TITLE:

Modulation of retinoic acid receptor signaling pathway *via* all trans retinoic acid in Merkel cell carcinoma cells

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The biological activity of retinoic acid or all-trans retinoic acid (ATRA) is mediated by retinoic receptors, which are ligand-dependent transcription factors that activate genes crucial for cell differentiation. Dysregulations of retinoic receptor signaling pathway lead to carcinogenesis. A strong *in vitro/in vivo* antitumor activity of ATRA by modulating the retinoic pathway has been proved in carcinoma of different histotypes. However, the effect of this molecule in Merkel cell carcinoma (MCC), a rare but aggressive skin neoplasm of viral origin in 80% of cases, is unknown.

Herein, we investigated the antineoplastic effect of ATRA in Merkel cell polyomavirus (MCPyV)-positive/-negative MCC cells and in human fibroblasts, as control.

The antineoplastic effect of ATRA was evaluated at day 3 of treatment by testing MCC cell proliferation, migration and clonogenicity. Apoptosis/cell death and cell cycle were evaluated *via* Annexin-V/propidium iodide (P.I.) and TALI assays, respectively. Apoptotic and retinoic pathways were evaluated by RT^2 Profiler PCR mRNA array, that allows the analysis of pro/anti-apoptotic and retinoic pathway genes (84+84 genes), and by western blot (WB) analysis.

ATRA treatment led to a strong reduction in MCC cell proliferation, migration and clonogenicity, while inducing cell cycle arrest and promoting apoptosis/death in MCC cells, with a more pronounced effect in MCPyV-positive MCC cells. A significant overexpression of various pro-apoptotic markers in ATRA-treated MCC cells compared to untreated cells was determined by gene expression array and WB analyses. No phenotypic and molecular effects were identified in ATRA-treated fibroblast control cells. Upon ATRA treatments in MCC cells, numerous retinoic signaling genes, such as *BMP2*, *FOXA1*, *MAFB*, *RBP4*, *OLIG2*, *UCP1* were found to be differentially expressed compared to untreated cells.

Our *in vitro* data indicate that ATRA is effective in reducing MCC cell growth, while presenting strong pro-apoptotic effects and favoring cell cycle arrest/death *via* retinoic receptor signaling pathway regulation.