Abstract

Myelodysplastic syndrome (MDS) with deletion of the long arm of the chromosome 5 (5q- syndrome, del(5q)) can be characterized by anemia, macrocytosis, a normal or high platelet count, and hypolobulated megakaryocytes in the bone marrow. 5q- syndrome belongs to low-risk MDS, which means low risk to transform to acute myeloid leukemia. 5q- syndrome is associated with female predominance and older age. Another sign is transfusion burden that is treated by erythropoiesis-stimulating agents (ESA) as erythropoietin (EPO). Moreover, the response of MDS patients is around 30-60% with the median of the response being ~24 months. The second line of treatment is lenalidomide (LEN) which is a derivate of teratogenic analog thalidomide. LEN increases erythropoiesis and inhibits the growth of del(5q) erythroid progenitors in vivo and it does not have a significant effect on the growth of normal CD34+ progenitors or cytogenetically normal progenitors in MDS with del(5q) clones.

LEN is used as therapy in multiple myeloma, myelodysplastic syndrome, and lymphoma. LEN is an expensive agent and not every MDS patient responds to this therapy. This is a reason why is a need to find a biomarker for the determination of successful treatment. Some multiple myeloma studies showed that cereblon can be the biomarker that is needed. Cereblon (CRBN) is part of the E3 ubiquitin ligase CRL4 complex (cullin 4-RING E3 ubiquitin ligase complex), containing cullin-4A (CUL4A), damaged DNA binding protein (DDB1), and regulator of cullins 1 (ROC1). The function of CRBN in the CRL4 complex as a substrate receptor is to attach proteins, determined for polyubiquitination and subsequent degradation in 26S proteasome. This E3 ubiquitin ligase $CRL4^{CRBN}$ ubiquitinates CRBN, DDB1, and some endogenous proteins in the absence of lenalidomide. LEN binds to a specific hydrophobic pocket in the exons 10 and 11 of CRBN and changes the specificity of the $CRL4^{CRBN}$. In multiple myeloma, CRBN targets Ikaros and Aiolos proteins for ubiquitination, and in myelodysplastic syndrome is the target casein kinase 1A1.

In studies in multiple myeloma was proven that high CRBN expression leads to a good response to LEN therapy and improved patients´ life. The lower CRBN expression leads to the failure of therapy and progresses disease to high-risk MDS (MDS-EB-1 or MDS-EB-2) or acute myeloid leukemia. Furthermore, some patients stop responding to LEN therapy, and in these cases is added EPO or EPO and prednisone (PRED) that show temporally the effect of therapy.

In my thesis, we mainly focused on confirmation CRBN as the prognostic factor in human MDS samples at mRNA and protein levels. In the first part of the study, we verified that the high level of CRBN mRNA and protein CRBN in mononuclear cells isolated from the

peripheral blood and bone marrow is associated with good response, and the lower level of CRBN mRNA and protein CRBN means the failure of LEN therapy or its combination (LEN+EPO or LEN+EPO+PRED). We validated our results with doctors and with the level of hemoglobin. We found out that CRBN protein in 5q- syndrome patients predict a quicker response on LEN therapy than CRBN mRNA. We confirmed that LEN binds to CRBN at exon 10 and 11. It was important to confirm the binding site of LEN because if exons 10 and 11 are missing, LEN cannot be bound and it also means the failure of treatment. Sardnal and his colleagues reported that A/G polymorphism has a prognostic signature in LEN treatment in MDS patients with normal karyotype. Unfortunately, we did not confirm this statement.

In the second part of this thesis, we focused on the role of nuclear factor erythroid-derived 2-like 2 (Nrf2). Nrf2 binds to a putative binding site in CRBN promoter and Nrf2 stimulates CRBN gene transcription under hypoxia/ reoxygenation conditions in neuronal cells. We confirm our assumptions that Nrf2 expression follows or overtakes the level of CRBN mRNA in the majority of MDS samples on EPO or LEN+EPO therapy. The level of Nrf2 mRNA did not follow CRBN mRNA in causes who failure LEN+EPO therapy.

The third part was experimental on MDS-L and SKM-1 cell lines when we monitored the effectiveness of arsenic trioxide (ATO). According to publications, ATO is effective with other agents. We made 19 groups, where we followed LEN, EPO, PRED, and ATO alone or its combination. Moreover, our result did not correspond at nucleotide and protein levels.