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"Characterization of Adenosine Receptors in Human and Mouse Melanoma Cells"

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Melanoma is one of the most aggressive malignant tumors due to its high metastasis incidence and resistance to multiple forms of treatment (1). It has been reported that adenosine (ADO) has tumour promoting activities mediated by adenosine receptors (ARs) activation (2). However, activation of A_3 adenosine receptor (A_3AR) by synthetic agonists induces inhibitory effects on melanoma cell growth (3).

The present work aimed at evaluating the expression of ARs in human and mouse melanoma cells and the proliferative and/or cytotoxic effects induced by ARs activation.

The presence of ARs in human A375 and C32 and mouse K1735-M2 melanoma cells was determined by immunocytochemistry. After cell fixation, cells were incubated with the correspondent primary anti-AR overnight (4ºC). Next, cells were incubated with Alexa Fluor 488 and Hoechst 33258. Visualization was performed by fluorescence microscopy. The proliferative/cytotoxic effects of ADO and selective ARs agonists were assessed using the MTT assay (A_{570}). Cells were incubated with ADO or selective AR agonists for different time periods (1, 24, 48 or 72 h). Moreover, when MRS 1220 or NBTI were used these were added 30 and 60 min, before the onset of the experiment, respectively.

Results are expressed as mean±SEM; *n* denotes the number of experiments (each condition in triplicate); p<0,05 was taken to reflect statistically significant differences (Student's *t* test). Results showed the presence of the four ARs on melanoma cells. However, A_3R evidenced the lowest staining pattern. CPA, CGS 21680 and Cl-IB-MECA (0,1–100 nM; 24h) did not affect cell viability of A375 and K1735-M2 cells. For C32 cells, ADO concentrations (0,1–100 μM; 24 h) promoted cell proliferation up to 117,90±2,52 (*n*=15; p<0,001, from control). This effect was abolished by the selective A_3AR antagonist MRS 1220 (100 nM): 96,07±2,40 (n=3). Cl-IB-MECA (0,1–100 nM) showed a proliferative effect at lower concentrations: 100 nM (1h): 14,31%±2,04 (*n*=18; p<0,05); 10 nM (24h): 18,55%±5,08 (*n*=18; p<0,001). The proliferative effect was abolished by MRS 1220 (100 nM). Higher concentrations of Cl-IB-MECA (30-100 μM) caused cytotoxicity in C32 cells (EC_{50}(1h)=36 μM and EC_{50}(24h)=70 μM). Cytotoxicity effects were not abolished by MRS 1220. Moreover, these effects were not time dependent (1-72h). In the presence of NBTI (10 μM), the cytotoxic effect mediated by 50 μM of Cl-IB-MECA (-38,22±2,48 n=4; p<0,001) was increased (-55,35±2,13; n=4; p<0,001).

Our findings show for the first time the presence of ARs on human C32 and mouse K1735-M2 melanoma cells. A_3R showed the lowest staining pattern. In C32 cells, cell proliferation elicited by exogenous ADO seems to be mediated by A_3AR. Nanomolar Cl-IB-MECA concentrations increases C32 cell proliferation and micromolar concentrations causes a non time-dependent cytotoxicity, not mediated by A_3AR activation. Moreover, we have gathered evidence that endogenous ADO may protect human C32 melanoma cells from the cytotoxicity induced by high concentrations of Cl-IB-MECA.

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Acknowledgements: FCT(SFRH/BD/64911/2009)