD41 and the vWF-R (Ravid et al., 1991; Shivdasani, 2001). Furthermore, the binding of GATA-1 to enhancer elements of the IgERα chain was reported by Nishiyama et al., 1999. All these molecules or subunits thereof we identified as signature transcripts for $CD36^+$ progenitors. On the other hand, Nakahata and colleagues reported the expression of megakaryocytic marker molecules, such as CD41 and CD61 in BFU-E type progenitors (Nakahata and Okumura, 1994). Taking these facts into account, the existence of a bipotential erythro-megakaryocytic progenitor can be considered (Debili et al., 1996; Shivdasani, 2001). We speculate that the E-cad negative population of our investigated $CD36^+$ progenitors might represent this bipotential progenitor type.

4.4 Conclusions and future perspectives

In this study our efforts led us to the isolation of an Epo-independent red cell progenitor out of the cord blood derived SI2 cell population using the erythroid progenitor specific early surface markers E-cad and CD36. We amplified these Epo-independent $E\text{-}cad^+$ progenitors in serum free culture conditions using SCF, IL-3 and hyper-IL-6 to large numbers and demonstrate their erythroid properties and differentiating potential by various parameters. The $E\text{-}cad^+$ progenitors have been shown to be early BFU-E type progenitors, similar to the early TGFα-dependent

progenitors observed in the chicken system (Hayman et al., 1993; Schroeder et al., 1993; Briegel et al., 1996; Wessely et al., 1997).

We compared the gene expression profile of the early Epo-independent E-cad$^+$ progenitor cells to the profile of the more mature Epo-dependent SCF/Epo progenitor cells, using the DNA chip technology. The aim of the comparison was not only to define differences but also to find genes of additional potential regulators of growth, commitment and differentiation, such as cytokine receptors or transcription factors. By the comparison of gene profiles, we could demonstrate that the Epo-dependent and Epo-independent progenitors are very similar but show also distinct differences. Additionally, the analysis of molecular differences resulted in the determination of several candidate genes.
For further analysis, the different roles of these genes, e.g. galectin-3, AMHR, PDF and IGF-1 in conjunction with IGFBP4, have to be determined. In particular, the special support of E-cad$^+$ progenitors by AMH will be an interesting question to be answered.

Furthermore, we demonstrate that the CD36$^+$ progenitors, separated by immunomagentic bead selection and described as Epo-independent erythroid progenitors (Freyssinier et al., 1999), contained at least 2 subpopulations. One subpopulation contained the previously described E-cad$^+$ progenitors. The second subpopulation did not express E-cad. The comparison of the gene expression profiles of CD36$^+$ and E-cad$^+$ progenitors led us the assumption that the second population has megakaryocytic features.

In future experiments our hypothesis, that CD36$^+$/E-cad$^-$ cells might be bipotential erythroid-megakaryocytic progenitors, remains to be proven. This requires a more effective method for the separation of this subpopulation, e.g. the sorting by flow cytometry methods. Based on our studies we generated the following model of erythroid development (Fig.4.1).

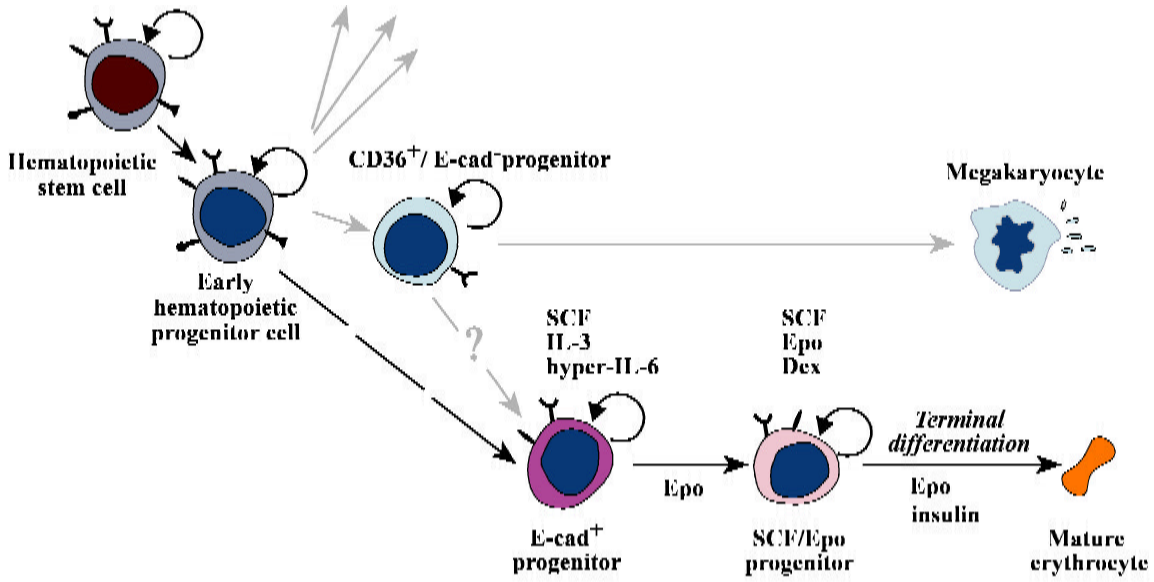

Figure 4.1 : Schematic representation of erythroid development

Different types of self renewing hematopoietic progenitor cells are represented. E-cad$^+$ progenitors and SCF/Epo progenitors are committed for the erythroid lineage. CD36$^+$/E-cad$^-$ progenitors represent either erythroid–megakaryocytic bipotential progenitors or are committed for the megakaryocytic/platelet lineage. Listed growth factors correspond to the cell culture conditions.

Originating from a multipotent hematopoietic stem/progenitor cell population via a stage of bipotential erythroid-megakaryocytic precursors, E-cad$^+$ progenitors start the erythroid development, already committed as Epo-independent red cell progenitors. It is still not clear whether the CD36$^+$/E-cad$^-$ progenitors represent the bipotential erythroid-megakaryocytic progenitor or whether they are already committed megakaryocytic progenitors.

This new knowledge, obtained from previous work, might lead to more efficient *in vitro* culture conditions for red cell progenitor amplification. Additionally, the more advanced *in vitro* model for erythropoiesis could help to answer prospective medical questions.

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6 APPENDIX

6.1 Abbreviations

µCi	micro curie
^{3}H	tritium
AMH	anti-müllerian hormone
AMHR	anti-müllerian hormone receptor
AR	amphiregulin
BFU-E	burst forming unit-erythroid
BSA	bovine serum albumin
BTC	betacellulin
CapG	gelsolin-like capping protein
CD	cluster of differentiation antigen
cDNA	complementary desoxyribonucleid acid
CFSE	5,6-carboxylfluorescein diacetate succinimidyl ester
CFU-E	colony forming units-erythroid
CFU-GEMM	colony-forming units-granulocyte, erythrocyte, monocyte, macrophage
CFU-GM	colony-forming units-granulocyte, macrophage
CHO	chinese hamster ovary
cRNA	complementary ribonucleid acid
CRYBA4	beta crystalline–A4
CV	coefficient of variation
DC	dendritic cell
Dex	dexamethasone
DNA	desoxyribonucleid acid
E-cad	E-cadherin
EGF	epidermal growth factor
EKLF	erythroid krüppel-like factor
Epo	erythropoietin
EpoR	Epo receptor
EtBr	ethidium bromide
EtOH	ethanol
f.c.	final concentration
FACS	fluorescence activated cell sorting
FCS	fetal calf serum
FGF	fibroblast growth factor
Fig.	figure
FITC	flourescein isothiocyanate
Flt3L	Fms-like tyrosine kinase 3 ligand
FOG	friend of GATA
GAPDH	glycerolaldehydphosphate dehydrogenase
GH	growth hormone
glyc	glycophorin
GM-CSF	granulocyte macrophage-colony stimulating factor
GP	glycoprotein
HB-EGF	heparin binding EGF
HCl	hydrochloric acid
HER	human estrogen receptor
HLH	basic helix-loop-helix
HPC	hematopoietic progenitor cell

HRGβ	heregulin β
HRP	horseradish peroxidase
HSC	hematopoietic stem cell
hyper-IL-6	IL-6/soluble IL-6 receptor fusion protein
Id1	inhibitor of DNA binding/differentiation 1
Ig	immunglobulin
IGF	insulin-like growth factor
IGFBP4	IGF binding protein 4
IL	interleukin
JAK	Janus kinases
kD	kilo Dalton
LENG4	leukocyte receptor cluster 4
Mac	macrophage
MAPK	mitogene-activated protein kinase
ME	mercaptoethanol
MeOH	methanol
MHC	major histocompatibility complex
min	minute
MNC	mononuclear cells
mRNA	messanger ribonucleid acid
MW	molecular weight
ND	not determined
NF-E2	nuclear factor erythroid 2
NTP	nucleotid triphosphate
OD	optical density
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDF	prostate differentiation factor
PDGF	platelet derived growth factor
PE	phycoerythrin
PECAM-1	platelet endothelial cell adhesion molecule 1
Pen/Strep	penicillin/streptomycine
Pen	penicillin
PF4	platelet factor 4
PI	propidium iodide
PI3K	phosphatidylinositol 3´kinase
PLC g	phospholipase Cγ
prog.	progenitor
Rh	rhesus
rhu	recombinant human
RNA	ribonucleid acid
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
Sca-2	stem cell antigen 2
SCF	stem cell factor
SDS	sodium dodecyl sulfate
sec	second
SI_2	culture conditions: SCF, IL-3, hyper-IL-6
β-ME	β-mercaptoethanol
STAT	signal transducer activator of transcription

STIF	culture conditions: SCF+Tpo+hyper-IL-6-Flt3L
Strep	streptomycin
TBE	tris boric acid EDTA buffer
TBS	tris buffered saline
TGF	transforming growth factor
Tpo	thrombopoietin
U	unit
UV	ultra violet
v/v	volume per volume
vWF-R	von-Willebrandt-factor receptor
w/v	weight per volume

6.2 Signature gene lists

- Id v2, refers to the indentity number on Affymetrix chip HG_U95Aver2
- Flags: “absent” and “present” calls determined by GeneChip Analysis Suit Software (MAS v. 5, Affymetrix, Inc.)
- Acc no, refers to accession number

A: Signature of $CD36^+$ progenitors

B: Signature of $E\text{-}cad^+$ progenitors

C: Signature of SCF/Epo progenitors

A: Signature genes of CD36+ progenitors

	CD36_1		CD36_3		CD36_4			
Id v2	Normalized	Flags	Normalized	Flags	Normalized	Flags	Acc no	Function
1115_at	5,748	P	23,353	P	25,148	P	M25897	receptor, chemokine, CXCL04, PF4
40644_g_at	1,977	P	2,692	P	2,273	P	M34480	surface protein, CD041
34023_at	6,697	P	2,461	P	2,808	P	X06948	surface protein, IgE R alpha, FcR
37952_at	7,582	P	5,605	P	3,887	P	M35999	surface protein, CD061, GPIIIa, platelets
40455_at	4,581	P	2,508	P	1,348	P	AB020637	KIAA0830
38968_at	2,123	P	2,663	P	1,450	P	AB005047	SH3BP5
41266_at	1,053	M	2,607	P	2,315	P	X53586	surface protein, CD049f, integrin, alpha6
36192_at	3,045	P	1,449	A	1,378	P	D83777	unknown
33063_at	2,551	P	4,523	P	4,724	P	J02940	surface protein, CD042b
1494_f_at	0,825	P	3,073	P	2,202	P	M33318	enzyme, cytochrome
36773_f_at	2,973	P	1,270	A	2,024	P	M81141	surface protein, MHC II, HLA-DQ
37908_at	4,256	P	3,980	P	2,971	P	U31384	G protein 11
37243_at	2,958	P	4,495	P	5,692	P	X66533	enzyme
1480_at	2,926	P	2,541	P	0,683	A	L12723	heat shock
120_at	2,891	P	2,585	P	0,834	A	X68742	surface protein, CD049a, (vla-1)
268_at	1,203	A	1,924	P	2,723	P	L34657	surface protein, CD031
479_at	1,816	P	2,007	P	2,023	P	U53446	miscellaneous
37969_at	2,062	P	2,365	P	1,365	A	M59979	enzyme
33753_at	1,158	P	2,982	P	1,648	P	AB014566	KIAA0666
39456_at	2,243	P	1,989	P	1,527	M	AF090100	unknown
32288_r_at	1,337	M	2,321	P	2,080	P	AJ001685	receptor, NK, NKG2E, lectin
37534_at	0,705	A	2,679	P	2,304	P	Y07593	receptor, CAR
36334_at	1,738	M	1,843	P	2,089	P	L42621	surface protein, CD229, Ly9
852_at	1,541	P	2,174	P	1,798	A	S70348	surface protein, CD061, integrin, beta 3
39956_at	2,466	P	0,986	A	2,050	P	AF041853	KIF3A
1797_at	2,369	P	1,659	P	1,442	P	U40343	cell cycle, CDK inhibitor
41723_s_at	2,593	P	1,126	P	1,634	P	M32578	surface protein, MHC II, HLA-DR
39610_at	2,426	P	1,715	P	1,165	P	X16665	transcription, homeo box, HOX2B
33535_at	2,779	P	1,483	P	1,027	P	U45448	receptor, purinoceptor
32877_i_at	2,055	P	0,828	A	2,352	P	AA524802	unknown
33210_at	1,987	P	2,231	P	0,988	A	AA709159	transcription, YY1
32940_at	0,931	P	2,831	P	1,429	P	AJ007014	miscellaneous
36414_s_at	2,551	P	1,108	A	1,456	P	AF032119	CASK
36878_f_at	2,763	P	0,752	A	1,549	P	M60028	surface protein, MHC II, HLA-DQ
33925_at	1,145	A	2,183	P	1,734	P	X99076	signalling
1570_f_at	2,546	P	1,032	P	1,433	P	L49219	cell cycle, retinoblastoma, Rb
336_at	1,191	A	1,844	P	1,958	P	D38081	receptor, hormone, thromboxane A2 R
34975_at	2,757	P	0,838	A	1,381	P	AF097358	mast cell, KLRG1
37115_at	0,996	A	2,019	P	1,929	P	AL031666	kinase, PKC binding
743_at	2,155	P	0,819	A	1,907	P	D50370	nucleosome assembly protein
40643_at	1,374	P	1,836	P	1,535	P	M34480	surface protein, CD041
41288_at	2,385	P	1,250	P	1,103	P	AL036744	unknown
37689_s_at	1,552	P	1,270	M	1,907	P	X68090	receptor, Fc, Fc-gamma-RIIA
32770_at	2,074	P	1,201	M	1,452	P	AB018298	structural protein
39200_s_at	1,868	P	1,860	P	0,947	A	W28760	GDF11

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
38833_at	2,017	P	1,022	M	1,594	P	X00457	surface protein, MHC II, HLA-DP1A
1882_g_at	0,917	A	2,089	P	1,622	P	U14520	Oncogene Aml1-Evi-1
36821_at	1,001	P	2,412	P	1,167	P	AL050367	unknown
39298_at	2,376	P	0,845	A	1,349	P	AB022918	enzyme
32962_at	0,792	A	2,258	P	1,510	P	S52028	enzyme, CTH
34665_g_at	1,641	P	1,627	P	1,282	P	X62573	surface protein, FcR
33969_at	1,868	P	1,664	P	1,017	A	X58822	receptor, IFN, IFNomega1
37150_at	2,249	P	1,161	P	1,132	P	AB026190	unknown
2058_s_at	0,910	A	2,174	P	1,434	P	M35011	surface protein, integrin, beta05
35514_at	1,574	P	1,965	P	0,949	P	AA916905	KIAA0861
580_at	1,381	A	1,443	P	1,645	P	M60748	replication, histone
38549_at	2,507	P	0,973	P	0,988	P	AF026941	unknown
33260_at	1,429	P	1,137	M	1,871	P	L13857	signalling
40045_g_at	1,047	P	1,136	M	2,240	P	AF009425	unknown
153_f_at	1,983	P	1,695	P	0,743	A	X00088	replication, transcription, histone, H2B
37214_g_at	2,141	P	1,498	P	0,777	A	X90392	enzyme, DNAse
31804_f_at	1,154	P	2,049	P	1,206	P	X78283	enzyme, sulfotransferase (ST1A3)
40981_at	1,233	P	1,303	A	1,868	P	U00930	KIAA1564
41318_g_at	1,600	M	1,269	P	1,534	P	X12534	signalling, ras related, rap2
35886_at	1,665	P	1,529	P	1,190	M	AL049758	structural protein
36287_at	2,001	P	0,950	A	1,430	P	X83368	kinase, PI-3K, signalling
41286_at	1,492	A	1,417	P	1,472	P	X77753	surface protein
32037_r_at	1,213	A	1,498	P	1,652	P	AF001175	enzyme, ribonuclease
705_at	1,019	A	2,286	P	1,057	P	U09806	enzyme
41504_s_at	1,999	P	1,013	A	1,348	P	AF055376	transcription, maf, c-maf
37778_at	1,617	P	0,922	A	1,798	P	AJ005273	KIN
41390_at	1,522	P	1,278	A	1,527	P	X69086	structural protein, utropin
35547_at	1,975	P	1,298	P	1,046	M	AF058056	enzyme, transporter
271_s_at	0,578	A	1,756	P	1,977	P	J05036	proteolysis, cathepsin E, host defense
37873_g_at	0,732	A	2,117	P	1,404	P	AF072468	miscellaneous, jerky homolog
38894_g_at	2,220	P	0,984	P	0,988	P	AL008637	enzyme, p40hox
35788_at	1,679	P	1,570	P	0,938	A	W28994	structural protein, dynein
39540_at	1,631	P	1,748	P	0,786	A	AF000561	miscellaneous, FBI1
39966_at	1,672	P	1,270	A	1,214	P	AF059274	structural protein
40399_r_at	0,996	P	0,984	P	2,170	P	AI743406	unknown
38032_at	1,473	P	1,279	P	1,393	P	AB018279	unknown
37398_at	0,901	M	1,121	P	2,112	P	AA100961	surface protein, CD031, PECAM1
38347_at	1,400	P	1,741	P	0,971	A	W27779	unknown
36482_s_at	1,802	P	0,962	P	1,343	P	Y15724	ATPase
38221_at	1,492	P	1,080	M	1,528	P	AF100153	signalling
37344_at	1,406	P	1,198	A	1,479	P	X62744	surface protein, MHC II, HLA-DMA
41357_at	1,652	P	1,427	P	0,988	A	W27997	ATP5B
41761_at	1,114	P	1,129	A	1,820	P	M96954	apoptosis, nucleolysin
38418_at	2,234	P	0,840	P	0,988	A	X59798	cell cycle, cyclin D1
32355_at	0,965	A	1,955	P	1,138	P	AL050270	unknown
36371_at	1,122	P	1,136	A	1,799	P	L35251	structural protein
36705_at	0,509	A	2,063	P	1,479	P	AJ224538	kinase
33290_at	0,884	P	1,756	P	1,407	P	M74161	phosphatase, signalling

