

ARCHIVES OF THE HUNGARIAN MEDICAL ASSOCIATION OF AMERICA



The official publication of the Hungarian Medical Association of America, Inc.

melanoma

cells.

Methods: We used A2058 (derived from metastatic melanoma) and WM35 (established from in situ melanoma) melanoma cell lines in addition to epidermal melanocytes (primary cell cultures isolated from human skin samples) in our experiments. Based on proteomic analysis of cell surface proteins from our pigment cell cultures, we have selected three proteins for further examination: basigin (CD147) was identified in melanoma cell lines only, while tyrosinase related protein 1 (TYRP1) and gasdermin (GSDM) were present in melanocyte samples only. Firstly, we separated plasma membrane fractions of the cells and we analysed protein expressions. After that we performed immunocytochemistry reactions followed by confocal microscopic evaluation.

Results: Western blots confirmed our proteomic findings in terms of basigin and TYRP1, but GSDM was detected in all three cell cultures. While TYRP1 immunofluorescence corroborated the western blots, but surprisingly, signals for basigin were detected in all three cell lines, although the signals were increasingly stronger along with the degree of malignancy. GSDM immunopositivity was also examined in all three cultures but the expression was found to be weaker along with the degree of malignancy.

Conclusions: The performed experiments were essential for the verification of our proteomic findings. TYRP1 may be used in separating benign and malignant lesions, while the expression levels of basigin and GSDM may give us information on progression. Our results identified new targets that may serve as potential biomarkers in melanoma treatment.

INVESTIGATION OF THE ANTITUMOR EFFECTS OF TAMOXIFEN AND ITS FERROCENE-LINKED DERIVATE ON PANCREATIC AND BREAST CANCER CELL LINES

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Keywords: tamoxifen, drug development, GPER1, reactive oxygen species, cyt

Aims: Tamoxifen is a long-known antitumor drug, that belongs to the family of selective estrogen receptor modulators. It can alter several non-estrogen receptor-dependent cell physiological pathways (e.g.: angiogenesis, apoptosis, PKC inhibition). According to previous studies, the conjugation of the original tamoxifen molecule with different compounds can significantly improve its antitumor effect.

Methods: Tamoxifen and its ferrocene-linked derivate were tested on three cell lines: PANC1 (pancreatic adenocarcinoma), MCF7 (ER+ breast adenocarcinoma) and MDA-MB231 (ER- breast adenocarcinoma). The estrogen receptor expression profile was described with indirect immunocytochemistry and the Zeiss CellDiscoverer 7 platform. Protein expression changes were measured with Proteome Profiler Human XL Oncology Array kit. Production of reactive oxygen species was assessed with the ROSGlo assay.

Results: PANC1 cells were confirmed to express G-protein coupled estrogen receptor 1 (GPER1). Measurement of the protein expression profile revealed a change in the levels of several groups of proteins with different cell physiological functions (e.g. apoptosis, chemotaxis, cell growth, angiogenesis, metastasis development). In PANC1 cells elevated expression levels of tumorigenesis promoting proteins were observed upon treatment with tamoxifen and its derivate. In MCF7 and MDA-MB231 cells, the expression of these factors was elevated by tamoxifen, yet downregulated by its ferrocene-linked derivate. Assessment of the oxidative stress showed, that

the ferrocene-linked tamoxifen derivate significantly increases ROS production in a concentration-dependent manner.

Conclusions: According to previous studies, both tamoxifen and its ferrocene-linked derivate were proved to be cytotoxic on the studied cell lines. This effect is either the result of estrogen receptor inhibition, or estrogen receptor-independent mechanisms, such as the elevated oxidative stress. Tamoxifen is a known agonist of GPER1, a receptor that can promote the expression of tumorigenic factors. Increased ROS production by the ferrocene-linked derivate has a direct cytotoxic effect, that can counter GPER1 associated pathways.

Support: This project was supported by the National Research, Development and Innovation Office (NVKP_16-1-2016-0036) and by the Ministry of Innovation and Technology (ÚNKP-19-2-I-SE-37).

EXAMINING THE BRAIN DISTRIBUTION OF D3 DOPAMINE RECEPTOR USING A FLUORESCENT ANTIPSYCHOTIC

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Keywords: Antipsychotics, Schizophrenia, Superresolution Microscopy, Dopamine

Aims: Schizophrenia affects nearly 1 % of the population, and the treatment is still unresolved. Cariprazine is a new, third generation antipsychotic, which appears to be particularly efficient in the treatment of the negative symptoms of schizophrenia. Cariprazine has the highest affinity for the D3 dopamine receptor (D3R), and probably this receptor mediates its unique effects. The exact function and brain distribution of D3R is still unknown, partly because there is no selective antibody against it, so the specific anatomic examination is not solved yet. In collaboration with Professor György Keserű's lab we developed a fluorescent analog of cariprazine (fluo-cariprazine), which helps to visualize the binding of the drug in brain tissue.

Methods: We examined the binding of fluo-cariprazine with confocal and superresolution microscopy on D3R expressing HEK 293 cells. We also tested the specificity of binding by mutating the ligand binding site on the receptor. To map the brain distribution of the drug we prepared 50 µm thick brain slices from wild type and D3R KO mice. The slices were treated with fluo-cariprazine and were immunostained. We analyzed the samples by making a three-dimensional reconstruction from the fluorescence microscopy images.

Results: In our cell experiments fluo-cariprazine bound to D3R in a concentration-dependent manner, which could be inhibited by pretreatment with unlabeled D3R ligand and also by mutation of the binding site. Fluorescent cariprazine was enriched mainly in the neuronal nitric oxide synthase positive regions of the basal forebrain, which overlap with the anatomic region called Islands of Calleja. The fluorescent signal was missing in D3R KO animals.

Conclusions: D3R can be found primarily in the Islands of Calleja suggesting that the basal forebrain plays a so far unknown key role in mediating the effects of antipsychotics.

Support: 'Frontline' - Research Excellence Programme KKP_19 (KKP 129961); National Program in Brain Sciences (2017-1.2.1-NKP-2017-00002 for I.K. and Gy.M.K.) Hungarian Academy of Sciences Momentum Program (LP2013-54); National Institutes of Health award number R01NS099457 and NS089575.