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The influence of ectonucleotidases expression on ATP-dependent extracellular signalling

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The adenosine triphosphate is well known as an energy storage and signalling molecule. Function of ATP and its metabolites in purinergic signalling is important in many pathophysiological processes. ATP and other nucleotides may stimulate cell proliferation and increase the invasiveness of different tumour cell types [1]. Nucleoside triphosphates secreted from cells are degraded by the nucleotide metabolizing enzymes. Ectonucleotidases are the cellular surface-located enzymes, which hydrolyse extracellular nucleoside tri-, di- and monophosphates or dinucleoside polyphosphates [2]. Belonging to these class of enzymes, NPPs (Nucleotide Pyrophosphatases/ Phosphodiesterases) and NTPDases (nucleoside triphosphate diphosphohydrolases) are responsible for maintenance the proper level of extracellular nucleotides. Degradation of ATP leads to formation of metabolites, which bound to P2 or P1 (ATP and adenosine) receptors to control the cellular responses [3]. In this study, we investigated the expression of known ectoenzymes (NPP1-3 and NTPDases 1–3) on selected cell types and their impact on the cellular response to the extracellular ATP and its more stable analogues, like α-S-ATP and β,γ-methylene-ATP. Particularly the cell life parameters, such as proliferation rate, invasiveness and ability to migrate were analysed. Moreover, nucleotide-stimulating cell response after knockdown of NPP1 will be investigated. Additionally, molecular docking analysis of ATP and their analogues to NPP1-3 enzymes was investigated. Acknowledgment This project was financially supported by the grant 2017/26/D/ST5/01046 from National Science Centre in Poland. References [1] Martinez-Ramirez AS et al. (2018) Purinergic Signal. 13,1-12 [2] Zimmermann H et al. (2012) Purinergic Signal. 8,437–502 [3] Lim HM et al. (2018) Purinergic Signal. 14,157–166

P-08.2-39
Oncogenic BRAF protein as a molecular target of HDAC inhibitors in melanoma cells

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BRAF is a component of the mitogen-activated protein kinase (MAPK) signal transduction pathway and oncogenic V600E mutation affecting BRAF gene has been widely described in melanomas. Considering that pharmacological use of selective BRAF inhibitors, including Vemurafenib, is often limited by the development of resistance mechanisms, the purpose of the present study was to evaluate whether HDAC inhibitors, a well-known class of epigenetic and anti-tumor drugs, could affect BRAF oncogenic signal in melanoma cells. Here, evidence is provided that both SAHA and ITF2357, two pan HDAC inhibitors, reduce the viability of BRAF V600E-mutated SK-Mel 28 and A375 human melanoma cells and remarkably decrease the level of oncogenic BRAF protein. To investigate the involvement of BRAF signalling pathway, the MEKK inhibitor UO126 was used either alone or in combination with HDAC inhibitors. This compound was shown to strongly potentiate the antitumor effects of HDAC inhibitors and to pronounce the reducing effects on BRAF protein levels. Interestingly, we showed that BRAF has a nuclear localization in melanoma cells, which might account for its oncogenic function, and preliminary immunoprecipitation data seem to indicate that BRAF can bind to p53. Treatment with HDAC inhibitors reduced BRAF levels in both the nucleus and the cytoplasm and dramatically decreased p53 levels. These effects were particularly evident in SK-Mel 28 cells where p53 is present in a mutated form with a moderate oncogenic potential. We also found that HDAC inhibitors produce a switch from pro-survival autophagy to caspase-dependent apoptosis in melanoma cells and further studies aim to clarify the relationship between oncogenic BRAF, p53 and the autophagic process in melanoma. Taken together, our results suggest that HDAC inhibitors can target oncogenic BRAF and thus be considered good candidates in melanoma targeted therapy.

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The adenosine carbocyclic analogue and its impact on human squamous carcinoma cells

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Adenosine is the well known molecule present both, extracellularly and inside the cells and it plays crucial role in cell proper functioning. Numerous data shows, that adenosine level and its interactions with receptors might be relevant in the treatment of various disorders, including psychiatric diseases, inflammation or cancers [1]. The adenosine analogues containing cyclopentane ring structure instead of ribose moiety, were shown to have antiviral and antitumor activity in different cancer cell types [2]. In current work, carbocyclic adenosine analogue was investigated for their activity in squamous carcinoma (A431) cells. The A431 cells was previously shown, to be sensitive for extracellular adenosine-dependent signalling, which makes them potentially susceptible to adenosine analogues treatment [3]. In this type of cancer cells, adenosine concentration occurred to be crucial for the proliferation properties. Furthermore, observed regulation is a result of interaction with A1 or A2 adenosine receptors. The carbocyclic analogue of adenosine was tested for their cytotoxicity and the ability to induce extracellular signalling pathways. Obtained data shown, that tested compound has a huge impact on the cell cycle progression and induce strong toxic effect in human squamous carcinoma cells. Furthermore, it was revealed that it acts through the induction of programmed cell death. Acknowledgment: This project was financially supported by the grant 2017/26/D/ST5/01046 from National Science Centre in Poland. References: [1] Stagg J, Smyth MJ (2010) Oncogene 29, 5346–5358 [2] Chandra G et al. (2015) J Med Chem 58, 5108−5120 [3] Lukasik B, et al. (2020) RSCAdv 10, 31838-31847