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Host institution
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3D Organotypic Melanoma Model: a Potent Tool to Dissect the Influence of Tumor Microenvironment in Melanoma Progression

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Introduction

Cutaneous melanoma is one of the most diagnosed malignancies worldwide and the deadliest form of skin cancer(1). It harbours high levels of inter and intra-tumoral heterogeneity(2). Its plasticity at transcriptional and phenotypical level provides melanoma cells the ability to metastasize at distal organs and develop drug resistance mechanism(3-5). The tumour microenvironment contributes to melanoma aggressiveness altering cell-cell and cell-matrix adhesion molecules, fostering the secretion of pro-tumorigenic factors, and sustaining melanoma immune escape(3-5). Current melanoma models do not fully recapitulate the complexity of the TME, partially mimic patients' drug response and are often difficult to establish and poorly reproducible(2;7). There's a urgent need for a novel melanoma 3D model that could reproduce patients' characteristics, being reproducible, easy to handle and suitable for the analysis of the different component of melanoma ecosystem, as well as for drug testing analysis.

Aims of the project

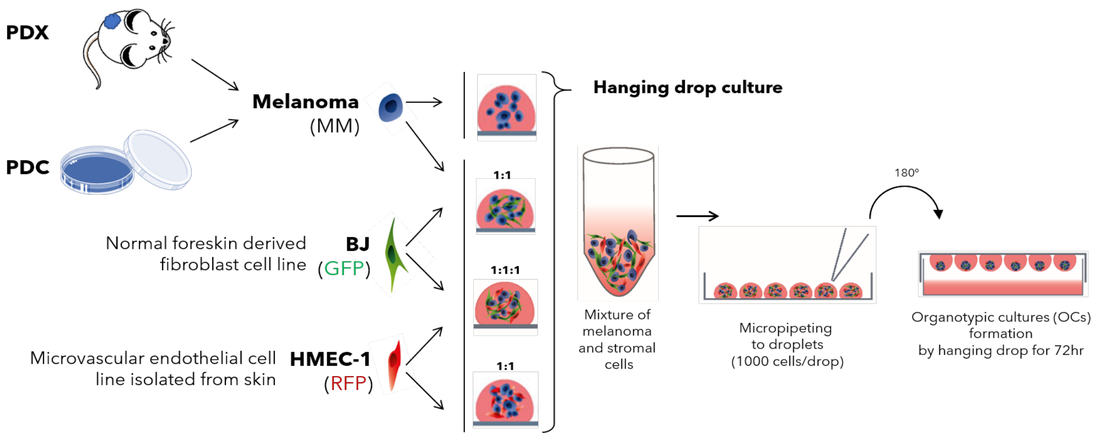
Set up of a novel melanoma 3D organotypic culture:

- ❖ to study how TME interacts with melanoma cells, modulating their phenotype and behaviour *in vitro*
- ❖ to assess how perturbations of the organotypic culture modulate melanoma development and dissemination *in vivo*

Matherials and Methods

Set-up of a plastic 3D system to study melanoma-TME interactions

To generate the 3D organotypic culture (OC) we co-cultured melanoma cells, from both patient-derived xenografts (PDXs) and primary cultures (PDCs), with the two major cellular components of the tumour microenvironment, fibroblasts and endothelial cells. We used the BJ fibroblasts, a cell line derived from normal foreskin, and the HMEC1, an endothelial cell line isolated from the microvasculature of the skin. Co-cultures were set up as hanging drops at 1:1 ratio for 72hr.

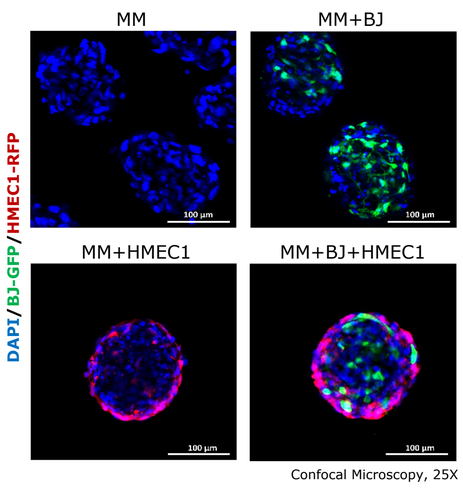


We established uniform and stable 3D OCs from 11 PDCs and 6 PDXs, with distinct genetic backgrounds, derived from both primary tumours and metastases.