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
1441_s_at	1,372	P	1,180	P	1,481	A	X83492	surface protein, CD095, Fas, Apo-1
1321_s_at	1,426	P	1,238	A	1,359	P	U43916	surface protein
35102_at	0,992	A	1,077	P	1,930	P	L32164	transcription, zinc finger
1379_at	1,394	P	0,986	A	1,610	P	M59371	kinase, tyrosine kinase, ECK
32687_s_at	1,726	P	0,993	M	1,270	P	X83857	receptor, prostaglandin E R
37210_at	0,958	P	1,298	P	1,720	P	S78296	structural protein
41358_at	1,757	P	0,929	P	1,289	P	AI827730	cell cycle, cyclin M2
31529_at	0,841	A	0,994	P	2,138	P	X99141	structural protein, keratin
888_s_at	1,500	P	1,376	P	1,094	P	M62302	receptor, GDF-1
41545_at	0,987	P	1,843	P	1,131	P	X66365	cell cycle, CDK06
35600_at	1,306	P	1,276	P	1,372	P	AB023967	ROD1
36784_at	1,446	P	1,500	P	0,988	P	J03071	receptor, growth hormone, GH
33071_at	0,996	P	1,514	P	1,419	P	Z98744	replication, transcription, histone 4
370_at	1,623	P	1,455	M	0,839	P	Z35102	kinase, Ndr
35974_at	0,802	P	1,718	P	1,390	P	U10485	structural protein
35139_at	1,082	A	1,057	P	1,768	P	AL049341	unknown
40925_at	1,913	P	0,983	P	1,012	P	AA554945	unknown
1221_at	1,058	P	1,287	A	1,556	P	X54871	G protein
33271_r_at	1,188	P	1,204	P	1,510	A	AJ005821	DMXL1
38830_at	1,325	A	1,307	P	1,252	P	U66685	enzyme
34443_at	1,528	P	0,967	A	1,387	P	R61362	unknown
32733_at	0,872	A	1,515	P	1,493	P	AA045160	unknown?
35494_at	0,988	P	1,407	P	1,479	P	M55536	enzyme, transporter
35434_at	1,763	P	1,258	P	0,852	M	L16794	transcription, MEF2
35440_g_at	0,996	A	1,438	P	1,438	P	D26121	miscellaneous
37536_at	1,662	P	0,656	A	1,551	P	Z11697	surface protein, CD083
41039_at	1,575	P	1,306	P	0,988	A	AL022476	enzyme, TTLL1
37537_at	1,429	P	1,337	P	1,098	P	L04510	enzyme
40084_at	1,655	P	1,193	P	1,013	P	U03494	transcription, CP2
40983_s_at	1,746	P	1,161	P	0,951	A	W28830	enzyme
31775_at	1,106	A	1,522	P	1,227	P	X65018	surface protein, lectin
32065_at	0,968	P	1,787	P	1,092	A	S68134	transcription, CREM
33347_at	0,873	P	1,547	P	1,426	P	AA883868	miscellaneous, ring finger, RNF5
36958_at	2,101	P	0,986	P	0,745	A	X95735	structural protein, zyxin
31943_g_at	1,308	P	1,360	P	1,162	P	AF045583	transcription, tub, TULP3
39402_at	1,441	P	0,979	M	1,404	P	M15330	receptor, IL-01b
34375_at	1,864	P	1,122	P	0,833	P	M28225	receptor, chemokine, CCL02, MCP-1
37310_at	2,046	P	1,079	P	0,682	A	X02419	proteolysis
39267_at	0,915	M	1,391	P	1,496	P	AF102265	enzyme
32987_at	1,176	P	1,035	A	1,584	P	X97249	leucine-rich primary response protein 1
35441_at	1,460	P	1,345	P	0,988	P	AF058922	structural protein
37750_at	0,999	P	1,301	P	1,476	P	AF049140	proteolysis
40294_at	1,416	P	1,435	P	0,916	P	U66676	enzyme, transporter
34757_at	1,765	P	1,341	P	0,661	A	AA595596	enzyme, transferase
31961_r_at	0,876	M	1,229	P	1,652	P	AF070579	unknown
548_s_at	1,674	P	0,986	M	1,088	P	S80267	kinase, syk
160033_s_at	1,214	P	1,617	P	0,914	P	M36089	replication, DNA repair
35465_at	0,655	A	1,658	P	1,426	P	AC003083	unknown

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
41240_at	1,233	P	0,986	P	1,520	P	AA772359	unknown
41024_f_at	0,724	P	1,783	P	1,224	P	X53004	surface protein, glycophorin B, CD235b
40191_s_at	0,886	P	1,901	P	0,937	P	AI761647	unknown
1293_s_at	1,801	P	0,925	P	0,988	P	L11701	enzyme, signalling, phospholipase D
34052_at	1,018	P	0,994	P	1,702	P	D14582	receptor, epimorphin
1438_at	1,334	P	1,401	P	0,965	P	X75208	receptor, ephrin R, HEK2
1000_at	1,345	P	1,373	M	0,979	P	X60188	kinase, ERK1
31810_g_at	1,607	P	1,282	P	0,788	A	Z21488	kinase, contactin
40604_at	1,653	P	1,406	P	0,612	M	Y13493	kinase, Dyrk2
40806_at	0,983	P	1,901	P	0,778	P	U15128	enzyme, MGAT2
33982_f_at	0,872	P	1,136	P	1,652	P	X59244	transcription, zinc finger, ZNF043
31944_at	1,182	P	1,487	P	0,988	M	AI028290	transcription, tub, TULP3
38538_at	1,393	P	1,229	A	1,028	P	AB014602	enzyme, channel
35385_at	1,454	P	1,436	P	0,752	P	AI766078	enzyme
36465_at	1,782	P	0,792	A	1,060	P	U51127	transcription, IRF-5
35436_at	1,187	P	1,442	P	1,002	M	L06147	miscellaneous
38512_r_at	1,490	P	1,294	P	0,843	M	D26158	miscellaneous
36765_at	1,296	P	1,334	P	0,988	P	AL080154	unknown
37425_g_at	1,028	P	1,216	P	1,369	P	AB029343	miscellaneous
32036_i_at	1,094	P	1,501	P	1,018	M	AF001175	enzyme, ribonuclease
37816_at	0,996	A	1,298	P	1,318	P	M57729	miscellaneous, host defense, inflammation
38259_at	1,595	P	1,229	P	0,786	P	AB002559	structural protein, syntaxin binding
34112_r_at	1,587	P	1,034	P	0,988	A	AL050065	unknown
39018_at	1,143	P	1,393	P	1,063	P	AF026977	enzyme, GST 3
37416_at	1,571	P	0,523	A	1,500	P	Z35227	G protein
32716_at	1,266	P	0,986	M	1,340	P	X62535	kinase, DAGK1
33432_at	0,964	A	1,637	P	0,988	P	AI547308	unknown
36357_at	0,962	P	0,774	M	1,849	P	AC004381	unknown
39263_at	1,389	P	0,991	P	1,204	A	M87434	enzyme
33024_at	1,482	P	1,576	P	0,525	A	M69136	enzyme, host defense?
39954_r_at	1,209	P	1,378	P	0,988	A	AB014528	unknown
37957_at	1,063	P	1,508	A	1,004	P	AL031177	proteolysis
39424_at	1,757	P	0,986	P	0,821	P	U70321	receptor, TNFR superfamily 14
704_at	1,115	A	1,694	P	0,745	P	U18759	transcription, NF-1
32577_s_at	1,626	P	1,000	P	0,927	P	L38933	unknown
33463_at	1,398	P	0,708	A	1,438	P	U39487	enzyme
40858_at	1,234	P	1,562	P	0,747	M	M34715	structural protein, PSG1
38220_at	1,886	P	0,662	M	0,988	P	U20938	enzyme
36101_s_at	0,568	A	1,726	P	1,237	P	M63978	receptor, VEGF
39323_at	1,458	P	1,082	M	0,988	P	U45974	phosphatase, signalling
32132_at	0,994	P	0,907	A	1,625	P	AL046649	unknown
35967_at	1,142	P	1,068	A	1,315	P	M69238	transcription, nuclear receptor, HLH
31927_s_at	0,996	A	1,359	P	1,169	P	D86062	miscellaneous
1694_s_at	1,467	P	0,836	A	1,218	P	D13413	miscellaneous
37590_g_at	0,869	P	1,230	P	1,420	M	AL109698	unknown
36152_at	1,760	P	0,846	M	0,909	P	X79353	G protein, signalling
39308_r_at	0,996	A	1,250	P	1,266	P	X81637	structural protein, clathrin
35346_at	1,152	M	1,069	P	1,289	P	AB007856	miscellaneous, FEM1B

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
41044_at	0,973	A	0,986	P	1,546	P	U43374	unknown
38882_r_at	1,056	M	1,244	P	1,203	P	AF096870	transcription, nuclear receptor, ER
34069_s_at	1,354	P	1,081	P	1,049	P	S79325	miscellaneous, SSX1
34994_at	1,429	P	0,652	A	1,401	P	AJ002572	unknown
37507_i_at	1,276	P	1,213	P	0,988	P	AA675900	unknown
39430_at	0,846	A	1,508	P	1,121	P	AF082557	enzyme
33601_at	0,757	P	1,505	P	1,206	P	AF052145	unknown
34093_at	1,430	P	0,936	A	1,102	P	AI829701	unknown
37486_f_at	1,311	P	1,308	P	0,848	M	U68385	transcription, homeo box, MRG2
642_s_at	1,537	P	0,719	A	1,210	P	L76528	proteolysis
34237_at	0,736	A	1,491	P	1,236	P	AB028961	unknown
38303_at	1,191	P	1,034	P	1,235	P	AB001523	enzyme,channel
39855_at	1,113	P	1,197	P	1,149	P	AC005787	cell cycle, fzr
33092_at	0,747	A	1,134	P	1,575	P	AC005946	unknown
38962_at	0,639	A	1,410	P	1,404	P	AB002296	unknown
36900_at	1,346	P	1,174	P	0,930	P	U52426	miscellaneous
33754_at	1,054	P	1,265	P	1,118	P	U43203	transcription, TTF1
1912_s_at	0,747	A	1,475	P	1,209	P	M74088	signalling, APC, E-cadherin
37432_g_at	1,062	P	0,986	M	1,381	P	AF077953	transcription, STAT, PIASx alpha
34544_at	1,483	P	0,986	P	0,959	P	X78925	transcription, zinc finger, ZNF267
37095_r_at	1,243	P	1,465	P	0,719	P	M84562	receptor, FMLP-R2, fmlp-related receptor I
38739_at	0,864	P	0,729	P	1,830	P	AF017257	transcription, ets2
36810_at	0,996	P	1,665	P	0,760	P	AB007954	unknown
39678_at	0,741	P	1,376	A	1,300	P	D10511	enzyme
37172_at	1,188	P	1,236	P	0,988	A	M75106	proteolysis
34427_g_at	1,124	P	1,087	P	1,196	P	U22963	surface protein, MHC I
34082_at	1,467	A	0,986	P	0,953	P	W28356	phosphatase
38491_at	0,996	A	1,211	P	1,194	P	U11732	transcription, ets, tel
33773_at	0,914	A	1,158	P	1,328	P	U13948	transcription, zinc finger
34131_at	1,267	P	1,127	M	1,005	P	AB026891	enzyme, transporter
2051_at	1,361	P	1,088	P	0,949	P	M31767	enzyme
210_at	1,304	P	0,984	M	1,107	P	M95678	enzyme, PLCB2
768_at	1,596	P	0,866	P	0,931	A	AF005361	importin alpha 6
36865_at	0,996	A	1,363	P	1,017	P	AB018302	KIAA0759
39710_at	1,090	P	1,169	P	1,117	P	U30521	miscellaneous
35229_at	0,996	P	1,116	P	1,256	P	L39211	enzyme
37384_at	1,148	A	1,106	P	1,109	P	D13640	phosphatase, protein phosphatase 2C
36211_at	1,027	P	1,141	P	1,194	P	D87461	apoptosis, regulator, Bcl
37053_at	1,165	P	1,279	P	0,917	M	L20977	ATPase
35411_at	1,247	P	1,178	P	0,936	M	AB018551	enzyme
37741_at	0,587	A	1,439	P	1,330	P	M77836	enzyme, carboxylate reductase
40965_at	0,454	A	1,242	P	1,657	P	AB019432	enzyme, channel, Cl-
37796_at	1,063	P	1,216	P	1,069	P	AF053356	miscellaneous
41689_at	0,801	P	1,278	P	1,265	M	R16035	structural protein
39286_at	1,645	P	1,035	P	0,661	A	D64109	signalling, erbB2, tob
1177_at	1,273	P	1,161	P	0,901	A	X77343	transcription, AP-2
35208_at	1,393	M	0,986	P	0,951	P	AB020681	KIAA0874
38942_r_at	0,854	A	1,453	P	1,023	P	W28610	unknown

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
37015_at	1,265	P	0,986	P	1,078	P	K03000	enzyme, aldehyde dehydrogenase
552_at	1,316	P	1,025	P	0,988	P	U02570	cell cycle, CDC42, G protein
40724_at	1,041	P	0,791	P	1,493	P	Y14443	transcription, zinc finger, ZNF200
35274_at	1,346	P	1,061	P	0,910	P	Y12226	structural protein, adaptin gamma
34280_at	0,736	A	1,376	P	1,202	P	Y09765	receptor, GABA R
35408_i_at	0,996	A	1,247	P	1,069	P	X16281	transcription, zinc finger, ZNF044, KOX7
39153_r_at	1,435	P	1,090	P	0,781	A	U06632	cell cycle, coilin
36717_at	0,852	A	1,226	P	1,220	P	AJ224162	enzyme
38723_at	1,121	P	0,986	P	1,189	P	AF052137	unknown
31787_at	1,088	M	1,062	P	1,142	P	AF068179	calcium modulating cyclophilin ligand
33779_at	0,919	A	1,048	P	1,321	P	AF060538	structural protein
34406_at	1,557	P	0,986	P	0,742	P	AB011174	KIAA0602
1230_g_at	0,972	P	0,950	A	1,361	P	U78556	unknown
35206_at	1,318	P	1,230	P	0,728	A	AF049105	cell cycle, CDC42, CDC42 effector, CEP2
178_f_at	1,141	P	1,219	P	0,907	A	U38964	replication, DNA repair, cell cycle, PMS3
39891_at	1,521	P	1,000	P	0,739	P	AI246730	unknown
41188_at	0,714	P	0,944	P	1,601	P	W28186	unknown
32282_at	1,240	P	1,012	A	1,000	P	U66047	unknown
40006_at	1,174	P	1,292	P	0,778	A	U63090	enzyme, Sialyltransferase
40232_at	1,320	P	0,892	A	1,022	P	U75370	transcription, RNA pol, mitochondrial
40536_f_at	1,397	P	1,108	P	0,728	A	AI254524	unknown
33850_at	1,078	P	1,229	P	0,925	A	W28892	translation
199_s_at	0,885	P	1,344	P	1,001	P	U33052	kinase
116_at	0,996	P	1,041	A	1,187	P	X14968	kinase, PRKAR2
732_f_at	1,104	P	0,703	A	1,416	P	M55406	surface protein, CD227, mucin
1673_at	1,137	P	0,864	M	1,222	P	M14764	receptor, TRK1, NGFR
41694_at	1,025	P	1,240	P	0,952	P	M17754	unknown
1243_at	0,937	P	1,181	P	1,095	P	U18300	replication
33470_at	0,927	A	1,136	P	1,147	P	AF052177	KIAA1719
31417_at	0,874	A	1,226	P	1,098	P	AF041240	receptor, orexin
40847_at	0,577	A	1,526	P	1,086	P	AB018293	unknown
40707_at	0,996	P	1,032	A	1,159	P	AA810792	unknown
35442_at	1,065	P	1,392	P	0,728	A	AB007958	KIAA0489
678_at	1,020	P	0,986	P	1,178	A	J04948	phosphatase
40756_at	0,870	M	1,138	P	1,174	P	AF081280	structural protein
35595_at	0,951	P	1,051	A	1,177	P	AI557374	receptor, calcitonin R component
41021_s_at	1,360	P	0,827	M	0,988	P	U36310	enzyme
39879_s_at	0,839	P	1,344	P	0,988	P	H16917	unknown
1642_at	0,899	A	1,170	P	1,097	P	U35113	miscellaneous
37225_at	0,762	P	1,158	P	1,245	P	D79994	KIAA0172
40495_at	1,087	P	1,184	P	0,888	P	AA306076	unknown
35116_at	1,018	P	1,083	P	1,054	P	X80821	ribosome
35536_at	0,996	P	1,145	P	1,012	M	AB011176	proteolysis, endothelin converting enzyme
1538_s_at	1,587	P	0,574	A	0,988	P	X00695	receptor, IL-02
34034_at	1,428	P	0,986	P	0,735	P	D80011	KIAA0189
35001_at	1,068	P	1,129	P	0,949	M	Z85986	kinase, Ndr
32810_at	0,914	P	0,913	P	1,316	P	AF019369	enzyme, transferase
40151_s_at	0,840	P	1,140	P	1,160	M	Z48054	structural protein

