In vivo MDS is mostly characterized by a normal or increased number of erythroblasts in the bone marrow. The defects in the erythroid development can be detected in a subpopulation of patients and is localized at CD34-/CD36- sorted cells with Epo and SCF demonstrated that an impaired proliferative response could be refractory anemia with excess of blasts or in transformation (RAEB, RAEB-1). Suspension cultures with anemia (RA) and refractory anemia with ringed sideroblasts (RARS) and in a minority of cases with disconnection in the process of proliferation and differentiation of the erythroid progenitors. CD34-/CD36 sorted bone marrow cells of myelodysplasia patients to differentiate along the erythroid lineage in the presence of erythropoietin (Epo) and stem cell factor (SCF) is analyzed. In normal erythropoiesis immature, but purely erythroid colonies are seen after 12-21 days of culture as large multicentered hemoglobinized colonies containing up to 10,000 cells, termed burst forming units-erythroid (BFU-E). In this study two subgroups of patients could be identified. In 43% of the patients a normal number of BFU-Es were cultured from CD34+/CD36- sorted cells. These cells had the capacity to differentiate to colony-forming units-erythroid (CFU-Es) progenitors in cell suspension cultures with Epo and SCF followed by Epo in the culture assay. The cells became CD34+/CD36-/Glycophorin A (GpA)+ after 7 days in culture, a pattern comparable to that of normal progenitors. In the second group of patients a different pattern was observed. No BFU-Es or low BFU-Es were cultured from the CD34+/CD36+ sorted cell fraction that was, in most of the cases, incapable to differentiate to CFU-E progenitors. Flow cytometric analysis showed the persistence of the CD34+/CD36+ expression in the majority of these cases after 7 days of suspension culture with Epo and SCF. Addition of interleukin-3 did not correct this defect. Furthermore, in 44% of the patients expression of EVI-1 was found at mRNA level. These results show that the defects in the erythroid development can be detected in a subpopulation of patients and is localized at an early stage of the erythroid differentiation. Additional studies have indicated that overexpression of EVI-1 affects the differentiation program of erythroid progenitors.

In chapter 2 the capacity of CD34+/CD36+ sorted bone marrow cells of myelodysplasia patients to differentiate along the erythroid lineage in the presence of erythropoietin (Epo) and stem cell factor (SCF) is analyzed. In normal erythropoiesis immature, but purely erythroid colonies are seen after 12-21 days of culture as large multicentered hemoglobinized colonies containing up to 10,000 cells, termed burst forming units-erythroid (BFU-E). In this study two subgroups of patients could be identified. In 43% of the patients a normal number of BFU-Es were cultured from CD34+/CD36- sorted cells. These cells had the capacity to differentiate to colony-forming units-erythroid (CFU-Es) progenitors in cell suspension cultures with Epo and SCF followed by Epo in the culture assay. The cells became CD34+/CD36+/Glycophorin A (GpA)+ after 7 days in culture, a pattern comparable to that of normal progenitors. In the second group of patients a different pattern was observed. No BFU-Es or low BFU-Es were cultured from the CD34+/CD36+ sorted cell fraction that was, in most of the cases, incapable to differentiate to CFU-E progenitors. Flow cytometric analysis showed the persistence of the CD34+/CD36+ expression in the majority of these cases after 7 days of suspension culture with Epo and SCF. Addition of interleukin-3 did not correct this defect. Furthermore, in 44% of the patients expression of EVI-1 was found at mRNA level. These results show that the defects in the erythroid development can be detected in a subpopulation of patients and is localized at an early stage of the erythroid differentiation. Additional studies have indicated that overexpression of EVI-1 affects the differentiation program of erythroid progenitors.

