CHARACTERIZATION OF PHOSPHODIESTERASE 6 AS A CANCER-RETINA ANTIGEN

Vojtěch TAMBOR

Abstract

The human phosphodiesterase 6, a cGMP specific enzyme, is physiologically expressed exclusively in the human retina and plays a key role in the phototransduction pathway. It was however recently discovered, that along with other proteins involved in the visual cycle, the rod phosphodiesterase 6α subunit is being expressed also in melanoma cells. This new group of proteins, which are expressed solely in retina and tumor cells and are capable of inducing antibody production in the patients sera, is called **cancer-retina antigens**.

The aim of my work was to investigate the rod phosphodiesterase 6β subunit (PDE6β) expression in melanoma tissues and cell lines with the aim to determine the photoreceptor holoenzyme PDE6 as a cancer-retina antigen. The goals of the work were the following:

1. Investigate the rod PDE6β subunit expression in melanoma cell lines and tissues, and in normal tissue both at mRNA and protein level.
2. Generate specific polyclonal rabbit antibodies against PDE6β.
3. Investigate the effects of PDE inhibitors and PDE6β antibodies on melanoma cells *in vitro*.

For this, standard molecular-biological methods were used. The mRNA expression was investigated using PCR, the protein production was examined with western blot analysis. Basic cell culture procedures were used for the PDE inhibitor assays and MTT test was used to evaluate the effect of these substances on the melanoma cells. During the work, also ELISA, RNA isolation and cDNA production, gel extraction, sequencing and *in silico* methods were used.

As for the results, the expression analysis revealed a tumor specific mRNA splicing variant of the rod PDE6β. Also, a full sized rod PDE6β protein was found to be expressed in 77% of the cell lines. Further research is carried out to explain this discrepancy and to get the full picture of this phenomenon.

The generation of the polyclonal rabbit antibody against rod PDE6β failed, most likely because a low immunogenity of the peptides used for immunization.

The PDE inhibitors surprisingly showed a stimulation of the cell proliferation. This effect is accompanied with cGMP level drop in the cells.

To fully understand all the factors involved to explain the above described results into detail, further research is being conducted. This work opens a new perspective for investigation of either the new tumor splicing variants of PDE6 mRNA, as well as the proliferative effect of the PDE inhibitors and its mechanism.