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
187_at	1,241	P	1,092	M	0,806	P	U07349	kinase, BL44
40521_at	1,133	P	0,971	A	1,028	P	AL050259	signalling, ras family, rab2
37278_at	1,139	P	1,043	P	0,951	P	X92762	structural protein
32612_at	1,408	P	0,706	P	1,011	P	X04412	structural protein
31594_at	1,246	P	0,986	P	0,889	A	Y16788	adhesion, keratin
32517_at	0,996	P	0,963	P	1,159	P	AB028639	proteolysis, CAPN7
875_g_at	1,111	P	0,944	P	1,063	P	M26683	receptor, chemokine, CCL02, MCP-1
39530_at	1,254	P	1,167	P	0,683	A	L35240	ENIGMA
34367_at	0,744	A	1,369	P	0,988	P	AF006043	Phosphoglycerate dehydrogenase
41572_r_at	0,637	A	1,354	P	1,109	P	X75042	transcription, NF-kB, c-rel
37256_at	0,571	P	1,214	A	1,313	P	AI829890	unknown
41458_at	0,779	M	1,157	P	1,160	P	AB007936	KIAA0467
1892_s_at	1,358	P	1,146	P	0,585	A	X03541	receptor, TRK1, NGFR, kinase
1993_s_at	0,775	P	1,420	P	0,894	M	U14680	miscellaneous, BRCA1
35833_at	0,996	P	1,055	P	1,036	P	AL080184	unknown
39968_at	1,433	P	0,907	P	0,744	P	U50136	enzyme
1116_at	0,748	A	1,314	P	1,018	P	M28170	surface protein, CD019
31535_i_at	1,358	P	1,096	P	0,624	P	W27858	unknown
1939_at	0,706	M	1,091	P	1,277	P	M22898	signalling, p53
31876_r_at	0,739	P	1,124	A	1,208	P	U92014	unknown
1142_at	0,996	P	1,290	P	0,784	A	Z69641	receptor, FGFR2
1790_s_at	0,913	P	1,136	P	1,019	P	L33264	cell cycle, kinase
36403_s_at	0,892	P	0,886	M	1,289	P	AI434146	unknown
40535_i_at	0,935	P	1,042	A	1,086	P	AI254524	unknown
34903_at	1,267	P	1,027	P	0,759	P	AI017382	KIAA1218
38122_at	1,020	P	0,986	A	1,043	P	D87075	unknown
41062_at	0,892	P	0,962	P	1,190	P	AA037278	unknown
473_g_at	0,908	A	0,952	P	1,177	P	U48730	transcription, STAT5B
538_at	0,789	P	0,986	A	1,261	P	S53911	surface protein, CD034
31456_at	0,513	A	1,558	P	0,965	P	AL008627	ribosome
1976_s_at	1,241	P	0,617	A	1,170	P	X06292	kinase, tyrosine kinase, fes
35026_f_at	0,512	A	1,211	P	1,302	P	X78932	transcription, zinc finger, ZNF273
32081_at	0,508	P	1,372	P	1,137	P	AB023166	kinase
34933_at	1,040	P	0,922	P	1,054	M	AJ238381	transcription, PAX9
41695_at	1,471	P	1,204	P	0,339	A	AB007874	transcription, zinc finger, ANF297b
40075_at	0,566	A	1,290	P	1,156	P	M55047	structural protein, synapse
1047_s_at	0,714	A	1,301	P	0,991	P	U37055	receptor, hepatocyte growth factor, HGF-like
35250_at	0,876	M	1,141	P	0,988	P	AL031670	miscellaneous, ring finger, RNF24
41134_at	1,148	P	1,221	P	0,633	A	AB023181	KIAA0964
32181_at	1,141	P	0,863	P	0,995	P	M60922	surface protein
41334_r_at	1,422	P	0,991	A	0,581	P	AA398463	unknown
963_at	1,139	P	1,120	P	0,730	P	X83441	replication, DNA ligase
41500_at	0,801	A	1,118	P	1,069	P	AI761818	unknown
35219_at	0,839	P	1,160	P	0,988	P	AL050202	unknown
1163_at	1,282	P	0,852	P	0,851	P	S67334	kinase, PI-3K, signalling
35305_at	0,842	P	0,986	P	1,150	P	X95762	proteolysis, aminopeptidase-like
518_at	1,042	P	1,257	P	0,679	A	U07132	transcription, nuclear receptor, LXRb, Ner-1

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
32133_at	1,252	P	0,986	P	0,732	M	AB011161	KIAA0589
32434_at	1,126	P	0,855	A	0,988	P	D10522	myristoylated alanine-rich c-kinase substrate
35345_at	0,827	P	1,196	P	0,943	A	X83618	enzyme, HMGCS2
32474_at	1,001	P	1,102	P	0,858	M	X96744	transcription, P AX7
39920_r_at	0,867	P	1,048	P	1,043	P	AF095154	receptor, C1q related factor
37869_at	1,211	P	0,818	P	0,916	P	AB029004	KIAA1081
32599_at	1,219	P	1,059	P	0,664	P	AF013168	structural protein, hamartin
33027_at	0,996	P	1,046	M	0,898	P	W27906	unknown
35768_at	0,861	P	1,184	P	0,893	P	AB014561	cell cycle, RB, RBP95
32045_at	0,437	A	1,180	P	1,321	P	AB002331	transcription, death associated TF, DIO-1
34260_at	1,049	P	0,897	A	0,988	P	AB014583	KIAA0683
1274_s_at	1,106	P	1,034	P	0,787	P	L22005	proteolysis
41137_at	0,717	P	1,218	P	0,988	P	AB007972	KIAA0503
36458_at	0,422	A	1,283	P	1,215	P	AB023235	KIAA1018
214_at	0,674	A	1,092	P	1,149	P	M97676	transcription, homeo box, HOX7
37411_at	0,997	P	0,926	A	0,988	P	D30758	KIAA0050
40050_at	0,852	P	1,059	P	0,998	P	AF069747	transcription, CBF-A2, CBFA2T2
31822_at	0,655	A	1,263	P	0,988	P	L12579	transcription, CDP, CCAAT
1391_s_at	0,873	P	0,986	P	1,042	P	L04751	enzyme, cytochrome
36375_at	0,996	P	1,022	P	0,882	M	X74614	structural protein
40235_at	1,059	P	1,173	P	0,667	P	L13738	cell cycle, CDC42, p21 kinase, ACK
38224_at	0,996	P	0,835	P	1,066	P	U71300	transcription, general, SNAP50
31443_at	1,174	P	0,730	A	0,988	P	S76346	transcription, RUNX1, AML1
36731_g_at	1,175	P	0,875	P	0,840	A	U66684	unknown
41526_at	0,914	P	0,986	P	0,988	P	AF072836	transcription, HMG, HMG20B
39421_at	0,941	P	0,986	P	0,958	P	D43969	transcription, CBF-A2, RUNX
634_at	0,785	A	1,030	P	1,069	P	L41351	proteolysis
39317_at	0,762	P	1,222	P	0,899	P	D86324	enzyme
38945_at	1,080	P	1,003	P	0,785	P	X78710	transcription, MTF-1
38284_at	0,825	A	0,774	P	1,253	P	AJ007041	transcription, homeo box, trithorax homologue
38523_f_at	1,404	P	0,689	A	0,753	P	D49677	splicing
1048_at	1,223	P	0,797	A	0,825	P	U38480	transcription, nuclear receptor, RXRg
1724_at	0,962	P	0,895	M	0,986	P	S75174	transcription, E2F, E2F-4
39831_at	0,837	A	1,018	P	0,988	P	AI972631	unknown
1375_s_at	0,984	P	1,049	P	0,801	M	M32304	proteolysis, metalloproteinase
41843_r_at	1,169	P	0,323	A	1,331	P	W28275	unknown
31861_at	0,973	P	0,860	P	0,988	M	L14754	transcription, SMBP2
40299_at	0,877	M	0,944	P	0,988	P	AF091890	receptor, G protein, GPCR, orphan
40549_at	0,744	P	1,002	P	1,054	P	L04658	kinase
35815_at	0,602	A	1,133	P	1,055	P	AL049470	unknown
33448_at	0,996	P	0,902	A	0,890	P	AB000095	proteolysis
36460_at	0,877	P	0,745	P	1,166	P	AF008442	transcription, general, RNA pol I
38467_at	0,483	A	1,244	P	1,037	P	U96721	structural protein
39306_at	0,824	P	0,817	M	1,113	P	AF052514	proteolysis, protease serine 16 (thymus)
33494_at	0,638	M	1,486	P	0,625	P	S69232	enzyme, oxidoreductase
37280_at	0,637	A	1,290	P	0,809	P	U59912	transcription, SMAD1, TNF
37466_at	1,322	P	0,402	A	1,008	P	D84488	G protein

Id v2	CD36_1 Normalized	Flags	CD36_3 Normalized	Flags	CD36_4 Normalized	Flags	Acc no	Function
33333_at	0,996	P	0,473	A	1,255	P	AB007863	KIAA0403
38575_at	1,317	P	0,462	A	0,938	P	AF070528	unknown
31948_at	0,733	P	1,071	P	0,906	P	X79563	ribosome
38409_at	1,289	P	0,346	A	1,074	P	M61199	surface protein
104_at	0,996	P	0,877	A	0,828	P	Z21966	transcription, homeo box, POU
37556_at	0,928	P	0,753	A	1,011	P	M81637	proteolysis
38358_at	0,952	P	0,827	P	0,908	A	AJ010840	replication, helicase
40005_at	0,601	A	0,830	P	1,249	P	X91992	replication, DNA repair, alkB
41015_at	1,112	P	0,986	P	0,555	A	AB022017	kinase
40020_at	1,000	P	0,986	P	0,663	P	AB011536	miscellaneous, MEGF2
41429_at	0,728	P	1,027	P	0,892	P	M65254	phosphatase
36072_at	1,069	P	0,517	A	1,058	P	AF025770	transcription, zinc finger, ZNF189
35913_at	1,005	P	0,807	M	0,815	P	U88047	transcription, DRIL1
32608_at	0,969	P	0,982	P	0,665	A	AF000560	miscellaneous
34282_at	1,184	P	0,635	A	0,797	P	AB010812	transcription, NF-E2, NRF3
35950_at	0,489	A	1,138	P	0,988	P	U90841	transcription, SSX4
551_at	0,825	P	1,003	P	0,779	A	U01877	transcription, CBP/p300
40817_at	1,101	P	0,986	P	0,488	M	M96824	miscellaneous, nucleobindin
1686_g_at	0,551	A	0,986	P	1,028	P	X82554	cell cycle, cyclin related
41406_at	0,609	A	0,962	P	0,988	P	AL080172	unknown
906_at	0,700	P	0,997	P	0,858	A	L78440	transcription, STAT4
366_s_at	1,103	P	0,986	P	0,459	P	Z29066	kinase, nek2
38109_at	0,569	A	1,032	P	0,929	P	AF020544	enzyme
34876_at	0,900	P	0,836	P	0,790	P	U65090	enzyme
1582_at	0,849	P	1,118	P	0,555	A	M29540	surface protein, CD066e
34827_at	0,693	A	0,773	P	1,051	P	AF045458	kinase
35212_at	0,959	P	0,864	P	0,677	A	AF064801	receptor, TRC8
33864_at	1,082	P	0,352	A	1,053	P	X86098	transcription, zinc finger
40556_at	0,425	A	1,005	P	1,055	P	D42073	enzyme
36551_at	0,553	A	0,701	P	1,205	P	AL049382	unknown
35999_r_at	0,536	A	0,977	P	0,934	P	AB018324	KIAA0781
32955_at	0,810	P	0,645	M	0,988	P	AL021546	enzyme, cytochrome
34780_at	0,840	P	0,773	P	0,807	P	AB002313	KIAA0315
34779_at	0,481	A	1,010	P	0,924	P	R90942	enzyme, transferase
37470_at	0,940	P	0,775	A	0,687	P	AF013249	surface protein, LAIR1, monocytes
35430_at	0,649	A	0,986	P	0,766	P	U78082	transcription, general, RNA pol II, MED6
32140_at	0,423	A	1,011	P	0,964	P	Y08110	surface protein
33281_at	0,840	P	0,869	P	0,681	A	D63485	transcription, NF-kB, IKKi
38964_r_at	0,978	P	0,508	M	0,901	P	U12707	signalling,WASP, GTPase
32321_at	0,743	P	0,864	P	0,779	P	X56841	surface protein, MHC I, HLA-E
40672_at	0,563	A	0,708	P	1,068	P	U57721	enzyme, hydrolase
39251_at	0,272	A	1,033	P	0,981	P	M74091	cell cycle, cyclin C
36672_at	0,493	A	0,772	P	0,988	P	L13977	enzyme, proteolysis
36028_at	1,066	P	0,667	P	0,507	P	U45285	enzyme, transporter, ATPase
34777_at	0,996	P	0,303	A	0,678	P	D14874	receptor, adrenomedullin
33767_at	0,231	A	0,673	P	0,796	P	X15306	structural protein, NF-H

B: Signature genes of E-cad+ progenitors

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
1017_at	1,000	P	1,247	P	U73737	miscellaneous
1042_at	0,889	P	1,062	P	U27185	transcription, nuclear receptor, RAR, responsive
1126_s_at	2,542	P	4,437	P	L05424	surface protein, CD044
1140_at	2,580	P	2,134	P	L25851	surface protein, CD103, integrin, alphaE
1195_s_at	1,115	P	1,203	P	AF012024	surface protein, integrin, associated protein
1201_at	1,046	P	1,518	P	D14889	GTPase, RAB33a
1207_at	1,076	P	0,796	P	X66365	cell cycle, CDK06
1212_at	0,691	P	1,065	P	U86529	enzyme, GSTZ1
122_at	0,852	P	0,988	P	X74331	replication
1223_at	0,917	P	0,939	P	X66362	kinase, PCTAIRE3
1231_at	0,822	P	1,023	P	M74525	G protein
1239_s_at	1,171	P	1,158	P	U13021	apoptosis, ICH-1
1257_s_at	0,617	P	0,811	P	L42379	receptor, BPGF-1
1264_at	0,897	P	1,018	P	M25393	phosphatase
1273_r_at	1,000	P	0,718	P	L22005	proteolysis
1307_at	1,254	P	1,063	P	D14533	replication, DNA repair
1432_s_at	1,281	P	0,839	P	D16105	kinase, tyrosine kinase, tyk1
1458_at	1,256	P	1,327	P	M64572	phosphatase
1462_s_at	0,914	P	0,851	P	M80397	replication
1469_at	1,406	P	1,359	P	U12779	kinase, MAPKAPK2
1519_at	1,181	P	1,729	P	J04102	transcription, ets2
1523_g_at	1,343	P	1,404	P	U43408	kinase, tyrosine kinase, tnk1
1528_at	0,933	P	1,586	P	U50531	miscellaneous
1529_at	2,351	P	2,087	P	U50534	miscellaneous
1559_at	0,610	P	0,688	P	U24153	kinase, signalling, p21
1567_at	0,912	P	1,214	P	S77812	receptor, VEGF, kinase, tyrosine kinase
1573_at	0,748	P	1,368	P	M12783	receptor, PDGF2
1598_g_at	1,123	P	0,747	P	L13720	cell cycle, gas, growth arrest
160025_at	0,897	P	0,770	P	X70340	receptor, TGF alpha
160042_s_at	1,329	P	1,159	P	X58431	transcription, homeobox
1623_s_at	1,633	P	2,410	P	J03358	kinase, tyrosine kinase, Fer
1647_at	1,674	P	1,925	P	U51903	G protein
168_at	0,871	P	1,641	P	U50196	kinase
177_at	1,547	P	1,514	P	U38545	enzyme, lipase
180_at	1,649	P	1,918	P	S82470	LENG4/BB1
1842_at	1,954	P	1,467	P	S75762	miscellaneous
1895_at	1,489	P	1,547	P	J04111	transcription, AP-1, c-jun
1901_s_at	1,360	P	1,796	P	M12036	receptor, EGF R, erbB2, HER2
1909_at	1,004	P	2,140	P	M14745	apoptosis, bcl2
2061_at	1,346	P	1,619	P	L12002	surface protein, CD049d, integrin, alpha 4
2062_at	1,078	P	1,000	P	L19182	receptor, IGF-1, IGFBP7
2086_s_at	0,998	P	1,136	P	D17517	receptor, axl family, tyro3
228_at	2,930	P	1,974	P	M35416	G protein
267_at	1,084	P	1,032	P	L34075	signalling, FKBP, cyclophilin
31491_s_at	1,156	P	2,192	P	X98175	proteolysis

