Institutionen för medicin, Enheten för hematologi

Molecular pathogenesis of refractory anemia with ring sideroblasts (RARS): Role of the mitochondrial iron transporter gene ABCB7

AKADEMISK AVHANDLING

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Refractory anemia with ring sideroblasts (RARS) is characterized by anemia, erythroid apoptosis, and mitochondrial ferritin (FTMT) accumulation. Granulocyte-colony-stimulating factor (G-CSF) inhibits some of these features in vitro and in vivo and can in combination with erythropoietin normalize hemoglobin levels. The focus for this thesis was to investigate ABCB7 gene expression levels and mutational status in CD34+ cells and erythroblasts from MDS patients in order to understand mechanisms underlying the pathogenesis of RARS as well as the anti-apoptotic effects of G-CSF. Furthermore, we wanted to test the hypothesis that ABCB7 is a key mediator of aberrant iron accumulation in acquired RARS.

To dissect these mechanisms, the CD34+ compartment of RARS bone marrow, as well as erythroblasts derived from CD34+ cells in an erythroblast culture system were subjected to gene expression analysis (GEP). Erythroblasts were also analyzed after incubation with G-CSF. The mutational and DNA methylation status of ABCB7 and other key down-regulated genes was assessed. To study the ABCB7 role in aberrant iron accumulation in RARS erythroblasts, we modulated the expression of ABCB7 in several cellular systems.

ABCB7 is not mutated in RARS. However, CD34+ ABCB7 expression level was significantly lower compared to other MDS subtypes. Furthermore, there was a significant inverse relation between ABCB7 expression and the percentage of ring sideroblasts. In contrast to normal bone marrow, ABCB7 expression decreased during erythroid differentiation of RARS CD34+ cells. Other down-regulated key genes included MFN2, STAT5B, FANCC and the negative apoptosis regulator MAP3K7. Neither ABCB7, nor other down-regulated key genes in RARS showed hypermethylation. Several genes involved in erythropoiesis were significantly over-expressed in RARS CD34+ cells but showed normal or decreased expression in differentiating erythroblasts. Deregulated pathways in RARS erythroblasts included apoptosis and mitochondrial function.

Interestingly, the mitochondrial pathway including MFN2 was significantly modified by G-CSF, and several heat shock protein genes were up-regulated, as evidence of anti-apoptotic protection of erythropoiesis. However, G-CSF had no effect on the expression of iron-transport or erythropoiesis-associated genes.

ABCB7 down-regulation led to marked up-regulation of FTMT in K562 cells, while inhibiting growth and erythroid differentiation. In normal bone marrow, ABCB7 silencing reduced erythroid colony growth, and induced erythroid apoptosis and a gene expression pattern similar to that observed in RARS day 7 erythroblasts. Importantly, down-regulation led to the accumulation of mitochondrial iron, in the form of FTMT. ABCB7 up-regulation potentiated erythroid differentiation in K562 cells, and restored erythroid colony growth and decreased FTMT expression level in RARS CD34+ BM cells. Mutations in the SF3B1 gene, a core component of the RNA splicing machinery, were recently identified in a high proportion of patients with RARS. Of the nine RARS patients included in our study, 7 carried SF3B1 mutations. Interestingly, SF3B1 silencing resulted in down-regulation of ABCB7.

Our findings support an essential role of ABCB7 in the phenotype of acquired RARS and suggest a relation between SF3B1 mutations and ABCB7 down-regulation that warrants further investigation.