Association of TXNDC5 and calreticulin with decreased ER stress, allowing resistant multiple myeloma cells to bypass proteasome inhibition

Multiple myeloma is a lymphoid malignancy affecting around 5.5/100 000 people in Europe, mainly the elderly patients - median age at onset is 72 years old. Multiple myeloma is derived from terminally differentiated lymphocytes B that, in most of the cases, produce characteristic monoclonal protein, which is either a complete immunoglobulin or a free light chain of an immunoglobulin. Symptoms of the disease are caused by malignant cells hyperplasia in bone marrow and by high serum concentration of the monoclonal protein and consist mainly of: lytic bone lesions that causes pathological fractures, anemia, immunodeficiency and renal failure.

There has been a huge breakthrough in multiple myeloma therapy in the last 15 years. It is associated with the introduction of many novel drugs, containing as different classes of medications as proteasome inhibitors, immunomodulatory agents, histone deacetylase inhibitors or monoclonal antibodies. The advances in the therapy allowed to prolong patients median overall survival time, counting from the moment of diagnosis, from previous 3 to current 6 years. Unfortunately, multiple myeloma remains incurable, despite the fact that most of the patients respond to the first line treatment, implemented after diagnosis of symptomatic disease, with a significant decrease in malignant cells burden and achieve state of remission. However, after different periods of time, attributed to the disease's heterogeneity, an inevitable relapse occurs. After several lines of treatment, the relapsed disease becomes refractory to all available drugs and patient dies from an active disease. It is the main reason why studies on acquired drug-resistance appear to be crucial in terms of further progress of the myeloma therapy. Despite many efforts, we still lack a widely accepted description of this process that could lead us to breaking the resistance.

One of the most important class of the new drugs, present in almost every therapeutic regimen, are proteasome inhibitors - bortezomib and carfilzomib. Proteasome is an enzymatic aggregate responsible for cleavage of specifically tagged proteins previously directed to degradation pathway. Drug-induced proteasome inhibition causes accumulation of the defective proteins, leading to the induction of apoptotic mechanisms and finally death of a malignant cell. According to one of the most promising theories describing the acquired resistance to proteasome inhibitors, resistant cells become independent from the proteasome-based protein degradation pathway. It is characterized by lower activity of unfolded protein response (UPR) pathway, activated in response to accumulation of misfolded proteins causing endoplasmic reticulum (ER) stress. This mechanism, although primarily playing a pro-survival role, after crossing the threshold of adaptive abilities is responsible for induction of cellular apoptosis. It is therefore an important way by which the proteasome inhibitors destroy myeloma cells.

In this project, mechanisms responsible for lowering the activity of the UPR pathway will be investigated. We assume that this effect is a consequence of increasing the effectiveness of processes involved in appropriate proteins folding in the endoplasmic reticulum. Basing on previous publications and results of our own studies we have chosen proteins which increased activity may facilitate this phenomenon. First, TXNDC5 (*Thioredoxin domain-containing protein 5)*, is a particle from protein disulfide isomerases group, playing important role in processes of protein folding and involved in maintaining appropriate redox conditions. The second one is calreticulin, member of produced-proteins quality-control system, responsible for calcium homeostasis, which is essential for protein folding in the endoplasmic reticulum.

In the first part of the project, research will be performed on multiple myeloma cell lines, resistant to bortezomib and carfilzomib. Their aim will be to prove impact of TXNDC5 and calreticulin on decreasing ER stress and as a consequence, on proteasome inhibitors resistance. Subsequently, the obtained results will be validated on clinical samples from multiple myeloma patients. If the assumptions are correct, plasma cells derived from the proteasome inhibitors-resistant patients will be characterized by higher concentration of TXNDC5 and calreticulin than proteasome inhibitors-sensitive patients' plasma cells.

The results of this project allow to extend our understanding of mechanisms responsible for lowering the UPR pathway activity causing acquired proteasome inhibitors resistance. This will lead to defining new therapeutical targets that, in future, could help to break the process of acquiring resistance and improve treatment's efficiency. Moreover, analysis of the patients samples will allow to use TXNDC5 and calreticulin concentration as potential predictor markers of response to treatment with bortezomib or carfilzomib.