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
31521_f_at	1,437	P	1,437	P	X60484	replication, transcription, histone, H4
31522_f_at	1,683	P	1,670	P	Z80779	replication, transcription, histone, H2B
31528_f_at	1,978	P	0,978	P	Z83738	replication, transcription, histone, H2B
31631_f_at	0,762	P	1,852	P	AF095288	miscellaneous
31768_at	1,815	P	1,577	P	AL009179	replication, transcription, histone, H2B
31773_at	1,299	P	2,312	P	U06715	enzyme, cytochrome
31783_at	0,849	P	1,109	P	U52112	enzyme, renin binding protein
31840_at	0,891	P	1,233	P	M21188	proteolysis
31843_at	5,393	P	5,864	P	AB020639	KIAA0832
31854_at	1,510	P	3,383	P	AF035582	kinase
31878_at	1,030	P	1,000	P	AJ005016	enzyme, transporter
31917_at	1,046	P	1,114	P	AC005609	unknown
31923_f_at	0,712	P	1,411	P	U60269	miscellaneous
32041_r_at	1,314	P	1,933	P	AB007892	KIAA0432
32066_g_at	0,996	P	1,549	P	S68134	transcription, CREM
32083_at	1,703	P	1,866	P	AF027826	receptor, 7TM
32104_i_at	1,000	P	0,843	P	U66063	kinase, CaM kinase II
32122_at	0,871	P	0,969	P	L31573	enzyme, sulfite oxidase
32137_at	1,312	P	1,190	P	AF029778	receptor, jagged2, JAG2, notch
32146_s_at	1,072	P	0,869	P	L07261	structural protein, adducin
32163_f_at	3,617	P	2,606	P	AA216639	unknown
32164_at	2,213	P	1,733	P	S79639	enzyme, structural protein
32253_at	2,248	P	2,185	P	AB007927	KIAA0458
32271_at	1,000	P	1,165	P	X16707	transcription, AP-1, Fra-1
32329_at	1,059	P	1,249	P	X99142	structural protein, keratin
32350_at	2,409	P	2,207	P	AB026118	MALT1
32535_at	2,123	P	1,766	P	X63556	fibrillin
32536_at	2,120	P	1,322	P	Z37986	miscellaneous
32603_at	0,687	P	0,724	P	W27118	replication
32624_at	1,051	P	0,713	P	AL050050	unknown
32680_at	1,586	P	1,829	P	AB011123	kinase
32717_at	0,782	P	1,256	P	AF029729	miscellaneous
32742_s_at	1,282	P	1,000	P	AF098638	signalling
32776_at	3,471	P	1,978	P	M35416	G protein, RALB
32791_at	2,279	P	1,616	P	L19183	miscellaneous
32819_at	4,336	P	3,687	P	AJ223352	replication, transcription, histone, H2B
32888_at	0,737	P	0,953	P	X52213	kinase, TYK1, ltk
32959_at	0,690	P	0,845	P	M25809	ATPase
33023_at	1,762	P	1,654	P	AI095013	replication, transcription, histone, H2A
33035_at	1,717	P	1,748	P	AL021397	enzyme, oxidoreductase
33080_s_at	0,955	P	1,438	P	AB007943	KIAA0474
33148_at	1,384	P	1,041	P	AI459274	unknown
33150_at	2,643	P	2,628	P	AI126004	unknown
33164_at	2,260	P	2,925	P	AJ132545	kinase
33167_r_at	0,509	P	1,216	P	X66503	enzyme, adenylosuccinate synthetase
33193_at	1,330	P	1,288	P	AW052084	unknown
33212_at	1,858	P	1,779	P	AF006751	ribosome
33254_at	0,837	P	1,000	P	AF008915	miscellaneous

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
33321_r_at	3,757	P	2,452	P	M33494	proteolysis
33323_r_at	0,791	P	0,911	P	X57348	signalling, 14.3.3
33328_at	1,821	P	1,123	P	W28612	unknown
33345_at	1,222	P	2,000	P	AF035621	structural protein
33349_at	1,295	P	0,971	P	AL049378	unknown
33358_at	3,142	P	3,507	P	W29087	unknown
33369_at	1,481	P	2,357	P	AI535653	enzyme, oxidase
33370_r_at	1,000	P	2,464	P	U60205	enzyme, oxidase
33385_g_at	2,000	P	1,729	P	U31346	enzyme, calpastatin
33389_at	3,038	P	4,108	P	U23942	enzyme, cytochrome
33413_at	1,725	P	1,343	P	AF051160	phosphatase
33421_s_at	2,100	P	2,401	P	AB016247	enzyme, sterol-C5-desaturase
33435_r_at	1,129	P	1,011	P	AI525962	unknown
33736_at	1,020	P	0,991	P	Y16522	structural protein, stomatin
33737_f_at	1,162	P	1,158	P	AI871359	unknown
33794_g_at	1,429	P	1,323	P	U19345	transcription, TCF20
33809_at	1,851	P	4,064	P	AL049933	G protein
33823_at	1,219	P	1,148	P	D12676	surface protein, CD036 like2
33895_at	0,983	P	1,370	P	AL050373	unknown
33898_at	1,087	P	0,788	P	AF015308	structural protein
339_at	2,116	P	1,948	P	AF035752	structural protein, caveolin 2
33921_at	1,021	P	0,741	P	Y15409	enzyme, translocase
33935_at	0,801	P	0,837	P	AL035305	enzyme
33985_s_at	1,721	P	1,028	P	W28616	heat shock, HSP090
34027_f_at	1,200	P	1,538	P	AA010078	replication, transcription, histone, H4D
34063_at	0,781	P	0,611	P	AB006533	transcription, chromatin
34091_s_at	1,769	P	2,275	P	Z19554	structural protein, vimentin
34107_at	1,287	P	1,209	P	AJ005577	enzyme
34145_at	0,755	P	0,777	P	AI184710	unknown
34157_f_at	0,927	P	0,678	P	AI200373	replication, transcription, histone, H2A
34210_at	4,992	P	4,645	P	N90866	surface protein, CD052, CDw52
34220_at	0,851	P	1,416	P	AL080140	unknown
34230_r_at	2,012	P	1,266	P	D84454	enzyme, UDP-galactose translocator
34255_at	1,088	P	0,848	P	AF059202	enzyme, transferase
34258_at	1,336	P	1,022	P	W28205	signalling
34259_at	0,974	P	0,912	P	AB014564	translation
34344_at	3,559	P	3,683	P	AF044195	transcription, NF-kB, IKAP
34395_at	0,885	P	1,039	P	AB002352	KIAA0354
34403_at	4,298	P	3,504	P	U58516	miscellaneous, BA46
34446_at	2,785	P	1,950	P	AL049701	unknown
34484_at	1,220	P	1,458	P	AI961669	G protein, GTPase
34577_at	1,267	P	1,303	P	U10694	miscellaneous, MAGE9
34594_at	1,445	P	1,125	P	D13644	KIAA0019
34628_at	1,572	P	1,823	P	Y09321	unknown
34634_s_at	1,102	P	1,364	P	U68487	receptor, serotonin R
34672_at	2,076	P	1,577	P	AB011175	KIAA0603
34676_at	1,167	P	2,307	P	AB029022	KIAA1099
34687_at	0,769	P	1,509	P	AF052167	unknown

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
34708_at	0,879	P	0,612	P	D88587	surface protein, Hakata antigen
34767_at	1,670	P	2,328	P	AI670788	unknown
34790_at	2,115	P	1,490	P	S70154	enzyme, thiolase
34848_at	2,652	P	2,119	P	X69141	enzyme, cholesterol
34930_at	1,332	P	2,082	P	U95218	T cell-death associated protein
34952_at	0,414	P	0,742	P	X95826	enzyme, transferase
34966_at	1,110	P	1,179	P	AJ001699	transcription, brachyury
35079_at	1,152	P	1,006	P	AB003592	adhesion
35121_at	1,212	P	1,318	P	Z18956	enzyme, transporter
35124_at	1,305	P	1,178	P	M62982	enzyme, 12-LOG
35133_at	2,605	P	3,112	P	J03358	kinase, tyrosine kinase, Fer
35176_at	0,951	P	0,916	P	Z82022	enzyme, GlcNac-1-P transferase
35181_at	1,131	P	1,463	P	AI539361	unknown
35182_f_at	1,016	P	1,055	P	W25874	unknown
35198_at	1,004	P	1,355	P	AF070596	unknown
352_at	1,356	P	1,063	P	D30036	signalling
35238_at	1,875	P	2,278	P	AB000509	receptor, TNF, TRAF5
35269_at	0,714	P	0,885	P	AF093420	heat shock, HSP070
35355_at	1,087	P	1,029	P	AB020697	KIAA0890
35361_at	0,933	P	0,751	P	W28299	unknown
35367_at	3,812	P	2,588	P	AB006780	surface protein, lectin, galectin 3
35403_at	1,045	P	0,940	P	AB029017	KIAA1094
35419_g_at	1,032	P	2,018	P	J04178	enzyme, hexosaminidase
35453_at	0,957	P	1,000	P	U59111	surface protein, proteoglycan
35492_at	0,972	P	1,188	P	AC004523	enzyme, cytochrome
355_s_at	0,819	P	1,393	P	D38037	signalling, FKBP, cyclophilin
35502_at	2,884	P	3,055	P	U29700	receptor, anti-mullerian hormone type II
35503_at	0,784	P	1,088	P	M81590	receptor, serotonin R
35545_at	1,030	P	0,831	P	AB018282	enzyme, transporter
35621_at	0,966	P	0,933	P	L77213	kinase, enzyme, cholesterol
35657_at	0,874	P	1,035	P	U08998	transcription, TRBP2
35687_at	0,912	P	1,452	P	Z24459	miscellaneous, MTCP1
35697_at	1,200	P	0,924	P	L76259	enzyme
35715_at	1,003	P	1,011	P	AL080071	unknown
35789_at	1,430	P	1,457	P	AB028965	KIAA1042
35820_at	2,745	P	2,783	P	X62078	miscellaneous
35831_at	1,626	P	3,132	P	AB014511	KIAA0611
35844_at	1,097	P	0,817	P	D79206	structural protein
35960_at	1,207	P	0,998	P	AF031416	transcription, NF-kB, IKB beta
35975_at	1,676	P	2,327	P	L13744	unknown
35984_at	0,966	P	1,181	P	AF041381	transcription, E2F, E2F-6
35985_at	5,476	P	5,260	P	AB023137	kinase
35992_at	1,000	P	0,489	P	AF087036	transcription, HLH, musculin, ABF-1
35996_at	0,989	P	1,083	P	X98261	cell cycle, MPP05
36011_at	1,281	P	1,203	P	AF035531	structural protein
36030_at	1,101	P	0,873	P	AL080214	unknown
36066_at	1,142	P	1,091	P	AB020635	KIAA0828
36071_at	1,646	P	1,388	P	AF070633	unknown

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
36084_at	0,987	P	1,378	P	D38548	KIAA0076
36113_s_at	1,313	P	1,608	P	AJ011712	structural protein
36119_at	2,294	P	2,991	P	AF070648	unknown
36177_at	1,212	P	0,779	P	X78627	replication
36196_at	0,863	P	1,000	P	U24183	enzyme, phosphofructokinase
36202_at	0,740	P	1,078	P	S76965	kinase, PKI
36221_at	1,833	P	1,191	P	X84195	phosphatase
36237_at	1,293	P	0,952	P	AB009698	enzyme, transporter
36262_at	3,015	P	2,422	P	Z12173	enzyme, sulphatase
36263_g_at	3,213	P	2,559	P	Z12173	enzyme, sulphatase
36294_at	0,851	P	0,704	P	U60207	kinase
36298_at	0,942	P	0,934	P	L14565	structural protein
36330_at	1,000	P	1,205	P	Y17448	enzyme
36360_at	0,715	P	0,977	P	AB007976	KIAA0507
36490_s_at	1,290	P	0,859	P	X15331	enzyme
36530_g_at	1,149	P	0,889	P	AI885381	unknown
36594_s_at	1,233	P	0,610	P	U72263	structural protein
36644_at	2,067	P	1,196	P	D29963	surface protein, CD151, 4TM
36651_at	1,297	P	1,028	P	X15525	enzyme, phosphatase
36664_at	1,581	P	1,338	P	M60091	enzyme, GALT
36703_at	1,085	P	1,503	P	U86358	receptor, chemokine, CCL25
36720_at	0,996	P	1,060	P	AA873266	kinase, enzyme
36804_at	1,312	P	0,989	P	M34455	receptor, IFN, IFN gamma inducible
36818_at	1,232	P	1,355	P	AF052100	unknown
36830_at	2,676	P	1,645	P	U80034	proteolysis
36835_at	0,632	P	1,132	P	U33052	kinase
36870_at	0,837	P	1,118	P	AB018347	KIAA0804
36920_at	2,555	P	3,180	P	U46024	myotubularin
36925_at	1,558	P	1,882	P	L26336	heat shock
36948_at	1,758	P	2,610	P	AL109701	unknown
36956_at	0,959	P	1,132	P	L20852	receptor, GLVR1
37024_at	9,831	P	4,337	P	AF010312	transcription, TNF, LPS induced
37026_at	2,945	P	2,891	P	AF001461	transcription, zinc finger, KLF6
37027_at	1,972	P	2,798	P	M80899	signalling
37049_g_at	1,165	P	1,291	P	U58970	structural protein, translocation
37050_r_at	1,801	P	3,178	P	AI130910	structural protein, translocation
37157_at	6,875	P	6,500	P	X56667	miscellaneous, Ca binding
37259_at	1,428	P	1,706	P	Z81326	proteolysis, inhibitor
37270_at	0,897	P	1,052	P	AF007876	ATPase
37276_at	1,456	P	1,943	P	U51903	G protein
37323_r_at	4,288	P	2,977	P	X82460	enzyme, prostaglandin dehydrogenase
37374_at	3,364	P	4,515	P	M82809	structural protein, annexin
37380_at	0,771	P	1,118	P	X59268	transcription, general, TFIIB
37409_at	1,869	P	2,290	P	U88666	kinase
37424_at	0,684	P	0,827	P	AB029343	miscellaneous
37434_at	1,506	P	0,758	P	W28907	unknown
37459_at	0,837	P	1,000	P	X57527	alpha 1(VIII) collagen
375_at	1,031	P	1,347	P	Z84718	enzyme

