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Keywords: Cannabinoid Receptor CB1, Protein Biosynthesis, Plasmid, Leptin

Aims: Endocannabinoids (ECs) enhance food intake via pleiotropic actions of the CB1Rs, whereas leptin, on the other hand, decreases EC levels in the hypothalamus and this effect contributes to leptin's anorexigenic actions. We hypothesized that leptin not only reduces EC levels but also interferes with CB1R signaling. Therefore, we aimed to study leptin's effect on CB1R signaling in cells expressing recombinant receptors.

Methods: Human embryonic kidney cells (HEK293T) were used. Expression of CB1R and leptin receptor (LepR) along with ERK phosphorylation was measured by western blot.

Results: Transfecting HEK293T cells simultaneously with LepR and CB1R construct resulted in drastically reduced LepR expression as compared to LepR transfection alone. We presumed that this reduction of LepR expression was due to the overload of the cell's protein synthesis system by large amounts of recombinant CB1R. In order to overcome this hindrance, we replaced the cytomegalovirus (CMV) promoter with the human herpes virus thymidine kinase promoter (TK) within our CB1R construct. The expression of the TK-CB1R was reduced approximately by two orders of magnitude when using the same amount of plasmid DNA. However, reduced receptor expression did not interfere with signaling efficiency as CB1R agonist – ERK phosphorylation dose response curves were virtually identical with both CMV-CB1R and TK-CB1R constructs. (Nonetheless, TK-CB1Rs attenuated LepR expression to the same extent as CMV-CB1Rs.)

Conclusions: Our results indicate that reduced CB1R expression driven by weak promoters may be sufficient to saturate the cell's endogenous signal transduction machinery and such may be beneficial when examining receptor function.

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F/4 INVESTIGATION OF THE ANTI-TUMOR EFFECTS OF 19 NOVEL TAMOXIFEN DERIVATIVES ON VARIOUS NEOPLASTIC CELL LINES

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Keywords: tamoxifen, cytotoxicity, impedimetry, cell cycle, apoptosis

Aims: The selective estrogen receptor modulator tamoxifen is a well-known anti-tumor compound, which can modulate multiple cellular physiological pathways. Previous investigations of the drug documented, that structural modifications of the parent molecule may result significantly increased activity in different models.

Methods: Tamoxifen and 19 analogues were investigated in 5 tumor cell lines (PANC-1, COLO205, A2048, EBC-1, MCF7). The antiproliferative effects (IC50) were evaluated by impedimetry (xCELLigence SP) and colorimetric alamarBlue assay. Effects on the cell cycle elicited by the analogues were observed with flow cytometry after propidium iodide staining.

Results: Tamoxifen performed antitumor effects in each cell line (IC50: PANC-1 – 33.7 uM, Colo205 – 12.0 uM, A2058 – 13.5 uM, EBC-1 – 8.2 uM). Incorporation of ferrocene as well as halogenation resulted significantly increased anti-tumor effect in PANC-1 (IC50: T15 – 15.0 uM, T6 – 29.6 uM) and in COLO205 (IC50: T15 – 2.5 uM, T6 – 9.9 uM) cells. Halogenation could increase the anti-tumor effects even in the MCF-7 cells. Cell cycle analysis confirmed that presence of ferrocene residue (subG1: 40.7%) can enhance the basic apoptotic character of

tamoxifen (subG1: 7.9%), while the halogenated derivate resulted G2/M phase arrest in PANC-1 cells.
Conclusions: Modification of the tamoxifen parent molecule with ferrocene or halogene residue results potentially increased anti-tumor character. The observed effect could be the result of the induction of apoptosis or a cell cycle arrest.

F/5 INVESTIGATING THE ROLE OF PHOSPHOLIPASE C γ 2 IN AUTOANTIBODY-INDUCED DERMATITIS

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Keywords: autoimmune, antibodies, inflammation, skin, phospholipase

Aims: Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering skin disease characterized by autoantibody formation against collagen type VII (C7). Phospholipase C γ 2 (PLC γ 2) is an important member of the immune receptor signaling pathway. We aimed to find and optimize a proper method for the isolation of anti-C7 antibodies, then study the role of PLC γ 2 in the pathogenesis of EBA-like dermatitis model. Methods: Rabbits were immunized with GST-C7 fusion protein. We tried two methods to isolate anti-C7 antibodies from its blood-serum, Melon Gel and Protein G based affinity chromatography. The efficiency of these methods was controlled by gel electrophoresis. Next we injected the antibodies into wild type and PLC γ 2 deficient (PLC γ 2 $^{-/-}$) bone marrow chimeras. We observed the size of affected skin area and the severity of the symptoms every second day until 14 days. We made ear histological sections.
Results: We could isolate nearly equal amount of protein with the two methods, but we found more contamination in the samples, when we used Melon Gel. In the dermatitis model, wild type mice developed skin lesions, upon anti-C7 antibody treatment. Inflammatory infiltration was observed in histological samples. On the other hand PLC γ 2 $^{-/-}$ animals remained protected from the disease, we couldn't find any differences between this and the control group.

Conclusions: Protein G based antibody purification method appeared to be more capable of the isolation of anti-C7 antibody than the Melon Gel in respect of efficiency and purity. We found that the PLC γ 2 has a crucial role in the development of autoimmune dermatitis model.

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F/6 GENETIC BACKGROUND INFLUENCE THE PROGRESSION OF RENAL FIBROSIS IN TGF-BETA TRANSGENIC MICE

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