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Human 3D Metastatic Melanoma Models for in vitro Evaluation of Targeted Therapy Efficiency

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Abstract : Targeted therapy molecules are used as a first-line treatment for metastatic melanoma with B-Raf mutation. Nevertheless, these molecules can cause side effects to patients and are efficient on 50 to 60 % of them. Indeed, melanoma cell sensitivity to targeted therapy molecules is dependent on tumor microenvironment (cell-cell and cell-extracellular matrix interactions). To better unravel factors modulating cell sensitivity to B-Raf inhibitor, we have developed and compared several melanoma models: from metastatic melanoma cells cultured as monolayer (2D) to a co-culture in a 3D dermal equivalent. Cell response was studied in different melanoma cell lines such as SK-MEL-28 (mutant B-Raf (V600E), sensitive to Vemurafenib), SK-MEL-3 (mutant B-Raf (V600E), resistant to Vemurafenib) and a primary culture of dermal human fibroblasts (HDFn). Assays have initially been performed in a monolayer cell culture (2D), then a second time on a 3D dermal equivalent (dermal human fibroblasts embedded in a collagen gel). All cell lines were treated with Vemurafenib (a B-Raf inhibitor) for 48 hours at various concentrations. Cell sensitivity to treatment was assessed under various aspects: Cell proliferation (cell counting, EdU incorporation, MTS assay), MAPK signaling pathway analysis (Western-Blotting), Apoptosis (TUNEL), Cytokine release (IL-6, IL-1α, HGF, TGF-β, TNF-α) upon Vemurafenib treatment (ELISA) and histology for 3D models. In 2D configuration, the inhibitory effect of Vemurafenib on cell proliferation was confirmed on SK-MEL-28 cells (IC50=0.5 µM), and not on the SK-MEL-3 cell line. No apoptotic signal was detected in SK-MEL-28-treated cells, suggesting a cytostatic effect of the Vemurafenib rather than a cytotoxic one. The inhibition of SK-MEL-28 cell proliferation upon treatment was correlated with a strong expression decrease of phosphorylated proteins involved in the MAPK pathway (ERK, MEK, and AKT/PKB). Vemurafenib (from 5 µM to 10 µM) also slowed down HDFn proliferation, whatever cell culture configuration (monolayer or 3D dermal equivalent). SK-MEL-28 cells cultured in the dermal equivalent were still sensitive to high Vemurafenib concentrations. To better characterize all cell population impacts (melanoma cells, dermal fibroblasts) on Vemurafenib efficacy, cytokine release is being studied in 2D and 3D models. We have successfully developed and validated a relevant 3D model, mimicking cutaneous metastatic melanoma and tumor microenvironment. This 3D melanoma model will become more complex by adding a third cell population, keratinocytes, allowing us to characterize the epidermis influence on the melanoma cell sensitivity to Vemurafenib. In the long run, the establishment of more relevant 3D melanoma models with patients' cells might be useful for personalized therapy development. The authors would like to thank the Picardie region and the European Regional Development Fund (ERDF) 2014/2020 for the funding of this work and Oise committee of "La ligue contre le cancer".

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