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
37502_at	0,707	P	1,000	P	AF092563	structural protein
37571_at	1,772	P	2,017	P	AB028981	KIAA1058
37628_at	1,915	P	1,674	P	M69177	enzyme, monoamine oxidase
37631_at	0,860	P	1,654	P	U14391	structural protein, myosin
37654_at	1,180	P	1,125	P	D31764	KIAA0064
37655_at	1,126	P	1,383	P	X75304	structural protein
37676_at	0,936	P	0,820	P	AF056490	enzyme, phosphodiesterase
37681_i_at	0,701	P	1,125	P	AB018266	KIAA0723
37684_at	6,488	P	4,095	P	AB020687	KIAA0880
37692_at	3,598	P	3,809	P	AI557240	unknown
37728_r_at	0,858	P	1,196	P	X78669	structural protein
37753_at	0,807	P	0,916	P	U66617	transcription, chromatin, SWI/SNF, BAF60a
37768_at	1,199	P	0,886	P	M74905	enzyme, glycosylase
37855_at	1,044	P	1,428	P	M95767	enzyme
37888_at	1,384	P	1,482	P	D87449	KIAA0260
37902_at	2,169	P	3,205	P	L13278	enzyme, reductase
37914_at	0,861	P	1,757	P	AB002303	structural protein, endofin
37952_at	1,820	P	1,837	P	M35999	surface protein, CD061, GPIIIa, platelets
37961_at	1,862	P	1,492	P	U90907	miscellaneous
38000_at	0,969	P	1,436	P	S72370	enzyme, pyruvate carboxylase
38007_at	1,678	P	1,331	P	L11353	structural protein
38027_at	1,327	P	1,309	P	X53742	miscellaneous, ECM
38042_at	2,346	P	2,962	P	X03674	enzyme, glucose-6-phosphate dehydrogenase
38062_at	0,996	P	1,602	P	D87467	G protein, GTPase
38066_at	1,161	P	1,000	P	M81600	enzyme, oxireductase
38098_at	3,064	P	1,978	P	D80010	KIAA0188
38099_r_at	1,767	P	3,839	P	AF030555	enzyme, acyl-CoA synthetase
38126_at	1,232	P	1,226	P	J04599	surface protein, proteoglycan
38151_at	3,776	P	3,935	P	AF002672	miscellaneous, BCSC-1
38152_at	2,836	P	4,302	P	AI632589	unknown
38170_at	1,055	P	1,705	P	AF070595	unknown
38218_at	2,990	P	2,312	P	M97347	enzyme, acetylglucosaminyltransferase
38227_at	1,245	P	1,860	P	AB006909	transcription, MITF
38233_at	3,163	P	1,848	P	AF093265	signalling
38264_at	1,050	P	1,076	P	U74324	G protein, GTPase
38278_at	1,407	P	0,911	P	M62324	transcription, MRF1
38311_at	1,258	P	0,867	P	AF055012	unknown
38332_at	1,354	P	1,635	P	U83993	receptor, purinoceptor, P2X4
38381_at	3,705	P	3,862	P	U32315	structural protein, syntaxin
38391_at	3,969	P	2,824	P	M94345	structural protein, myeloid
38403_at	3,570	P	3,199	P	X77196	surface protein, CD107b
38425_at	0,767	P	0,886	P	U49719	enzyme, lyase
38440_s_at	1,820	P	2,003	P	AA015605	unknown
38495_s_at	1,034	P	1,331	P	U27328	enzyme, fucosyltransferase
38513_at	0,817	P	1,153	P	D31765	KIAA0061
38528_at	1,108	P	1,028	P	U19822	enzyme, carboxylase
38552_f_at	1,000	P	1,055	P	AI984786	surface protein, Ig, BAP29
38553_r_at	1,102	P	1,336	P	AI984786	surface protein, Ig, BAP29

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
38555_at	1,708	P	3,436	P	AB026436	phosphatase
38563_at	0,861	P	1,292	P	AF039652	enzyme, RNAse H
38621_at	1,410	P	1,444	P	AJ012008	miscellaneous
38622_at	1,049	P	1,085	P	W28953	unknown
38624_at	1,199	P	1,115	P	AF054506	enzyme, transporter
38759_at	1,386	P	2,284	P	U97502	butyrophilin
38816_at	1,680	P	1,618	P	AF095791	miscellaneous
38871_at	1,000	P	1,128	P	AJ006288	apoptosis, bcl10
38931_at	1,406	P	1,353	P	X59739	transcription, zinc finger, ZFX
38941_s_at	1,016	P	1,028	P	W28610	unknown
38948_at	0,738	P	1,176	P	Y18206	phosphatase
38973_at	0,807	P	0,891	P	AB028943	KIAA1020
38979_at	1,178	P	1,656	P	AI302176	enzyme
38989_at	1,671	P	1,498	P	AF035296	unknown
38997_at	1,524	P	1,342	P	X96924	structural protein
39013_at	0,843	P	1,145	P	Y11588	apoptosis
39019_at	2,604	P	2,500	P	D14696	KIAA0108
39059_at	2,753	P	2,026	P	AF034544	enzyme, sterol reductase
39105_at	1,435	P	1,468	P	Z46389	structural protein, motility
39140_at	0,651	P	0,810	P	AL079292	replication, helicase
39244_at	0,938	P	1,040	P	M28211	G protein, GTPase
39252_at	1,139	P	1,413	P	AF058954	enzyme, succinyl-CoA synthetase
39262_at	0,810	P	0,808	P	U79266	miscellaneous
39292_r_at	2,247	P	3,153	P	AF009353	transcription, nuclear receptor, coactivator, TIF1
39345_at	1,909	P	1,975	P	AI525834	unknown
39351_at	2,235	P	2,054	P	M84349	surface protein, CD059
39420_at	2,323	P	1,191	P	S62138	miscellaneous
39464_at	1,844	P	1,038	P	W28493	unknown
39500_s_at	0,774	P	0,994	P	AL049299	unknown
39520_at	1,168	P	1,060	P	AI924382	unknown
39541_at	1,065	P	1,008	P	W52003	unknown
39547_at	1,383	P	1,143	P	AB008515	G protein, GTPase, cell cycle, RAN BP 9
39552_at	0,929	P	1,208	P	U92436	phosphatase, PTEN
39555_at	1,418	P	1,270	P	AB012853	transcription, chromatin binding
39570_at	1,011	P	1,238	P	W22289	unknown
39620_at	1,152	P	1,590	P	Z21966	transcription, POU
39621_at	0,908	P	1,367	P	AB007928	KIAA0459
39650_s_at	0,896	P	0,982	P	AB007895	KIAA0435
39706_at	2,731	P	3,246	P	AB014536	structural protein, copine III
39743_at	1,190	P	1,250	P	AI862521	unknown
39752_at	1,876	P	1,297	P	AF040704	cell cycle
39766_r_at	0,825	P	1,117	P	AI744294	transcription, general, RNA pol II, RPB10A
39772_at	0,888	P	1,045	P	AF007157	unknown
39773_at	1,586	P	1,400	P	W28235	unknown
39835_at	0,977	P	0,702	P	U93181	phosphatase
39882_at	0,805	P	0,998	P	U66035	structural protein
39910_at	1,000	P	0,889	P	AA663800	unknown
39942_at	3,090	P	2,366	P	AF016898	transcription, ATF, B-ATF

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
39965_at	1,055	P	0,873	P	AI570572	G protein, GTPase
39975_at	1,515	P	1,314	P	AC002400	unknown
39986_at	0,802	P	1,180	P	AL050100	unknown
40025_at	0,717	P	1,029	P	AF052186	unknown
40040_at	1,355	P	1,915	P	AC002542	unknown
40131_at	1,282	P	0,766	P	D89937	receptor, TGF, BMP, follistatin related
40147_at	3,036	P	2,983	P	U18009	miscellaneous
40166_at	2,440	P	2,523	P	AA923149	unknown
40182_s_at	0,651	P	0,756	P	AF055027	unknown
40196_at	1,651	P	1,626	P	D88153	miscellaneous, HYA22
40208_at	2,623	P	2,711	P	R37702	unknown
40234_at	1,100	P	0,899	P	X96484	miscellaneous
40266_at	0,958	P	1,602	P	AB028959	KIAA1036
40268_at	1,469	P	1,184	P	X16706	transcription, AP-1, Fra-1, fos jun, LZ
40322_at	2,964	P	2,638	P	D12763	receptor, IL-01R like, IL1RL1
40335_at	0,777	P	1,006	P	AF052117	unknown
40337_at	1,035	P	1,103	P	M35531	enzyme
40343_at	1,000	P	2,240	P	AJ005814	transcription, homeo box, HOX1
40345_at	0,928	P	0,656	P	U20180	enzyme, IRP2
40396_at	6,923	P	3,942	P	U49395	receptor, purinoceptor, P2X5a
40432_at	3,784	P	3,006	P	AA522891	unknown
40433_at	3,907	P	2,866	P	W25921	proteolysis, lysosomal, GL6S, n-acetylglucosamine-6-sulfatase precursor
40461_at	1,989	P	2,508	P	AB007855	KIAA0395
40462_at	1,240	P	1,338	P	AF055022	unknown
40474_r_at	1,162	P	1,311	P	S75295	structural protein
40477_r_at	1,395	P	1,731	P	U58198	transcription, ILF-3
40510_at	2,208	P	3,057	P	AB007917	enzyme
40513_at	0,938	P	1,517	P	M30773	phosphatase
40567_at	9,191	P	5,270	P	X01703	structural protein, tubulin
40588_r_at	1,343	P	2,150	P	AF054186	translation
40592_at	1,437	P	1,109	P	L13329	enzyme, sulfatase
406_at	2,219	P	1,218	P	X53587	surface protein, CD104, integrin, beta 4
40607_at	2,478	P	2,719	P	U97105	enzyme
40608_at	1,227	P	1,136	P	AA013087	ribosome, ribosomal protein S13
40633_at	1,000	P	1,733	P	U79246	miscellaneous
40635_at	1,000	P	0,983	P	AF089750	structural protein
40640_at	1,091	P	1,062	P	U62317	miscellaneous
40652_at	0,767	P	1,075	P	D50925	kinase, pim-1 related
40720_at	1,977	P	2,839	P	AL022398	transcription, IRF-6
40722_at	1,000	P	1,212	P	AA431822	unknown
40740_at	1,221	P	0,997	P	M93650	transcription, PAX6
40792_s_at	1,233	P	1,335	P	AF091395	kinase
40798_s_at	1,611	P	1,354	P	Z48579	proteolysis, ADAM10
40807_at	0,913	P	0,638	P	X86018	miscellaneous
40813_at	2,031	P	2,170	P	AI768188	unknown
40815_g_at	1,196	P	1,167	P	L40586	enzyme
40833_r_at	1,665	P	1,244	P	AL050126	unknown
40843_at	1,316	P	1,409	P	AF012023	surface protein, integrin, associated protein

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
40852_at	0,975	P	0,949	P	AB025254	miscellaneous
40866_at	2,927	P	2,324	P	AJ001258	miscellaneous
40880_r_at	1,094	P	1,504	P	X78520	structural protein
40913_at	1,000	P	1,010	P	W28589	unknown
40953_at	2,995	P	3,431	P	S80562	structural protein, actin binding
40966_at	0,734	P	1,614	P	AF099989	kinase
40991_at	1,129	P	1,834	P	AW044649	unknown
40994_at	0,511	P	1,167	P	L15388	G protein, GTPase, kinase
41018_at	0,743	P	0,858	P	AL050015	unknown
41034_s_at	0,991	P	0,831	P	U92315	enzyme, transferase
41059_at	4,039	P	3,103	P	AF020314	surface protein
41083_at	1,126	P	0,923	P	AC006276	unknown
411_i_at	1,906	P	2,349	P	X57351	receptor, IFN, IFN inducible
41139_at	1,471	P	2,013	P	W26633	unknown
41142_at	1,850	P	1,978	P	U62961	enzyme, CoA transferase
41149_at	1,607	P	0,875	P	AC004381	unknown
41154_r_at	1,127	P	2,282	P	AF102803	adhesion, catenin A1
41195_at	1,152	P	1,174	P	U49957	structural protein, translocation
41228_r_at	0,704	P	1,429	P	X60221	ATPase
41257_at	2,027	P	2,963	P	D16217	proteolysis, calpastatin
41306_at	1,000	P	1,013	P	AA004795	unknown
41310_f_at	1,025	P	1,000	P	X12794	transcription, nuclear receptor, ear2, erbA, NR2F6
41362_at	1,451	P	2,119	P	X91249	enzyme, transporter, ABC
41410_at	0,712	P	0,745	P	W29042	unknown
41431_at	1,491	P	1,417	P	AB023153	kinase
41437_at	1,762	P	2,532	P	AL080118	unknown
41472_at	1,112	P	0,983	P	AL078641	unknown
41478_at	2,822	P	2,728	P	AL033538	unknown
41484_r_at	1,968	P	1,199	P	X56681	transcription, AP-1, junD
41493_at	1,366	P	1,824	P	AI094610	ATPase
41528_at	1,593	P	0,891	P	W72239	unknown
41533_at	2,347	P	1,972	P	U79298	miscellaneous
41590_at	0,962	P	1,047	P	AI652660	unknown
41596_s_at	0,943	P	1,011	P	U43572	enzyme
41648_at	0,949	P	0,890	P	X78706	enzyme, acetyltransferase
41656_at	3,752	P	2,414	P	AF043325	enzyme
41755_at	2,620	P	3,871	P	AB023194	KIAA0977
41771_g_at	2,128	P	2,008	P	AA420624	enzyme, EC 1.4.3.4
41772_at	1,850	P	1,858	P	M68840	enzyme, monoamine oxidase
41781_at	1,242	P	1,072	P	U22815	phosphatase
41790_at	2,706	P	4,276	P	AL031230	enzyme
41793_at	1,282	P	1,112	P	AI288757	unknown
41799_at	1,734	P	1,088	P	W28595	unknown
41831_at	1,449	P	1,432	P	AF077820	receptor, LDLR
422_s_at	1,339	P	1,317	P	X66867	transcription, myc, Max
438_at	1,208	P	1,056	P	X07767	kinase
449_at	1,073	P	0,946	P	U66469	apoptosis, p53, p53-induced
487_g_at	1,465	P	1,000	P	U60521	proteolysis

Id v2	E-cad_4 Normalized	Flags	E-cad_5 Normalized	Flags	Acc no	Function
496_s_at	1,063	P	1,102	P	U32324	receptor, IL-11R
520_at	1,000	P	1,566	P	U07358	kinase
537_f_at	1,706	P	1,439	P	U07000	kinase, tyrosine kinase, abl
539_at	3,954	P	2,518	P	S59184	kinase, tyrosine kinase, ryk
556_s_at	1,048	P	1,063	P	M96233	enzyme, glutathione transferase
575_s_at	2,595	P	4,160	P	M93036	carcinoma-associated antigen
582_g_at	1,000	P	1,419	P	M29960	transcription, nuclear receptor, TR2
605_at	2,296	P	2,001	P	L78833	miscellaneous
622_at	0,812	P	1,165	P	M28212	G protein, GTPase
709_at	1,572	P	1,930	P	J00314	structural protein, tubulin
721_g_at	1,072	P	1,262	P	D87673	transcription, heat shock
789_at	1,407	P	2,021	P	X52541	transcription, EGR1, KROX-24
844_at	1,489	P	1,258	P	U48707	phosphatase, inhibitor
866_at	0,881	P	1,452	P	U12471	receptor, thrombospondin 1
938_at	0,763	P	1,064	P	S71404	receptor, IL-09R
940_g_at	0,969	P	1,000	P	D12625	structural protein

C: Signature genes of SCF/Epo progenitors

	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2			
id v2	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Acc no	Function
1000_at	0,626	P	0,511	A	1,482	P	0,998	P	0,945	P	1,000	P	X60188	kinase, ERK1
1034_at	2,567	P	4,342	P	1,691	P	1,587	P	1,757	P	1,515	P	U14394	proteolysis, metalloproteinase, TIMP-3
106_at	2,520	P	1,146	A	2,797	P	1,543	P	1,887	P	1,401	P	Z35278	transcription, CBF-A3, runt domain
1116_at	1,576	P	1,163	P	0,722	P	1,080	P	0,407	P	0,869	P	M28170	surface protein, CD019
112_g_at	0,436	A	2,202	P	1,439	P	1,645	P	1,308	P	0,774	P	X07024	enzyme, red cell
116_at	1,085	P	1,425	P	0,731	P	1,185	P	0,823	P	0,551	A	X14968	kinase, PRKAR2
1163_at	0,809	P	0,713	P	1,084	P	0,929	P	1,139	P	1,181	P	S67334	kinase, PI-3K, signalling
1165_at	1,591	P	1,053	P	2,117	P	1,261	P	0,956	P	0,754	A	D49950	receptor, IL-18
1170_at	1,161	P	1,348	P	1,271	P	1,000	P	0,836	P	1,181	P	X05825	receptor, M-CSF, CSF-1
1356_at	2,010	P	1,896	P	0,663	P	0,631	P	1,000	P	1,054	P	U18321	miscellaneous
1583_at	2,527	P	0,753	P	0,965	P	0,956	P	0,943	P	0,903	P	M32315	receptor, TNFRb, CD120b
160029_at	1,154	P	1,262	P	0,916	P	1,000	P	2,904	P	2,417	P	X07109	kinase, PKCb
1615_at	1,617	P	1,347	P	1,367	P	2,184	P	1,580	P	0,862	A	Z23115	apoptosis, bcl-xL
1642_at	1,814	P	1,177	P	0,871	P	0,903	P	1,010	P	0,648	A	U35113	miscellaneous
1649_at	2,417	P	1,392	P	2,053	P	1,918	P	0,896	P	1,062	P	U61836	cell cycle, cyclin G1 interacting
1650_g_at	3,908	P	1,000	A	4,556	P	3,050	P	1,249	P	1,429	P	U61836	cell cycle, cyclin G1 interacting
1717_s_at	0,986	P	0,887	P	1,986	P	2,522	P	1,078	P	3,138	P	U45878	apoptosis, IAP2, TRAF1+2 binding
1737_s_at	7,014	P	3,011	P	15,009	P	6,033	P	6,441	P	12,872	P	M62403	receptor, IGF-1, IGFBP4, insulin like growth factor
1779_s_at	1,016	P	0,876	P	1,353	P	1,628	P	1,930	P	1,736	P	M16750	kinase, pim-1
178_f_at	0,918	P	1,302	P	0,457	A	0,724	P	1,168	P	1,041	P	U38964	replication, DNA repair, cell cycle, PMS3
1790_s_at	0,963	P	0,858	M	0,738	P	1,070	P	0,798	P	1,420	P	L33264	cell cycle, kinase
1890_at	3,491	P	1,161	P	2,308	P	1,969	P	1,048	P	1,336	P	AB000584	receptor, TGF, PDF
1891_at	1,169	P	0,858	P	1,783	P	1,308	P	1,165	M	1,700	P	D14497	kinase, MAP3K8

