Insights into substrate specificity of the noncanonical cytoplasmic poly(A) polymerase TENT5C in B lymphocytes

Each mRNA molecule, constituting a template for protein synthesis, undergoes specific processing steps in order to become a mature molecule. One of them is polyadenylation - the process of addition of adenine nucleotides, which are not encoded in DNA to the 3' end of mRNA, the so-called " poly(A) tail". This process is performed by enzymes - canonical poly(A) polymerases. However, in cells, there is also a process of cytoplasmic noncanonical polyadenylation, which consists of elongating poly(A) tails by noncanonical poly(A) polymerases.

One such cytoplasmic poly(A) polymerase is a newly identified TENT5C protein. The gene encoding this protein is one of the most frequently mutated genes in multiple myeloma patients - an incurable cancer of plasma cells, i.e. terminally differentiated B cells producing antibodies. Our first studies have shown that TENT5C inhibits the development of this cancer's cells and this effect is dependent on its enzymatic activity. Therefore, in order to better understand the role of TENT5C, we wanted to investigate the exact mechanism of its action in the B cells model. So far, we have shown that TENT5C extends the poly(A) tails of immunoglobulin encoding mRNAs, elongating the life-time of these mRNAs and increasing efficiency of immunoglobulins production. Moreover, mice without this gene show a weakened immune response to the introduction to their organisms, the so-called antigen, a molecule that induces a reaction of the immune system. Additionally, TENT5C influences the rate of B cell division and differentiation to plasma cells. Overall, these results indicate the important role of TENT5C in immunity.

However, many questions about the mechanism determining how TENT5C works in B cells remain open. How does TENT5C choose which mRNA to polyadenylate? Does it interact only with its substrates or does it bind to RNA molecules in an unspecific way, carrying out polyadenylation of only selected ones? What is the role of TENT5C cellular localization and how does the loss of activity affect the location of this protein? The aim of this project is to find answers to these questions.

To achieve this, we will use a new model of a transgenic mouse expressing the catalytically inactive (thus unable to perform polyadenylation) TENT5C protein in fusion with a GFP (green fluorescence protein) tag. We will perform advanced molecular analyses using two alternative approaches to isolate the RNAs with which TENT5C interacts and then identify them using RNA sequencing. We will also use fluorescence microscopy to check how the cellular localization of the TENT5C protein differs in cells expressing an active or inactive form of this protein.

We expect that this project will provide answers to the outlined questions about the noncanonical poly(A) polymerase TENT5C mechanism of action. We will confirm the interaction of TENT5C with the mRNAs, which it polyadenylates, find out whether it interacts specifically with its substrates, and perhaps identify new, previously unknown substrates. We will also verify the role of the TENT5C cellular location. By deciphering these issues, we will get closer to an accurate understanding of the mechanism of action and the role of TENT5C in B lymphocytes.