In vivo MDS is mostly characterized by a normal or increased number of erythroblasts in the bone marrow. In contrast to these findings is the in vitro finding of an impaired erythroid colony formation. In chapter 3 we analyzed whether these conflicting results from the in vivo and in vitro data might be due to a disconnection in the process of proliferation and differentiation of the erythroid progenitors. CD34+/CD36- sorted bone marrow cells from MDS patients were cultured in a clonogenic and suspension culture assay in the presence of Epo and SCF. BFU-Es and CFU-Es were observed in the majority of cases with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) and in a minority of cases with refractory anemia with excess of blasts or in transformation (RAEB, RAEB-T). Suspension cultures with CD34+/CD36- sorted cells with Epo and SCF demonstrated that an impaired proliferative response could be seen in cases with less than 10 BFU-E/10^5 cells, especially in the group of RAEB/RAEB-T patients. Furthermore, in nearly all cases differentiation was demonstrated along the erythroid lineage by FACS and cytology. In addition evidence was obtained that the differentiating cells belonged to the abnormal MDS clone. Finally, in the cases with an impaired proliferative response an enhanced binding of Annexin-V, which is expressed in cells undergoing programmed cell death, was found early in the cell suspension culture. These results suggest that a defect in erythroid colony formation and in vivo by possible to differentiate between iron taken up by the marrow and extramedullary formation of red cells (ineffective erythropoiesis). In chapter 4 the defects in the erythroid development and the factors that affect the erythroid colony formation in vivo and in vitro are reported. Inhibitory regulators of erythroid colony formation are discussed. In a minority of cases with high risk disease as well as with an increased ETU values was observed in 51% and 25% of cases with RA and RARS, respectively. ETU values were observed between RA and RAEB-T and diminished ETU-value, which correlated with the presence of cytogenetic abnormalities. These observations suggest that erythroid colony formation. Transfusion dependent anemia and cytogenetic abnormalities. These observations have been correlated with bone marrow markers of iron. In chapter 5 the ultrastructural character of ineffective erythropoiesis is analyzed. In normal erythropoiesis immature, but purely erythroid colonies are seen after 12-21 days of culture as large multicentered hemoglobinized colonies containing up to 10,000 cells, termed burst forming units-erythroid (BFU-E). In this study two subgroups of patients could be identified. In 43% of the patients a normal number of BFU-Es were cultured from CD34+/CD36- sorted cells. These cells had the capacity to differentiate to colony-forming units-erythroid (CFU-Es) progenitors in cell suspension cultures with Epo and SCF followed by Epo in the culture assay. The cells became CD34+/CD36+/Glycophorin A (GpA)+ after 7 days in culture, a pattern comparable to that of normal progenitors. In the second group of patients a different pattern was observed. No BFU-Es or low BFU-Es were cultured from the CD34+/CD36+ sorted cell fraction that was, in most of the cases, incapable to differentiate to CFU-E progenitors. Flow cytometric analysis showed the persistence of the CD34+/CD36+ expression in the majority of these cases after 7 days of suspension culture with Epo and SCF. Addition of interleukin-3 did not correct this defect. Furthermore, in 44% of the patients expression of EVI-1 was found at mRNA level. These results show that the defects in the erythroid development can be detected in a subpopulation of patients and is localized at an early stage of the erythroid differentiation. Additional studies have indicated that overexpression of EVI-1 affects the differentiation program of erythroid progenitors.