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
1939_at	1,119	P	0,972	P	0,888	P	0,926	P	1,274	P	1,089	P	M22898	signalling, p53
1953_at	1,133	P	1,515	P	3,461	P	1,710	P	1,285	P	1,724	P	AF024710	receptor, VEGF-A
1976_s_at	1,275	M	0,970	P	1,000	P	0,814	P	0,901	P	1,049	P	X06292	kinase, tyrosine kinase, fes
199_s_at	0,275	A	1,476	P	1,065	P	1,608	P	0,735	P	0,613	P	U33052	kinase
1993_s_at	0,874	P	0,787	P	0,878	P	0,850	P	2,678	P	1,547	P	U14680	miscellaneous, BRCA1
2051_at	0,633	P	1,045	P	0,927	P	0,816	P	1,671	P	0,954	P	M31767	enzyme, methyltransferase
214_at	1,123	P	1,143	P	0,770	P	1,000	P	0,509	P	0,675	M	M97676	transcription, homeo box, HOX7
31439_f_at	0,696	P	0,776	P	2,105	P	1,622	P	2,085	P	1,212	P	X63095	surface protein, blood group, rhesus, CD240CE
31456_at	1,019	P	1,125	P	0,960	P	1,213	P	1,038	P	0,598	A	AL008627	ribosome
31535_i_at	1,269	P	1,000	P	0,980	P	1,533	P	0,588	P	0,828	P	W27858	unknown
31622_f_at	1,237	P	2,336	P	3,707	P	0,987	A	2,058	P	3,093	P	M10943	enzyme, MT1F
31695_g_at	0,630	P	0,765	P	0,831	P	1,184	P	1,562	P	1,000	P	X82877	enzyme, transporter
31822_at	1,474	P	0,846	A	0,847	P	1,445	P	0,984	P	1,243	P	L12579	transcription, CDP, CCAAT
31833_at	1,156	P	0,714	P	0,712	P	1,161	P	0,776	P	0,898	M	U78575	kinase, PIP-5K1A, signalling
31874_at	1,356	P	1,104	P	1,798	P	1,119	P	1,742	P	1,167	P	Y07846	cell cycle, gas, gas related, GAR22
31882_at	1,518	P	1,058	P	0,676	A	1,000	P	1,324	P	1,072	P	AJ001340	splicing
31901_at	1,426	P	1,353	P	1,287	P	1,485	P	0,999	P	1,214	P	AF044253	enzyme, channel, K+
31930_f_at	0,800	P	0,952	P	2,397	P	2,095	P	2,191	P	1,140	P	X63096	surface protein, blood group, rhesus, CD240CE
31931_f_at	0,928	P	1,013	P	2,349	P	2,008	P	2,044	P	1,075	P	AI632247	surface protein, blood group, rhesus, CD240CE
31961_r_at	0,940	P	1,231	P	0,868	P	0,864	M	0,644	P	0,546	P	AF070579	unknown
32021_at	1,925	P	1,255	P	1,195	P	0,988	A	1,019	P	1,091	P	AI560890	unknown
32034_at	1,098	P	1,772	P	1,234	P	2,123	P	1,851	P	1,891	P	AF041259	transcription, zinc finger, ZNF217
32036_i_at	2,726	P	0,946	P	0,978	P	1,020	A	1,000	P	1,476	P	AF001175	enzyme, ribonuclease
32045_at	1,065	P	0,874	P	1,100	P	1,274	P	0,936	P	1,113	P	AB002331	transcription, death associated TF, DIO-1, DATF1
32046_at	1,598	P	0,683	A	1,051	P	1,946	P	1,000	P	1,528	P	D10495	kinase, PKCd, signalling

id v2	SCF/Epo_01 Normalized	Flags	SCF/Epo_03 Normalized	Flags	SCF/Epo_4 Normalized	Flags	SCF/Epo_5 Normalized	Flags	SCF/Epo_kk_1 Normalized	Flags	SCF/Epo_kk_2 Normalized	Flags	Acc no	Function
32073_at	0,946	P	1,237	P	0,932	M	1,262	P	1,358	P	1,191	P	AB014577	KIAA0677
32081_at	0,975	P	1,162	P	1,397	P	1,262	P	1,204	P	0,915	P	AB023166	kinase
32097_at	1,263	P	0,685	P	1,361	P	1,357	P	1,225	P	0,552	P	AB007862	structural protein, perinectin
32181_at	0,776	P	0,944	P	0,655	P	1,109	P	1,166	P	1,231	P	M60922	surface protein
32202_at	1,870	P	1,606	P	1,137	P	1,340	P	0,832	P	1,373	P	U67322	miscellaneous, XAP4
32257_f_at	0,863	P	0,906	P	1,859	P	1,938	P	1,224	P	0,721	P	AF003001	cell cycle
32321_at	1,491	P	0,710	A	3,071	P	2,996	P	0,840	P	1,743	P	X56841	surface protein, MHC I, HLA-E
32506_at	1,134	P	0,584	A	0,628	P	0,792	P	1,422	P	1,083	P	AB029031	KIAA1108
32517_at	0,981	P	1,017	P	0,797	P	1,047	P	1,151	P	0,879	P	AB028639	proteolysis, CAPN7
32554_s_at	2,615	P	1,214	P	2,543	P	1,304	P	1,757	P	1,137	P	Y12781	miscellaneous, transducin, TBR1
32561_at	0,621	M	1,052	P	1,159	P	1,000	P	1,253	P	1,039	P	D63480	KIAA0146
32562_at	1,796	P	1,000	P	0,912	P	0,782	P	1,397	P	0,918	P	X72012	surface protein, CD105
32577_s_at	0,934	P	1,139	P	0,588	P	0,956	P	1,146	P	0,882	P	L38933	unknown
32593_at	1,961	P	2,326	P	1,309	P	0,893	P	1,000	P	1,415	P	D42043	KIAA0084
32612_at	0,613	P	0,593	P	1,579	P	0,813	P	1,398	P	1,429	P	X04412	structural protein
32635_at	2,415	P	1,000	P	1,231	P	0,604	P	1,772	P	1,571	P	AB029036	transcription, general, TIF1 gamma
32668_at	0,940	P	0,514	P	2,046	P	1,971	P	1,159	P	1,090	P	AL080076	miscellaneous
32739_at	0,945	P	1,066	M	1,193	P	0,898	P	0,781	P	0,690	P	AA001791	enzyme, N-acetylglucosamine-phosphate mutase
32747_at	3,377	P	1,230	P	1,328	P	3,226	P	1,034	P	1,055	P	X05409	enzyme, ALDH
32770_at	1,000	P	0,787	P	1,039	P	1,082	P	0,966	P	1,245	P	AB018298	structural protein
32955_at	1,279	P	0,951	P	1,848	P	0,803	P	1,898	P	1,326	P	AL021546	enzyme, cytochrome
32962_at	2,596	P	2,264	P	0,580	P	2,048	P	1,305	P	0,936	P	S52028	enzyme
32979_at	0,655	A	0,939	P	0,980	P	1,430	P	1,123	P	1,160	P	U43885	signalling, GRB2
32998_at	1,218	P	1,146	P	0,756	P	1,010	P	0,709	P	0,877	M	L19315	receptor, cholecystokinin A R
330_s_at	0,928	P	0,895	P	1,750	P	1,066	P	1,303	P	2,001	P	X06956	structural protein
33011_at	1,000	P	1,109	P	0,742	P	1,134	P	0,563	P	0,873	P	Y10148	receptor, NTR2R
33027_at	1,287	P	0,757	P	1,000	P	0,711	M	0,812	P	0,939	P	W27906	unknown

id v2	SCF/Epo_01 Normalized	Flags	SCF/Epo_03 Normalized	Flags	SCF/Epo_4 Normalized	Flags	SCF/Epo_5 Normalized	Flags	SCF/Epo_kk_1 Normalized	Flags	SCF/Epo_kk_2 Normalized	Flags	Acc no	Function
33134_at	1,163	P	1,452	P	1,085	P	0,924	P	2,043	P	1,169	P	AB011083	signalling, adenylate cyclase
33191_at	0,417	A	1,402	P	0,652	P	0,784	P	1,361	P	1,122	P	AW051579	unknown
33195_at	1,086	P	1,037	P	1,383	P	1,054	P	2,564	P	0,991	P	M94065	enzyme, dehydrogenase
33223_at	1,150	P	0,696	P	1,157	P	1,518	P	1,273	P	1,200	P	AB011133	kinase
33233_at	1,303	P	1,201	P	0,609	P	0,997	P	1,173	P	1,119	P	D38491	KIAA0117
33260_at	0,676	A	1,016	P	1,927	P	1,112	P	1,833	P	0,825	P	L13857	signalling
33269_at	1,000	P	0,701	M	1,255	P	0,754	P	1,468	P	1,070	P	AB003723	structural protein
33281_at	0,942	P	1,363	P	0,859	P	1,042	M	1,000	P	1,387	P	D63485	transcription, NF-kB, IKKi
33289_f_at	2,048	P	1,000	M	1,516	P	1,077	P	1,539	P	1,298	P	D88827	transcription, zinc finger, ZNF263
33290_at	0,881	P	1,232	P	1,020	P	1,132	P	1,354	P	0,717	P	M74161	phosphatase, signalling
33432_at	0,855	P	0,769	A	0,696	P	1,132	P	0,532	P	0,838	P	AI547308	unknown
33448_at	1,000	A	0,798	P	0,925	P	1,005	P	0,837	P	0,876	P	AB000095	proteolysis
33494_at	0,991	P	0,958	P	0,630	A	1,155	P	1,794	P	1,000	P	S69232	enzyme, oxidoreductase
33712_at	1,025	P	1,227	P	1,290	P	1,624	P	1,000	P	0,929	P	N63574	enzyme, transferase
33754_at	1,057	P	1,094	P	0,658	P	0,915	P	0,843	P	1,197	P	U43203	transcription, TTF1
33767_at	3,188	P	1,951	P	1,623	P	1,302	P	2,342	P	2,510	P	X15306	structural protein, NF-H
33773_at	0,722	P	1,125	P	1,219	P	0,915	P	1,239	P	0,972	A	U13948	transcription, zinc finger
33804_at	1,148	P	0,928	P	1,998	P	1,575	P	1,718	P	1,688	P	U43522	adhesion, kinase, CAK
33864_at	0,567	A	1,021	P	1,115	P	1,003	P	1,047	P	0,616	P	X86098	transcription, zinc finger
33916_at	1,822	P	0,974	P	1,008	P	1,175	P	1,606	P	1,439	P	AB023192	KIAA0975
33934_at	0,723	P	1,431	P	1,868	P	1,043	P	0,685	P	0,872	P	AB018340	KIAA0797
33982_f_at	0,895	P	2,095	P	1,563	P	1,705	P	0,735	P	0,719	P	X59244	transcription, zinc finger, ZNF043
34016_s_at	1,576	P	1,903	P	2,063	P	1,964	P	2,307	P	1,970	P	X78338	enzyme, MRP
34034_at	1,009	P	1,780	P	1,508	P	1,202	P	2,461	P	1,987	P	D80011	KIAA0189
34058_at	0,995	A	1,337	P	0,963	P	1,733	P	0,870	P	1,358	P	L15309	transcription, zinc finger
34059_at	1,491	P	1,254	M	2,554	P	1,643	P	1,460	P	0,949	P	AA586695	unknown
	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2			

id v2	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Acc no	Function
34077_at	1,239	A	1,450	P	1,174	P	1,208	P	1,370	P	1,880	P	X95876	receptor, chemokine, CXCR03, CD183, IP10/Mig R
34131_at	1,054	P	1,753	P	0,853	P	1,152	P	0,808	P	1,116	P	AB026891	enzyme, transporter
34260_at	0,897	P	0,948	P	0,719	P	0,835	P	1,564	P	1,092	P	AB014583	KIAA0683
34282_at	0,740	P	1,574	P	1,351	P	1,359	P	1,270	P	1,627	P	AB010812	transcription, NF-E2, NRF3
34367_at	3,270	P	1,255	P	1,354	P	1,756	P	0,780	P	1,121	P	AF006043	enzyme, dehydrogenase
34406_at	1,288	P	1,024	P	0,992	P	0,663	P	1,097	P	1,265	P	AB011174	KIAA0602
34427_g_at	0,834	P	1,020	P	1,000	P	1,042	P	1,444	P	1,226	P	U22963	surface protein, MHC I
34510_at	1,646	P	1,198	P	2,778	P	0,998	P	1,933	P	1,222	P	AF070552	unknown
34544_at	1,046	P	1,403	P	0,644	P	0,869	P	1,549	P	1,443	P	X78925	transcription, zinc finger, ZNF267
34659_at	1,155	P	1,399	P	0,937	P	1,000	P	2,566	P	1,743	P	AB018334	structural protein, nucleoporin
34731_at	1,774	P	1,309	P	0,758	M	0,955	P	1,860	P	1,330	P	D80007	KIAA0185
34740_at	2,488	P	0,748	P	2,861	P	1,907	P	1,642	P	1,282	P	AF032886	transcription, forkhead
34757_at	1,015	P	0,591	P	0,496	M	0,689	P	0,784	P	1,326	P	AA595596	enzyme, transferase
34759_at	1,198	P	0,884	P	1,323	P	1,488	P	1,006	P	1,000	P	U68494	miscellaneous
34779_at	0,961	P	0,907	A	0,930	P	1,104	P	1,212	P	0,908	P	R90942	enzyme, transferase
34780_at	0,955	P	0,803	P	1,000	P	0,841	P	1,119	P	1,225	P	AB002313	KIAA0315
34827_at	1,200	P	0,916	A	1,468	P	2,106	P	1,258	P	1,177	P	AF045458	kinase
34871_at	1,301	P	1,140	P	1,031	P	1,111	P	1,492	P	1,569	M	W30677	unknown
34875_r_at	0,966	P	1,166	P	1,109	P	1,292	P	0,979	P	0,818	P	D86958	KIAA0203
34876_at	0,959	P	0,911	P	1,180	P	1,353	P	1,068	P	0,885	P	U65090	enzyme, carboxypeptidase
34903_at	1,058	P	1,334	P	0,853	P	0,611	P	0,810	P	1,037	P	AI017382	unknown
34933_at	1,022	P	0,755	P	0,726	P	0,757	P	0,664	P	1,061	P	AJ238381	transcription, PAX9
34955_at	0,668	P	2,228	P	1,805	P	0,996	P	1,803	P	1,495	P	AF071202	enzyme, transporter
34981_at	1,212	P	1,292	P	0,968	P	1,073	P	0,618	A	1,127	P	M55513	enzyme, channel, K+
35001_at	0,637	A	1,370	P	0,861	P	1,002	P	1,502	P	1,000	P	Z85986	kinase, Ndr
35017_f_at	1,254	P	0,571	P	1,296	P	3,013	P	0,810	P	1,835	P	M80469	surface protein, MHC I, HLA-J
35026_f_at	0,735	P	1,394	P	2,327	P	1,023	P	1,211	P	0,983	P	X78932	transcription, zinc finger, ZNF273

