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

AKADEMISK AVHANDLING

För avläggande av medicine doktorsexamen vid Karolinska Institutet, som offentligen försvaras i Birke 1, Karolinska Universitetssjukhuset, Huddinge

Fredagen den 9 September, 2011, kl 10.00

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Stockholm 2011

ABSTRACT

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.

ISBN 978-91-7457-443-2