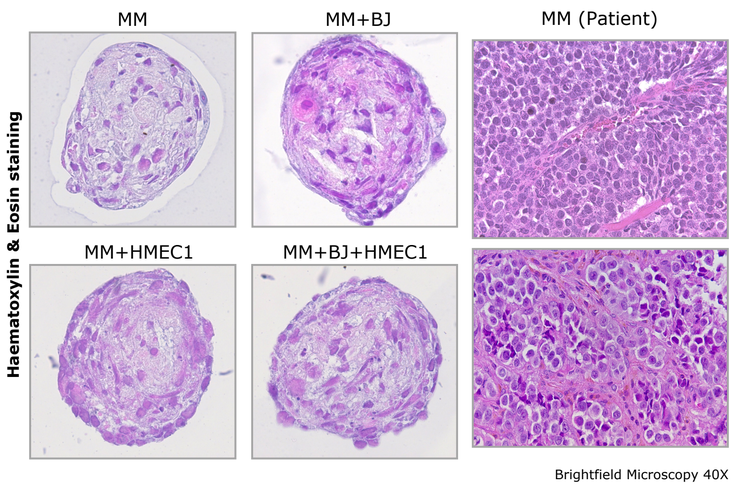
Results

1) 3D structure of organotypic cultures resembles patients' tissue architecture

OCs showed a round-shaped and compact structure, with a final diameter of ~150µm. Confocal microscopy demonstrated that BJ-GFP fibroblasts invade and aggregate in the internal part of the OCs, while HMEC1-RFP endothelial cells cover the surface, establishing capillary like structures.

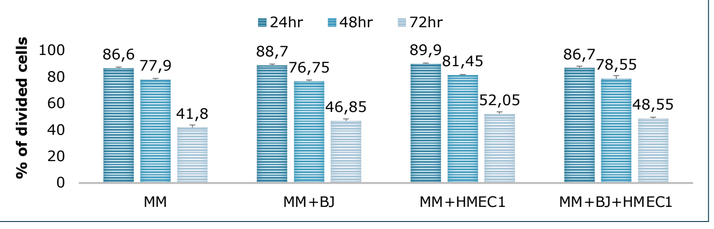


Confocal Microscopy, 25X

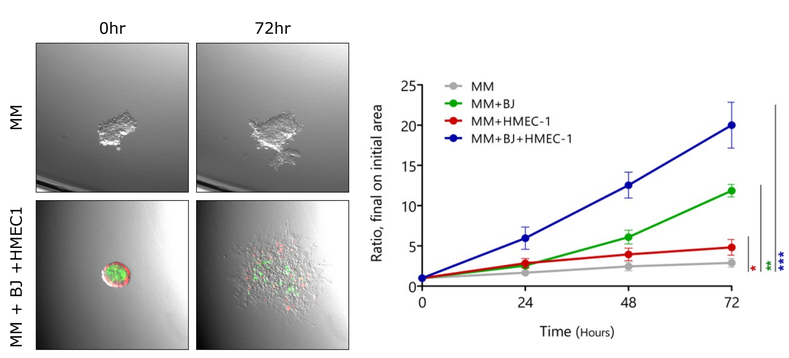


Brightfield Microscopy 40X

2) Melanoma proliferation is not affected by co-culture with stromal cells in hanging drops



3) Fibroblasts and Endothelial cells foster the capability of melanoma cells to invade collagen

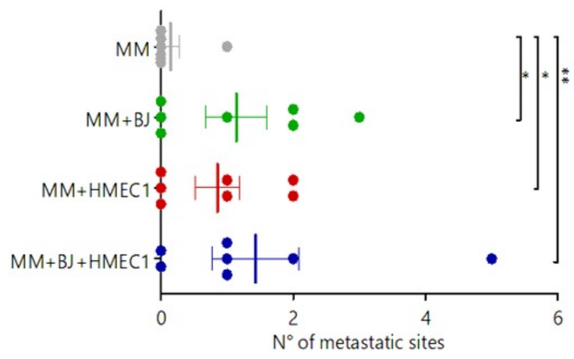


Collagen invasion was evaluated at 24, 48 and 72 hrs. Fibroblasts and endothelial cells can drastically increase the invasive potential of different melanoma cultures, indicating that melanoma cells display an active interplay with stromal cells in the OCs. Increasing concentration of stromal cells further enhances collagen invasion (data not shown).