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In the second part of the study, the authors review the defects in the erythroid development of MDS patients. In chapter 3, the defects in erythropoietin (Epo) and stem cell factor (SCF) signalling are discussed. In the majority of cases, activated RAS is observed in the differentiating cells of the erythroblasts. CD34-CD36- sorted cells. These cells had the capacity to differentiate to CFU-E progenitors. Flow cytometry studies have indicated that overexpression of EVI1 is found at mRNA level. These results show that impaired erythroid colony formation and in vitro data might be due to a defect in the proliferation of the erythroid progenitors. CD34+/CD36+Glycophorin A (GpA)-normal progenitors. In the second group of patients a CD36-negative subset was observed in the majority of cases. This subset could differentiate to CFU-E progenitors. Flow cytometry studies have indicated that overexpression of EVI1 is found at mRNA level. These results show that in a subpopulation of patients and is localized at the developing stage. In chapter 5, the ultrastructural characteristics of erythroblasts in MDS are investigated for a better understanding of the underlying defects. By using electron microscopy, 77% of the cases demonstrated nuclear abnormalities consisting of disrupted membranes and cytoplasmic perinuclear spaces. The ultrastructural abnormalities of the nucleus may represent dysplasia by dysregulated conservation of the nucleus and impairment of DNA metabolism or nuclear formation during mitosis. In 32% of the patients, a low percentage of apoptosis (mean 3.1 ± 1.6% vs. < 0.5% in normal controls) in the erythroid lineage could be noted, primarily in mature erythroblasts and significantly associated with spongioblastic nuclear features. In all patients extensive cytoplasmic vacuolization and myelin figures were demonstrated. In 55% of the cases, enlarged and abnormal mitochondria were observed, significantly associated with iron-accumulation. An inverse relation existed between the absence of apoptosis and the more advanced stages of MDS including high risk MDS and cytogenetic abnormalities. Mitochondrial abnormalities were correlated with high risk disease as well as with an increase in transfusion dependency. These data suggest that in MDS apoptosis might play a role in the early stages of disease. The overall prominent defects in the mitochondrion of the erythroblasts might be an additional defect that is involved in the ineffective erythropoiesis. In MDS the precise mechanism of ineffective erythropoiesis is not fully elucidated, but conflicting results are reported regarding the extent of apoptosis in the early phase of the disease. In chapter 6, TdT-mediated
dUTP-nick end labeling (TUNEL) staining of parafin embedded bone marrow specimens were used to assess the number of apoptotic cells in MDS patients. In a subset of MDS patients the TUNEL assay was performed in combination with Glycophorin A (GpA) staining to determine the degree of apoptosis in the mature erythroid compartment. Cell cycle analysis in MDS has shown a large number of myeloid, erythroid and megakaryocytic cells synthesizing DNA. Cell proliferative activity was assessed by means of immunostaining of the cell proliferation associated nuclear antigen Ki-67. The mean percentage of positive staining cells for apoptosis (apoptotic index (AI)) in MDS patients was not increased. Moreover, no difference in mean AI was observed in the GpA compartment between MDS versus normal controls. In addition the different FAB-classifications and the different International Prognostic Scoring System (IPSS)-risk groups showed no differences between the subgroups. The expression of Ki-67, as marker for proliferative activity, in the GpA compartment from MDS patients did not differ from normal controls. These results show that apoptosis of erythroid progenitors cannot only be responsible for the ineffective erythropoiesis in MDS, but might be a characteristic of the cells when cultured in vitro. These findings correspond with the ultrastructural analysis in chapter 5 demonstrating that apoptosis of erythroblasts was limited to patients with RA and low risk MDS. Our findings suggest that the observed increased apoptosis in in vitro culture assays is related to the detachment of the cells from the microenvironment due to an increased susceptibility to apoptosis.

Synthesis and future perspectives

In normal hematopoiesis, the development of the erythroid lineage is dependent on Epo and the presence of the Epo receptor (EpoR). The expression of the EpoR correlates with the biological responsiveness being low on primitive erythroid precursors, high on proerythroblasts and is down regulated in the late basophilic erythroblast stage. With Epo deprivation erythroid progenitors undergo apoptosis which might be regulated by the expression of apoptotic proteins such as Bcl-X1 and Bcl-2. Uregulation of Bcl-X1 leads to a "default" terminal differentiation of apoptosis-protected, committed erythroblasts, independent of any exogenous signal. A second important mechanism for regulating erythropoiesis is the Fas/Fas-ligand (Fas-L) system on erythroblasts. In normal hematopoiesis, Fas is rapidly upregulated in early erythroblasts and expressed at high levels throughout the terminal differentiation. In contrast, Fas-L is selectively induced in early differentiating Fas-insensitive erythroblasts. Fas-L bearing mature erythroblasts display a Fas-based cytotoxicity against immature erythroblasts which is abrogated by high levels of Epo. In addition upregulation of the Fas/Fas-L pathway can be accomplished by tumor necrosis factor (TNF)-α and interferon-γ. Upregulation of Fas-L can also be achieved by high levels of stromal derived factor (SDF)-1α produced by bone marrow stromal cells. At low concentrations SDF-1α enhances CD34+ cell proliferation. Based on these results it is conceivable that the interaction of Fas and Fas-L, regulated by different acting cytokines, represents an apoptotic control mechanism for erythropoiesis in normal circumstances, contributing to the regulation of red blood cell homeostasis.