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
35139_at	0,808	M	1,006	P	1,000	P	0,844	P	0,928	P	0,819	P	AL049341	unknown
35173_at	1,885	P	1,430	P	1,305	P	0,505	A	0,545	P	0,786	P	U03886	miscellaneous (unknown)
35212_at	0,782	P	0,766	P	1,035	P	1,254	P	1,198	P	1,080	P	AF064801	receptor, TRC8
35217_at	1,507	P	1,123	P	0,874	P	1,285	P	1,000	P	1,260	P	AL049404	structural protein, extracellular, MFAP3
35219_at	1,203	P	1,021	P	1,115	P	1,122	P	0,786	P	0,928	M	AL050202	unknown
35229_at	0,858	P	1,236	P	0,760	P	0,660	P	1,001	A	1,016	P	L39211	enzyme, carnitine palmitoyltransferase
35230_at	1,313	P	0,720	A	1,906	P	1,115	P	1,052	P	1,071	P	AF070530	hypothetical protein, clone 24751
35250_at	1,378	P	1,201	P	1,848	P	0,796	P	0,931	P	1,481	P	AL031670	ring finger, RNF24
35253_at	2,966	P	4,316	P	2,108	P	3,200	P	1,000	P	1,060	P	AB011143	signalling, GRB2 associated
35274_at	1,184	P	1,059	P	0,934	P	1,371	M	1,280	P	0,799	P	Y12226	structural protein, adaptin gamma
35282_r_at	2,189	P	0,893	P	1,052	P	1,359	P	1,756	P	1,909	P	M33680	surface protein, CD081, 4TM
35305_at	0,791	P	0,491	P	1,394	P	0,859	P	1,411	P	0,954	P	X95762	proteolysis, aminopeptidase-like
35313_at	1,459	P	1,000	A	0,714	P	1,026	P	1,730	P	1,774	P	AB002308	KIAA0310
35338_at	1,366	P	0,785	A	1,372	P	1,000	P	1,166	P	1,238	P	X17094	proteolysis
35346_at	0,923	P	1,035	A	1,131	P	1,048	P	0,943	P	1,008	P	AB007856	miscellaneous, FEM1B
35430_at	1,678	P	1,377	P	1,630	P	0,595	P	1,717	P	1,497	P	U78082	transcription, general, RNA pol II, MED6
35434_at	1,338	P	0,787	P	0,801	P	1,000	P	0,907	P	1,327	P	L16794	transcription, MEF2
35442_at	2,184	P	1,398	P	0,777	P	0,771	M	1,350	P	1,282	P	AB007958	KIAA0489
35505_at	1,000	P	0,869	P	1,359	P	1,078	P	1,538	A	1,122	P	AI290660	transcription, chromatin, SWI/SNF
35590_s_at	1,702	M	1,362	P	0,761	P	1,023	P	0,725	P	0,922	P	X81832	receptor, G protein, GPCR
356_at	1,005	P	0,912	P	0,608	P	1,000	P	2,075	P	1,177	P	AB017430	structural protein, kinesin
35650_at	1,197	P	1,108	A	1,196	P	1,104	P	0,976	P	1,284	P	AB002354	KIAA0356
35718_at	1,126	P	1,160	P	1,972	P	2,833	P	0,891	P	1,361	P	L22342	receptor, IFN, IFN inducible
35768_at	1,098	P	0,728	P	0,991	P	0,832	M	1,016	P	0,924	P	AB014561	cell cycle, RB, RBP95
35788_at	0,582	A	0,700	P	0,817	P	0,709	P	1,535	P	1,194	P	W28994	structural protein, dynein

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
35791_at	1,160	P	0,801	A	1,027	P	0,753	P	2,918	P	1,918	P	AF038961	miscellaneous
35807_at	1,630	P	0,969	P	0,796	P	2,826	P	1,066	P	1,804	P	M21186	enzyme, myeloid
35847_at	0,979	P	1,244	P	1,142	P	0,921	P	2,973	P	2,349	P	AB028980	KIAA1057
35886_at	0,889	P	0,937	P	0,926	P	1,622	P	1,731	P	0,729	A	AL049758	structural protein
35913_at	1,042	P	0,849	P	1,721	P	1,398	P	1,297	P	1,245	P	U88047	transcription, DRIL1
35919_at	6,461	P	2,433	P	1,922	P	4,835	P	1,501	P	4,920	P	J05068	enzyme, transcobalamin
35967_at	0,828	A	1,039	P	0,919	P	1,653	P	1,075	P	0,934	P	M69238	transcription, nuclear receptor, HIF1 beta, HLH
36028_at	1,132	P	0,751	A	1,105	P	1,166	P	1,227	P	1,252	P	U45285	enzyme, transporter, ATPase
36068_at	1,058	P	0,620	P	1,786	P	1,226	P	1,277	P	1,314	P	AF002210	enzyme, chaperone for superoxide dismutase
36072_at	1,925	P	1,137	P	1,899	P	1,840	P	1,474	P	1,056	P	AF025770	transcription, zinc finger, ZNF189
36089_at	0,261	A	1,000	P	1,177	P	1,132	P	2,252	P	1,194	P	AB023183	KIAA0966
36100_at	1,197	P	1,428	P	3,782	P	1,746	P	1,254	P	1,661	P	AF022375	receptor, VEGF-A
36115_at	1,023	P	0,585	A	3,143	P	1,381	P	1,429	P	1,531	P	L29217	kinase, clk3
36136_at	1,100	P	0,805	P	1,000	P	0,995	P	0,685	P	1,399	P	AF010315	apoptosis, p53, p53-induced
36152_at	1,208	P	0,901	P	1,296	P	1,097	P	1,147	P	1,272	P	X79353	G protein, signalling
36175_s_at	2,536	P	0,911	P	2,491	P	1,442	P	1,139	P	1,544	P	AL023584	transcription
36211_at	0,835	P	1,160	P	1,258	P	1,156	P	0,912	P	0,978	P	D87461	apoptosis, APOPTOSIS REGULATOR BCL
36303_f_at	0,526	P	1,689	P	0,828	P	1,220	P	1,000	P	1,137	P	U35376	transcription, zinc finger, ZNF085
36336_s_at	1,099	P	0,512	A	1,283	P	1,414	P	0,830	P	0,678	P	AC005390	enzyme
36357_at	0,530	M	1,600	P	0,880	P	1,121	P	0,906	P	0,890	P	AC004381	unknown
36403_s_at	0,865	P	0,910	P	0,844	P	0,819	P	1,323	P	1,265	P	AI434146	unknown
36458_at	1,207	P	1,161	P	0,836	P	1,059	P	0,980	P	1,274	P	AB023235	KIAA1018
36460_at	1,815	P	1,030	P	0,869	P	0,710	P	2,773	P	2,032	P	AF008442	transcription, general, RNA pol I
36478_at	1,574	P	1,123	A	3,208	P	1,372	P	1,327	P	1,000	P	X83973	transcription, TTF1
36482_s_at	1,068	P	2,083	P	1,000	P	0,794	P	1,771	P	1,861	P	Y15724	ATPase
36551_at	1,233	P	0,967	P	1,322	P	1,454	P	1,252	P	0,769	P	AL049382	unknown

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
36554_at	1,518	P	1,000	P	1,539	P	0,982	P	3,130	P	1,932	P	Y15521	enzyme, transferase
36591_at	0,874	P	1,003	P	2,348	P	1,147	P	1,151	P	1,915	P	X06956	structural protein
36617_at	6,340	P	1,799	P	1,660	P	3,488	P	3,471	P	1,654	P	X77956	transcription, HLH, Id1
36618_g_at	3,196	P	1,468	P	1,106	P	1,653	P	1,704	P	1,388	P	X77956	transcription, HLH, Id1
36672_at	1,169	P	0,484	M	1,037	P	1,000	P	1,289	P	1,150	P	L13977	enzyme, proteolysis
36704_at	0,860	P	1,596	P	1,537	P	0,628	A	0,742	P	0,709	P	AB030654	structural protein
36717_at	0,885	P	1,593	P	1,055	P	0,951	P	1,366	P	1,128	P	AJ224162	enzyme, synthetase
36731_g_at	1,009	P	1,281	P	0,856	P	0,906	P	1,067	P	1,405	P	U66684	unknown
36737_at	8,410	P	5,772	P	11,999	P	5,604	P	3,467	P	3,285	P	U59057	crystallin beta 4
36743_at	0,952	P	1,306	P	1,876	P	1,690	P	1,673	P	0,646	A	AL096739	unknown
36805_s_at	3,393	P	4,145	P	1,965	P	0,326	A	2,287	P	2,998	P	X03541	receptor, TRK1, NGFR, kinase
36821_at	1,380	P	1,620	P	3,331	P	0,611	P	2,449	P	1,158	P	AL050367	unknown
36865_at	0,802	P	0,659	P	0,853	P	0,436	A	1,015	P	1,049	P	AB018302	KIAA0759
36936_at	1,538	P	0,838	P	0,695	P	0,936	P	1,807	P	1,371	P	U58766	enzyme, red cell
36940_at	0,626	P	0,922	P	0,453	A	0,596	P	1,105	P	1,119	P	D86970	KIAA0216
370_at	0,481	A	1,142	P	1,287	P	0,751	P	1,187	P	0,926	P	Z35102	kinase, Ndr
37021_at	1,689	P	0,828	P	1,094	P	1,950	P	1,250	P	2,376	P	X16832	proteolysis, cathepsin H, host defense
37061_at	1,336	P	0,971	P	1,039	P	1,917	P	0,849	P	0,714	P	U29615	enzyme
37095_r_at	0,892	P	0,891	P	0,632	P	1,742	P	0,857	P	0,913	P	M84562	receptor, fmlp -related receptor, lipoxin a4 receptor
37105_at	0,775	P	0,631	P	0,762	P	1,741	P	0,290	A	0,944	P	M16117	proteolysis, cathepsin G, host defense
37115_at	0,298	A	1,324	P	0,413	P	0,968	P	1,263	P	0,600	P	AL031666	kinase, PKC binding
37165_f_at	0,897	P	0,838	P	1,734	P	1,461	P	1,995	P	1,060	P	X54534	surface protein, blood group, rhesus, CD240CE
37178_at	1,031	P	2,018	P	0,711	P	1,563	P	1,624	P	1,772	P	M74089	miscellaneous
37179_at	0,964	P	0,811	P	1,854	P	1,366	P	1,648	P	1,336	P	S77763	transcription, NF-E2
37188_at	1,858	P	1,154	P	0,621	P	1,090	P	1,809	P	1,288	P	X92720	enzyme, kinase
37192_at	1,103	P	0,564	P	1,878	P	1,257	P	1,089	P	1,510	P	U28389	surface protein, band 4.9, dematin

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
37210_at	1,222	P	0,977	P	0,964	P	0,664	P	0,514	P	1,000	P	S78296	structural protein
37225_at	0,891	P	1,925	P	0,224	A	0,982	P	1,205	P	0,969	P	D79994	KIAA0172
37245_at	0,720	P	1,211	P	1,603	P	0,768	A	1,414	P	0,947	P	U13044	transcription
37250_at	1,003	P	1,395	P	0,598	P	0,944	P	2,790	P	2,086	P	AB007191	transcription, myc, c-myc binding
37256_at	0,957	P	1,087	P	1,145	P	1,120	P	1,489	P	1,000	P	AI829890	unknown
37278_at	0,858	P	0,989	P	0,639	A	0,930	P	0,731	P	0,999	P	X92762	structural protein
37285_at	1,164	P	0,435	P	2,510	P	2,037	P	1,498	P	1,000	P	X60364	enzyme, heme biosynthesis, ALA-S erythroid
37299_at	0,881	A	0,954	P	1,910	P	1,290	P	1,359	P	1,206	P	J04501	enzyme, glycogen synthase
37311_at	1,131	P	0,793	P	0,836	P	0,844	P	2,077	P	1,634	P	AF010400	enzyme
37360_at	1,210	P	1,498	P	1,644	P	3,065	P	2,431	P	3,402	P	U66711	surface protein, Ly6E
37383_f_at	1,152	P	0,420	P	1,689	P	2,711	P	1,385	P	1,845	P	X58536	surface protein, HLA
37416_at	0,866	P	1,948	P	1,892	P	2,111	P	1,098	P	0,968	P	Z35227	G protein
37421_f_at	1,334	P	0,466	A	1,750	P	2,992	P	0,971	P	1,703	P	AL022723	surface protein, MHC I, HLA-F
37425_g_at	0,514	P	0,428	P	1,014	P	0,562	P	1,711	P	1,262	P	AB029343	miscellaneous
37464_at	0,704	A	1,358	P	1,602	P	1,016	P	1,009	P	1,171	P	AF048755	unknown
37470_at	1,091	P	0,794	P	0,690	P	1,841	P	0,409	A	0,866	P	AF013249	surface protein, LAIR1, monocytes
37486_f_at	1,265	P	1,121	P	0,722	P	1,170	P	0,581	P	1,632	P	U68385	transcription, homeo box, MRG2
37579_at	1,484	P	0,738	A	1,378	P	1,104	P	0,716	P	1,117	P	L47738	miscellaneous
37586_at	0,969	P	0,872	P	0,390	P	1,015	M	1,262	P	1,139	P	D87073	transcription, zinc finger
37649_at	1,000	P	0,652	P	1,763	P	1,593	P	1,744	P	1,658	P	M95623	enzyme, heme biosynthesis, HMB9
37674_at	3,643	P	2,166	P	4,891	P	4,805	P	1,000	P	1,359	P	Y00451	enzyme, heme biosynthesis, ALA-S, non-erythroid
37695_at	1,349	P	0,950	P	3,210	P	1,343	P	1,983	P	1,204	P	D79983	KIAA016
37741_at	1,542	P	0,853	P	0,512	A	0,893	P	0,963	P	0,650	P	M77836	enzyme
37750_at	1,401	P	0,899	P	0,514	A	1,130	P	0,902	P	0,590	P	AF049140	proteolysis
37869_at	0,296	A	1,758	P	1,290	P	1,448	P	1,131	P	1,457	P	AB029004	KIAA1081
38021_at	1,693	P	1,746	P	1,430	P	0,942	A	1,440	P	2,215	P	U53204	structural protein

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
38072_at	0,972	P	0,716	P	2,042	P	2,170	P	1,052	P	0,871	P	AL031432	unknown
38090_at	0,612	P	0,969	P	1,796	P	0,805	P	2,569	P	1,588	P	AL050371	enzyme, decarboxylase
38109_at	1,045	P	0,899	P	1,505	P	0,990	P	1,231	P	0,760	P	AF020544	enzyme, palmitoyl-protein thioesterase
38122_at	1,466	P	0,873	P	0,836	P	1,016	P	1,383	P	1,014	P	D87075	KIAA0238
38136_at	0,637	A	0,603	P	0,835	P	0,870	P	1,167	P	1,032	P	L76937	unknown
38192_at	1,356	P	1,620	P	1,360	P	1,755	P	1,402	P	0,921	P	AB014543	KIAA0643
38224_at	1,241	P	1,671	P	1,892	P	2,305	P	1,063	P	0,927	P	U71300	transcription, general, SNAP50
38241_at	0,846	P	1,120	P	0,965	P	1,150	A	1,589	P	1,177	P	U90548	butyrophilin
38284_at	1,191	P	1,083	P	0,976	P	1,088	A	1,099	P	0,971	P	AJ007041	transcription, homeo box, trithorax homologue
38354_at	1,171	P	0,909	P	1,022	P	2,549	P	0,742	P	1,068	P	X52560	transcription, C/EBP beta, NF-IL6
38358_at	1,030	P	0,920	M	1,124	P	1,397	P	1,255	P	1,291	P	AJ010840	replication, helicase
38404_at	2,226	P	1,246	P	2,247	P	3,740	P	1,190	P	0,800	P	M55153	enzyme, transglutaminase
38409_at	2,076	P	0,917	P	1,139	P	1,244	P	1,000	P	0,786	A	M61199	surface protein
38418_at	2,141	P	2,474	P	1,604	P	1,000	A	3,456	P	3,607	P	X59798	cell cycle, cyclin D1
38467_at	1,057	P	0,489	A	0,910	P	0,927	P	0,838	P	0,785	P	U96721	structural protein
38474_at	3,365	P	2,118	P	1,238	P	2,369	P	1,437	P	1,062	P	L00972	enzyme, cystathionine-beta-synthase
38491_at	0,852	P	1,825	P	1,101	P	1,592	P	0,985	P	1,203	P	U11732	transcription, ets, tel
38523_f_at	1,504	P	1,041	A	0,830	P	0,607	P	1,154	P	1,100	P	D49677	splicing
38559_at	1,289	P	1,577	P	1,729	P	1,628	P	1,968	P	0,988	P	AB007861	KIAA0401
38614_s_at	2,614	P	1,448	P	3,931	P	2,140	P	1,485	P	0,923	P	U77413	enzyme, GlcNAc transferase
38649_at	2,028	P	1,490	P	1,218	P	1,284	P	1,135	P	0,673	A	AB023187	KIAA0970
38662_at	1,675	P	0,970	P	0,699	P	3,004	P	2,250	P	2,773	P	AL047596	unknown
38723_at	1,031	P	0,921	P	0,751	P	0,805	P	1,280	P	1,452	P	AF052137	unknown
38739_at	0,941	P	0,953	P	1,748	P	1,420	P	1,061	P	1,000	P	AF017257	transcription, ets2
38788_at	1,653	P	1,000	A	1,303	P	1,706	P	0,778	P	1,146	P	M82827	transcription, zinc finger, PML-2
38942_r_at	1,455	P	1,153	P	0,657	P	0,890	P	0,600	A	1,000	P	W28610	unknown