Increasing concentration of stromal cells further enhances collagen invasion, suggesting that the niche plays a crucial role in driving melanoma invasion.

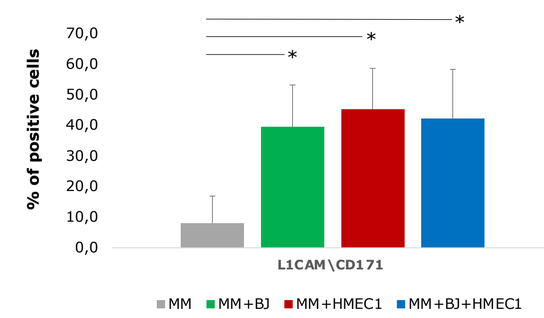
4) The presence of stromal components in the 3D organotypic cultures increase the metastatic potential of melanoma cells

OCs were transplanted intradermally in NSG mice, primary tumours resected and metastases formation assessed. Our data show that primary melanoma growth is not affected by stromal cells, while the number of metastases significantly increased in mice transplanted with OCs containing fibroblasts and/or endothelial cells.



4) NCAM-L1 (CD171) expression is significantly increased in melanoma cells upon interactions with BJ and HMEC-1

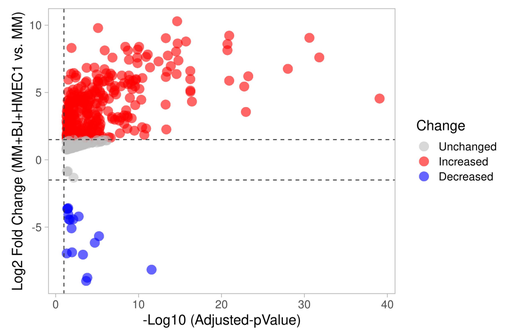
Flow cytometry analysis revealed that there are no major changes in common melanoma differentiation and cell-cell interaction markers in OCs. On the contrary, L1CAM (CD171) is significantly increased in melanoma cells upon co-culture with fibroblasts and endothelial cells.
Interestingly, NCAM-L1 has been associated with melanoma invasion and migration (8).



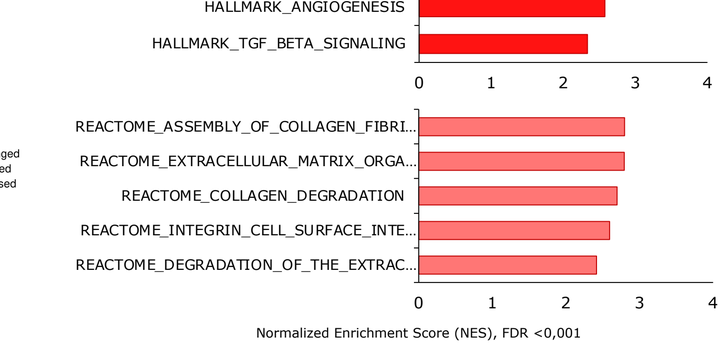
6) OCs RNAseq data show an enrichment of genes related to extracellular matrix remodelling and phenotype switch in melanoma cells co-cultured with fibroblasts and endothelial cells

MM+BJ+HMEC1 vs MM
Vucano Plot

Up- and down- regulated genes are reported as red and blue dots, respectively. (Adj-pVal threshold=0,05)



MM+BJ+HMEC1 vs MM Patway Enrichment:



Discussion and Conclusions

We report that in our OC model, the 3D cellular organization recapitulates patients' tissue structure and that the addition of TME components fosters melanoma invasion *in vitro* and metastatic potential *in vivo.*

We also show that NCAM-L1 is upregulated in melanoma cells in contact with fibroblasts and endothelial cells and that the cell-cell interaction reprograms melanoma cells toward a more aggressive phenotype. We will further investigate the transcriptional and proteomic profiles of the cultures, also comparing our results with existing databases.

We are now co-culturing healthy donor derived PBMCs with our 3D OCs. Increasing the system complexity will allow us to more faithfully and accurately test new therapeutic approaches and combination treatments.

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