Abstract

This thesis focuses on the role of G protein coupled receptors (GPCRs) in the regulation of the DNA damage response (DDR). DNA is constantly exposed to various factors that can damage it, and effective repair of this damage is crucial for maintaining cellular integrity and preventing mutations that can lead to cancer. GPCRs are a large family of cell surface receptors that regulate a variety of physiological processes through G protein-mediated signaling. This work investigates how activation of the TRH receptor (TRH-R), a member of the GPCR family, can affect the DNA repair process and the potential mechanisms by which these receptors can modulate DDR.

In the practical part of this thesis, we focused on the complexes formed by the 53bp1 protein with its binding partners (mainly MDC1 and NBS1) upon activation of TRH receptors and reduced amounts of β-arrestin 2. For this goal, it was necessary to optimize the procedures of fractionation and immunoprecipitation and to verify the efficiency of transfection using siRNA against β-arrestin 2. We achieved the set goals by using various biochemical and molecular biological methods. During fractionation, the detection of proteins using 1D-electrophoresis and immunoblotting served us as a check for the efficient division of cell compartments. Protein-protein interactions were monitored using methods such as co-immunoprecipitation and immunoblot.

In this work, we successfully obtained the nuclear fraction from GH1 cells, optimized the immunoprecipitation conditions and also verified the transfection efficiency using siRNA against β-arrestin 2. The interactions of 53bp1 with MDC1 and NBS1 and the interactions between MDC1 and NBS1 were identified, suggesting that the MDC1 protein forms two separate complexes with 53bp1 and NBS. An increased immunosignal was detected upon TRH treatment in both control and transfected cells. A similar effects was observed after treatment with taltirelin only in control cells. Thus, TRH receptor activation under certain conditions could play a role in the DNA damage response. However, a change in H2AX phosphorylation after treatment with taltirelin was not observed. Future research could focus on observing the effect of TRH receptor ligands at different cellular amounts of β-arrestin 2 on H2AX phosphorylation. For the detection of 53bp1 protein complexes, the immunofluorescence method could be used to confirm the obtained results. These new findings could clarify whether TRH receptor activation can influence the DNA damage response and repair.

Keywords

GPCR, TRH, β-arrestin 2, DNA damage