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
38949_at	1,250	P	1,853	P	2,339	P	0,841	P	1,987	P	1,888	P	L01087	kinase
38962_at	0,894	M	1,863	P	1,122	P	1,284	P	0,989	P	1,000	P	AB002296	KIAA0298
38964_r_at	1,354	P	1,427	P	1,130	P	1,483	P	1,104	P	1,036	P	U12707	signalling,WASP, GTPase
39058_at	1,498	P	1,125	P	1,028	P	1,380	P	1,307	P	1,602	P	U01147	G protein
39172_at	1,017	P	1,184	M	1,193	P	1,347	P	0,951	P	0,956	P	AL049319	unknown
39251_at	0,783	P	1,026	P	0,887	P	0,817	P	2,236	P	1,546	P	M74091	cell cycle, cyclin C
39261_at	1,220	A	0,890	P	1,140	P	1,000	P	1,293	P	1,511	P	L16896	transcription, zinc finger
39267_at	1,084	P	1,493	P	0,919	P	1,213	P	1,153	P	1,030	P	AF102265	enzyme, phosphate mutase
39285_at	0,685	A	1,199	P	1,256	P	1,337	P	1,612	P	0,916	P	L28957	enzyme
39304_g_at	0,856	M	1,000	P	1,749	P	1,384	P	2,287	P	1,413	P	Y14153	G protein, transducin
39317_at	0,569	A	1,488	P	0,640	P	1,206	P	0,892	P	0,987	P	D86324	enzyme, hydroxylase
39369_at	1,302	P	1,688	P	0,538	A	1,879	P	1,414	P	1,458	P	AB023152	KIAA0935
39523_at	0,823	M	1,519	P	1,150	P	2,064	P	1,430	P	1,293	P	AF038897	structural protein, syntaxin
39582_at	1,000	P	1,077	A	0,863	P	1,136	P	1,106	P	0,945	P	AL050166	unknown
39594_f_at	1,334	P	2,254	P	3,788	P	0,938	P	1,339	P	3,689	P	R93527	enzyme, MT1F
39636_at	2,228	P	2,021	P	1,272	P	1,375	P	1,197	P	0,871	P	AL079294	unknown
39664_at	0,729	P	1,013	P	0,784	P	0,981	M	1,298	P	0,766	P	U28413	miscellaneous
39703_at	1,094	P	1,227	P	1,043	P	0,466	A	0,798	P	0,887	P	AF000993	miscellaneous
39765_at	1,083	P	1,223	P	0,750	P	1,114	P	0,918	P	1,139	P	AB002318	KIAA0320
39770_at	1,175	P	1,253	P	0,742	P	1,079	P	1,562	P	1,501	P	D87437	KIAA0250
39781_at	5,602	P	3,917	P	15,825	P	6,722	P	11,400	P	17,244	P	U20982	receptor, IGF-1, IGFBP4, insulin like growth factor
39788_at	0,833	P	0,525	A	0,826	P	1,061	P	1,617	P	0,852	P	X81889	adhesion, catenin G, plakoglobin
39879_s_at	1,567	P	1,306	P	1,014	P	1,018	P	0,764	P	0,838	P	H16917	unknown
39891_at	1,243	P	0,781	P	0,520	A	0,948	P	0,807	P	1,000	P	AI246730	unknown
39920_r_at	0,667	M	0,724	P	1,123	P	0,985	P	0,870	P	0,883	P	AF095154	receptor, C1q related factor
40005_at	1,067	P	0,918	P	1,331	P	1,015	P	1,199	P	1,354	P	X91992	replication, DNA repair, alkB
40020_at	1,434	P	1,215	P	0,771	P	1,065	P	0,597	P	0,554	P	AB011536	miscellaneous, MEGF2

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
40045_g_at	1,230	P	0,785	P	0,474	P	1,258	P	0,857	P	0,641	M	AF009425	unknown
40050_at	0,995	P	0,917	P	1,109	M	1,226	P	1,102	P	1,071	P	AF069747	transcription, CBF-A2, CBFA2T2
40071_at	1,769	P	2,681	P	2,812	P	0,575	P	1,400	P	3,001	P	U03688	enzyme, cytochrome
40084_at	0,996	P	1,013	P	0,875	A	1,069	P	2,356	P	2,220	P	U03494	transcription, CP2
40191_s_at	0,646	P	1,078	P	1,984	P	0,813	P	1,156	P	0,925	P	AI761647	unknown
402_s_at	1,220	P	1,231	P	1,841	P	1,997	P	1,349	P	1,722	P	X69819	surface protein, CD050, adhesion, ICAM-3
40209_at	1,801	P	0,939	P	1,857	P	1,742	P	0,817	P	1,444	P	U72671	surface protein, ICAM5
40235_at	1,321	P	0,866	P	1,447	P	0,874	P	0,933	P	1,161	P	L13738	cell cycle, CDC42, p21 kinase, ACK
40246_at	0,737	P	1,592	P	0,898	P	1,447	P	1,000	P	0,824	P	U13897	Drosophila discs large protein
40282_s_at	1,182	P	1,000	P	1,694	P	1,894	P	0,908	P	1,115	P	M84526	proteolysis
40370_f_at	1,117	P	0,608	P	1,853	P	2,725	P	1,424	P	1,924	P	M90683	surface protein, MHC I, HLA-G
40556_at	1,739	P	1,363	P	1,032	P	1,398	P	1,390	P	0,992	P	D42073	enzyme, reticulocalbin
40575_at	1,358	P	1,461	P	1,899	P	0,606	P	2,327	P	1,755	P	AB011155	KIAA0583
40643_at	2,840	P	0,916	P	2,694	P	1,096	P	2,128	P	2,136	P	M34480	surface protein, CD041
40672_at	0,961	P	0,861	P	0,653	P	1,227	P	0,871	P	1,333	P	U57721	hydrolase
40686_at	1,432	P	0,888	P	1,000	P	0,973	P	1,295	P	2,130	P	AI985272	receptor, neuromedin B
40688_at	1,778	P	1,811	P	1,915	P	0,695	P	4,206	P	4,203	P	AJ223280	signalling
40707_at	0,831	P	1,182	P	1,109	P	0,948	P	1,190	P	0,835	M	AA810792	unknown
40708_at	1,854	P	2,375	P	0,785	P	0,755	A	0,716	P	1,000	P	AL096751	structural protein
40724_at	1,308	P	1,060	P	0,688	P	1,152	P	1,174	P	0,804	P	Y14443	transcription, zinc finger, ZNF200
40756_at	0,914	P	1,070	P	0,813	P	0,777	A	1,924	P	1,241	P	AF081280	structural protein
40806_at	1,012	P	1,081	P	1,226	P	0,913	P	0,984	P	0,861	P	U15128	enzyme, MGAT2
40817_at	1,323	P	0,422	A	1,014	P	0,788	P	1,377	P	1,080	P	M96824	miscellaneous, nucleobindin
40847_at	0,919	P	1,197	P	1,280	P	1,240	P	1,169	P	1,232	P	AB018293	KIAA0750
40848_g_at	1,584	P	1,677	P	3,821	P	1,757	P	3,107	P	2,318	P	AB018293	KIAA0750
40858_at	0,999	P	1,798	P	0,966	P	0,869	A	1,000	P	0,869	P	M34715	structural protein, PSG1

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
40877_s_at	0,970	P	1,786	P	3,349	P	1,921	P	1,236	P	0,857	P	AF041080	unknown
41015_at	0,394	A	1,670	P	1,992	P	1,473	P	1,565	P	1,097	P	AB022017	kinase
41024_f_at	0,911	P	0,588	P	1,000	P	2,363	P	1,287	P	0,703	P	X53004	surface protein, glycophorin B, CD235b
41044_at	1,360	P	1,124	P	0,458	A	0,902	P	1,461	P	1,408	P	U43374	unknown
41062_at	0,760	P	1,223	P	1,231	P	0,784	P	1,227	P	1,657	P	AA037278	unknown
41086_at	2,302	P	1,883	P	3,878	P	1,858	P	1,878	P	2,767	P	AF060877	G protein, GTPase
41095_at	1,428	P	1,000	P	0,668	M	1,065	P	1,086	P	1,174	P	X52221	replication, DNA repair, ERCC2
41137_at	0,918	P	1,421	P	1,093	P	1,192	P	0,817	P	0,375	P	AB007972	unknown
41177_at	1,987	P	1,132	P	0,556	P	1,556	P	0,917	P	1,060	P	AW024285	unknown
41188_at	1,669	P	1,325	P	1,512	P	1,030	P	1,217	P	0,966	P	W28186	unknown
41232_at	1,386	P	1,297	P	0,768	M	0,800	P	0,895	P	1,023	P	AL050022	unknown
41240_at	0,499	P	1,137	P	0,773	P	0,551	A	1,489	P	1,326	P	AA772359	unknown
41387_r_at	0,748	P	1,346	P	1,066	P	1,023	P	0,874	P	0,843	P	AB002344	KIAA0346
41393_at	1,439	P	1,318	P	0,797	P	1,057	P	1,231	P	0,890	A	AF003540	transcription, zinc finger
41409_at	0,600	P	0,497	P	0,651	P	1,398	P	1,120	P	0,723	P	AF044896	unknown
41429_at	0,918	P	1,548	P	0,832	P	1,012	P	1,774	P	1,248	P	M65254	phosphatase
41446_f_at	1,611	P	1,783	P	2,846	P	0,982	M	1,965	P	2,479	P	H68340	enzyme, MT1F
41458_at	1,027	P	0,831	P	0,858	P	1,139	P	0,800	P	0,767	M	AB007936	KIAA0467
41500_at	1,011	P	0,963	P	0,838	P	0,767	P	0,879	M	0,993	P	AI761818	unknown
41522_at	1,962	P	1,064	P	1,223	P	0,777	P	1,757	P	1,750	P	Z93096	unknown
41524_at	1,606	P	1,000	P	0,609	P	1,207	P	1,853	P	1,978	P	L08488	phosphatase
41526_at	1,107	P	0,838	P	1,860	P	0,936	P	1,542	P	2,443	P	AF072836	transcription, HMG, HMG20B
41561_s_at	1,000	P	1,232	P	1,129	P	0,935	P	0,795	P	0,778	P	AI651368	unknown
41654_at	1,411	P	0,638	A	0,847	P	1,603	P	0,537	P	1,529	P	X02994	enzyme, adenosine deaminase
41694_at	1,000	P	0,805	P	0,973	P	0,805	M	1,073	P	1,007	P	M17754	unknown
41702_r_at	0,812	P	1,219	P	1,000	P	0,677	P	0,625	A	0,724	P	U93869	transcription, RNA pol
41762_at	0,898	P	1,087	P	1,072	P	0,804	M	1,197	P	1,320	P	D64015	apoptosis, nucleolysin

id v2	SCF/Epo_01		SCF/Epo_03		SCF/Epo_4		SCF/Epo_5		SCF/Epo_kk_1		SCF/Epo_kk_2		Acc no	Function
	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags	Normalized	Flags		
41787_at	1,156	P	0,979	P	1,444	P	1,178	P	1,554	P	0,666	A	AI452442	unknown
41809_at	1,127	P	1,883	P	1,727	P	1,510	P	2,142	P	0,937	A	AI656421	unknown
41812_s_at	1,674	P	0,843	P	0,625	P	1,315	P	1,851	P	1,652	P	AB020713	KIAA0906
41843_r_at	0,893	P	0,851	P	1,091	P	1,440	P	1,322	P	1,110	P	W28275	unknown
473_g_at	0,700	P	0,661	M	1,054	P	1,472	P	1,408	P	0,925	P	U48730	transcription, STAT5B
495_at	1,002	P	1,139	P	0,920	P	1,248	P	0,784	P	1,010	P	U31628	receptor, IL-15RA
518_at	1,095	P	0,668	A	1,460	P	1,370	P	1,257	P	1,396	P	U07132	transcription, nuclear receptor, LXRb, Ner-1
551_at	1,475	P	0,543	A	0,995	P	0,963	P	1,062	P	1,664	P	U01877	transcription, CBP/p300
552_at	1,577	P	0,961	P	0,914	P	0,780	M	1,096	P	1,209	P	U02570	cell cycle, CDC42, G protein
634_at	1,068	P	1,044	P	0,815	A	1,044	P	0,754	P	0,886	P	L41351	proteolysis
642_s_at	1,063	P	1,112	A	0,851	P	1,330	P	1,114	P	1,076	P	L76528	proteolysis
755_at	1,890	P	1,664	P	1,907	P	1,427	P	1,348	P	1,607	P	D26070	signalling, receptor
825_at	1,129	P	1,408	P	0,893	P	0,791	P	1,467	P	0,982	P	U48736	kinase, PR4H
859_at	1,495	P	1,991	P	2,563	P	0,638	P	1,109	P	2,476	P	U03688	enzyme, cytochrome
883_s_at	1,007	P	0,732	P	1,118	P	1,342	P	1,482	P	1,441	P	M54915	kinase, pim-1
906_at	1,082	P	1,182	P	0,917	P	1,274	P	0,459	A	0,973	P	L78440	transcription, STAT4
992_at	2,214	P	1,116	A	1,348	P	1,043	P	1,775	P	1,541	P	X52221	replication, DNA repair, ERCC2

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6.3 Curriculum Vitae

Name	Britt Lemke
Born	at July 1th in 1970 in Waren (Müritz), Germany
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Education:

1977 –1979	Polytechnische Oberschule "Herbert Baum", Berlin-Friedrichshain, Germany
1979 –1987	Polytechnische Oberschule "Edgar Andre", Berlin-Friedrichshain, Germany, with extended Russian
1987 – 1989	Erweiterte Oberschule "Carl von Ossietzky", Berlin-Pankow, Germany, school-leaving examination (Matriculation), with extended Russian and English classes
1989 – 1990	Staatlich Veterinärmedizinsches Prüfungsinstitut, Berlin-Mitte, Germany, adult education, specialized training as laboratory assistant in biology, subject of the examination: *Comparison of the Bucuresti-VR2 bacterial strain from the vaccine production of different socialistic countries.* under the supervision of Dr. W. F. Schielow
1991 – 1997	University of Potsdam, Germany, faculty of Biology, department of cell biology, Bachelors Degree in Biology subject of the thesis: *Analysis of Filamin Isoforms in Skeletal Muscle.* under the supervision of Prof. Dr. D.O. Fürst
since 1998	Charité Berlin/ Max Delbrück Centre for Molecular Medicine, Berlin, Germany PhD student in the research team „Molecular Cell Biology of Hematopoietic Cells" (from 1998 to 2001, participation to the graduate program "Molecular basics of therapy") subject of the thesis: *Identification and Characterization of an Epo-independent Human Red Cell Progenitor – the E-cad$^+$ progenitor.* under the supervision of Prof. Dr. Martin Zenke

6.4 Publications

Articles:

Lemke B., Bartunek P., Hacker C., Koh K.R., Guilmette E.R., Neote K. and Zenke M. (2003) *The Moleculare Signature of Epo-independent Human Red Cell Progenitors*. Manuscript in preparation.

Koh K.R., Janz M., **Lemke B**., Stirling D., Dörken B., Zenke M. and Lentzsch S. (2004) *Immunomodulatory derivative of thalidomide IMiD CC-4047 inhibits red cell development concomitantly with down regulation of red cell transcription factors and modulation of cytokine secretion.* Manuscript in preparation.

Hacker C., Zagouras P., **Lemke B.**, Koh K., Brisette W., Guilmette E.R., Hambor J., Neote K. and Zenke M. (2003) *The Gene Expression Repertoir of Human Red Cells*. Manuscript in preparation.

van der Ven P.F.M, Oberman W.M.J, **Lemke B.**, Gautel M., Weber K. and Fürst D.O. (2000) *Characterization of Muscle Filamin Isoforms Suggests a Possible Role of –Filamin/ABP-L in Sarcomeric Z-Disc Formation*. Cell Motility and Cytoskeleton, 45: 149-162

Oral presentations:

Lemke B., Anzinger B., Bartunek P. and Zenke M.(1999) *Growth and Differentiation of Human Erythroid Progenitors in vitro*. Graduiertensymposium „Application of Molecular Methods for the Development of New Therapies“, 29.-31.03.1999, Gross Dölln, Germany

Lemke B., Anzinger B., Bartunek P. Hacker C., Ju X.-S.,and Zenke M.(2000) *Gene Expression in Red Blood Cell Progenitors*. Graduiertensymposium, 19.-21.09.2000, Gross Dölln, Germany

Poster presentations:

Lemke B., Anzinger B., Hacker C., Ju X.-S., and Zenke M.(2001) *Identification of an E-cadherin^{+}CD36^{+} Human Red Blood Cell Progenitor*. Keystone Symposium, Hematopoiesis, 3.-8.04.2001, Whistler, British Columbia, Canada

Lemke B., Anzinger B., Hacker C., Ju X.-S., and Zenke M.(2001) *Identification of an E-cadherin^{+}CD36^{+} Human Red Blood Cell Progenitor*. Gordon Research Conference, Red Cells, July.2001, Tilton School, New Hampshire

Lemke B., Anzinger B., Hacker C., Ju X.-S., and Zenke M.(2001) *Identification of an E-cadherin^{+}CD36^{+} Human Red Blood Cell Progenitor*. 7th MDC graduate Students' Symposium, 14.05.2001, Berlin; (Preis für Posterpräsentation)

Patent:

Zenke M., **Lemke B.**, Hacker C., Brisette W, Zagouras P., Neote K. *Therapeutics and Diagnostics for Disorders of Erythropoiesis.* application serial number 10/285,366, filed 10/31/2002/2002

6.5 Eidestattliche Erklärung

Hiermit erkläre ich, daß die vorliegende Promotionsarbeit an keiner anderen Hochschule eingereicht wurde, sowie von mir selbständig und ausschließlich mit den angegebenen Mitteln angefertigt worden ist.

Potsdam, 19.01.2004

Britt Lemke