However, additional control mechanisms exist which control the extent of proliferation and differentiation of the erythroid progenitors. One is tumor necrosis factor-related apoptosis inducing ligand (TRAIL) produced by bone marrow mononuclear cell. A second control mechanism is the receptor:ligand pair TRAIL-receptor-2 (TRAIL-R2) expressed on mature red blood cells. Epo deprivation and TRAIL-R2 expression were found to increase in MDS. Therefore, the imbalance of TRAIL and TRAIL-R2 might be subject for further research in understanding the pathological process. Furthermore, normal erythroid differentiation is regulated by other cytokines such as TNF-α, interferon-γ and stromal derived factor (SDF)-1, which are produced by bone marrow stromal cells. The interaction between macrophages and erythroblasts in the bone marrow microenvironment may also be involved in the process of apoptosis.

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In this process, the imbalance in programs of apoptosis and proliferation might be subject for further research in understanding the pathological process. Furthermore, normal erythroid differentiation is regulated by other cytokines such as TNF-α, interferon-γ and stromal derived factor (SDF)-1, which are produced by bone marrow stromal cells. The interaction between macrophages and erythroblasts in the bone marrow microenvironment may also be involved in the process of apoptosis.
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a subset of MDS patients the TUNEL assay was
aiming to determine the degree of apoptosis in the
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proliferative activity was assessed by means of
antigen Ki-67. The mean percentage of positive
MDS patients was not increased. Moreover, no
difference between MDS versus normal controls. In
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produced by bone marrow mononuclear cells that acts as a negative regulator of normal erythropoiesis.6 A
second control mechanism is the receptor-binding cancer antigen expressed on alveolar cells (RCAS1).7
RCAS1-receptor (RCAS1R) is expressed on erythroid colony-forming cells and upon binding it induces
apoptosis, including collapse of the mitochondrial membrane potential and activation of caspase 8 and
3. The degree of RCAS1 binding diminishes during erythroid maturation. Furthermore, RCAS1 is found in
the cytoplasm of bone marrow macrophages. This pathway may contribute to the interaction of
macrophages and erythroblasts in the bone marrow erythroid lobules. It is well defined that the
interaction between macrophages and developing erythroblasts promotes terminal maturation and
enucleation of maturing erythroid cells.8,9

Furthermore, normal erythroid differentiation requires the transient activation of several caspases.10
Caspase-6, which cleaves lamin B, is activated by caspase-3. The nuclear protein acinus, implicated in
chromatin condensation, is cleaved by caspase-3. Cleavage of these proteins may account for the nuclear
structural changes associated with maturation of erythroblasts. The reduction in mitochondrial
transmembrane potential leads to activation of caspase-9/3, effectors of the apoptotic machinery, but also
as caspase-3 affects cytoskeleton formation by binding to F-actin. In MDS mitochrondrial disruptions are
found with increasing incidence in advancing risk MDS. Upon Epo deprivation or Fas activation, the
transient caspase activity is amplified via either the mitochondrial or the caspase-9 or caspase-8 pathway
leading to apoptosis. Overexpression of Fas/Fas-L, as seen in 50% of the MDS patients, may interact with
this process. The imbalance in programs deployed during terminal maturation leading to enucleation,
might be subject for further research in understanding ineffective erythropoiesis in MDS. It is conceivable
that aggravation of mitochondrial disruptions in advanced stage of MDS may lead to increased levels of
uncleaved caspases, which might be of relevance in the hematopoietic control as seen in acute
myelogenous leukemia.11 Recently Claessens et al. reported a 2-step liquid culture assay with MDS CD34+
cells. In the presence of Epo, stem cell factor, insulin like growth factor and steroid hormones it was
possible to enhance erythroid proliferation whereas terminal erythroid differentiation was obtained with a
medium containing Epo and insulin.12 It is of interest to define these stimuli in the process of proliferation
and differentiation further in order to be able to define the stimuli present in in vivo conditions.


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