

Anticancer activity of anandamide in human cutaneous melanoma cells

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Cutaneous melanoma is one of the most aggressive human cancers and the most aggressive type of skin cancer with an increasing incidence worldwide (Blázquez et al., 2006). Metastatic melanoma is associated with poor prognosis, still limited therapeutic options and it is generally refractory to conventional chemotherapy (Wolchok and Saenger, 2007). Novel therapeutic strategies are thus required. Cannabinoids are implicated in the control of cell proliferation (Guindon and Hohmann, 2011; Flygare and Sander, 2008; Petersen et al., 2005), but little is known about the role of the endocannabinoid system in human malignant melanoma. The aim of this study was to characterize the *in vitro* antitumor activity of anandamide (AEA) in A375 melanoma cells. The mRNA expression profile of genes that code for proteins involved in the metabolism and in the possible mechanism of AEA action was assessed by RT-PCR. Cell viability was tested using WST-1 assay and the apoptotic cell death was determined by measuring caspase 3/7 activities. A375 cells expressed fatty acid amide hydrolase (FAAH), cyclooxygenase-2 (COX-2), cannabinoid receptor type 1 (CB1), transient receptor potential vanilloid type 1 (TRPV1) and G-protein-coupled receptor 55 (GPR55) genes. AEA induced a concentration-dependent cytotoxicity with an IC₅₀ of $5.8 \pm 0.7 \mu\text{M}$ and such an effect was associated to a caspase-dependent apoptotic pathway. Inhibition of FAAH and COX-2 enzymes potentiated (2-fold increase, $p < 0.05$) and mitigated (5-fold decrease, $p < 0.01$) the cytotoxicity of AEA, respectively. Blocking CB1 receptors partially decreased the AEA antitumor activity, whereas selective antagonism on the TRPV1 receptor subtype barely affected the mechanism of AEA action. Finally, methyl- β -cyclodextrin, a membrane cholesterol depletory, completely reversed the cytotoxicity induced by the selective GPR55 agonist, O-1602, and AEA. In conclusion, AEA induces cytotoxicity against A375 human melanoma cells in the micromolar range of concentrations through a complex mechanism mediated by caspase induction, COX-2-derived product synthesis and activation of CB1 and lipid raft modulation, the latter probably linked to GPR55 activation.

Blázquez et al. (2006). *FASEB J.* 20, 2633-35.

Flygare and Sander (2008). *Semin in Cancer Biol.* 18, 176-89.

Guindon and Hohmann (2011). *Br J Pharmacol.* 163, 1447-63.

Petersen et al. (2005). *J Neurochem.* 93, 299-309.

Wolchok and Saenger (2007). *Curr Opin Oncol.* 19, 116-20.