# 7 Summary

In the present study the remodelling of iron metabolism during megakaryocytic differentiation of K562 cells and some aspects of regulation of iron homeostasis in proliferating K562 cells were characterized. 48 hours of iron overload with 100 µM ferric ammonium citrate (FAC) provided evidence that under these conditions iron regulates steady-state levels of mRNAs of transferrin receptor-1 (TfR1), ferritin heavy chain (H-FT) and ferroportin (Fpn1), which correspond to iron-mediated posttranscriptional feed back mechanism of regulation of iron responsive element (IRE)-containing transcripts. Unchanged steady-state levels of transcripts of ferritin light chain (L-FT) in K562 cells were observed after 48 hours iron overload with 100 µM FAC or after 120 hours cell culture in the presence of 4 mg/ml ferri-transferrin. This result implicates that during proliferation of K562 cells, iron is predominantly incorporated into H-FT-rich ferritin-nanocages. Expression of Fpn1 mRNAs was constant after 120 hours incubation of K562 cells with iron-saturated human transferrin, indicating that just high increase of the labile iron pool (LIP) leads to iron responsive element / iron regulatory protein (IRE / IRP)-dependent regulation of these transcripts. In opposite, high iron concentrations were not able to alternate the expression of haemochromatosis protein (HFE).

K562 cells, which overexpressed the pre-pro-hepcidin cDNA, showed unaffected steady-state levels of TfR1, H-FT, L-FT or Fpn1 transcripts, implicating unchanged cellular iron metabolism in these cells. Overexpression of pre-pro-hepcidin cDNA in K562 cells resulted in reduction of expression of endogenous pre-pro-hepcidin transcripts, which suggests an autocrine regulation of hepcidin expression in erythroid cells. 72 hours of phorbol ester (PMA)-mediated megakaryocytic differentiation of K562 cells resulted in strong reduction of hepcidin-mRNA expression.

Megakaryocytic differentiation was accompanied not only by increased expression of mega-karyocytic marker CD61, diminished expression of erythroid marker γ-globin and increased acetylcholinesterase activity, but also by reorganisation of cellular iron metabolism. The haemoglobin content, which represents a part of erythroid characteristics, was alternated in differentiating cells similar to the cellular iron content. Increase of total cellular iron was caused by block of iron release, which correlated with strong reduction of expression of Fpn1 transcripts. The increase of total iron levels was accompanied by the increase of total

haemoglobin content, indicating not only increase of haemoglobin synthesis, but also incorporation of accumulated iron into haemoglobin molecules after 72 hours of PMA-treatment of K562 cells. This was possible, because in differentiated cells iron was able to restore diminished γ-globin expression and to induce in addition the expression of ferrochelatase (FECH) transcripts. The increased rate of protein synthesis and enlargement of the cytosolic compartment due to the differentiation process resulted under low iron conditions in loss of increase of the iron-to-protein ratio. Increase of iron availability (FAC in cell culture medium) restored low haemoglobin-to-protein ratios in differentiated cells to levels observed in proliferating cells, providing evidence that loss of erythroid characteristics during megakaryocytic differentiation represents an iron-dependent process. The down-regulation of heavy ferritin mRNA in differentiating cells was also iron-dependent and for that reason reflects the reduction of LIP during megakaryocytic differentiation of K562 cells. Transkriptional up-regulation of light ferritin mRNA and the resulting decrease in the H- to L-ferritin ratio during this process suggest changes of the ferritin molecule structure.

During *in vitro* megakaryopoiesis of K562 cells reduction of transferrin-dependent iron uptake due to iron-dependent transcriptional down-regulation of TfR1, down-regulation of TfR2 (transferrin receptor-2) and modulated up-regulation of HFE were also observed. In agreement with these results, reduced levels of isoform 1B of divalent metal transporter-1 (DMT1B) were detected at RNA level. Other processes, which could contribute to the diminished presence of TfR1 in cell membranes of K562 cells, such as shedding of TfR1 or sorting of TfR1 into exosomes, were not affected by the increase of protein kinase C activity during PMA-dependent megakaryocytic development. Down-regulation of isoform 1A of DMT1, which participates in non-transferrin-dependent iron uptake, correlates with diminished expression of other above mentioned iron transport proteins, indicating reduction of iron transport between megakaryocytic cells and their environment. This implicates that iron stored in these cells in ferritin molecules represents a main source for metabolic pathways.