New three-dimensional in vitro model to study tumour-stroma interactions in melanoma progression.

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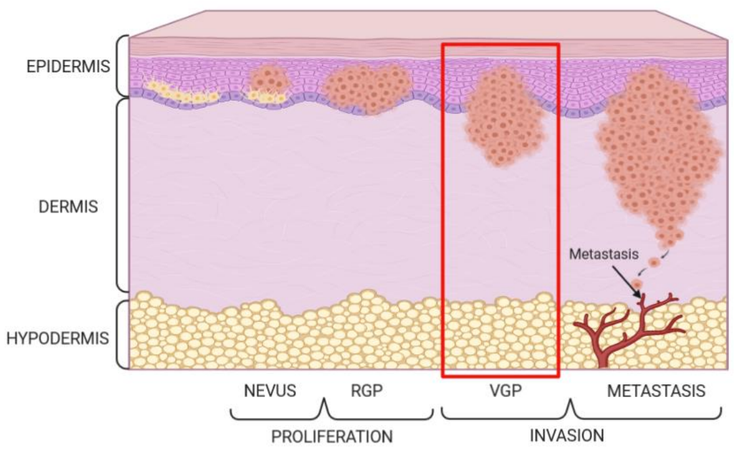
INTRODUCTION

Melanoma cells interact bidirectionally with the tumour environment, which is actively involved in tumour progression and represents an attractive therapeutic target. Despite the evolution of available antitumour therapies frequently appear resistance and recurrence, a reflection of their ineffectiveness in combating all tumour cell phenotypes. Our data indicate that human adipose tissue, present in the stroma of most tumours, induces melanoma primary cell invasion in 2D systems. It is essential to develop new in in vitro three-dimensional (3D) models that recreate more accurately tumoral in vivo structure, cell-cell and cell-extracellular matrix (ECM) interactions, to study the role of the tumour environment in tumour progression. This work focuses on the development of new in vitro melanoma 3D models.

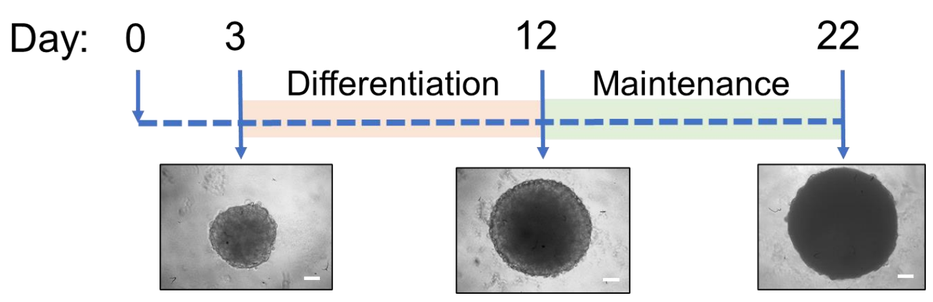


AIMS

To develop new *in vitro* three-dimensional (3D) models that recreate more accurately *in vivo* tumor structure and environment

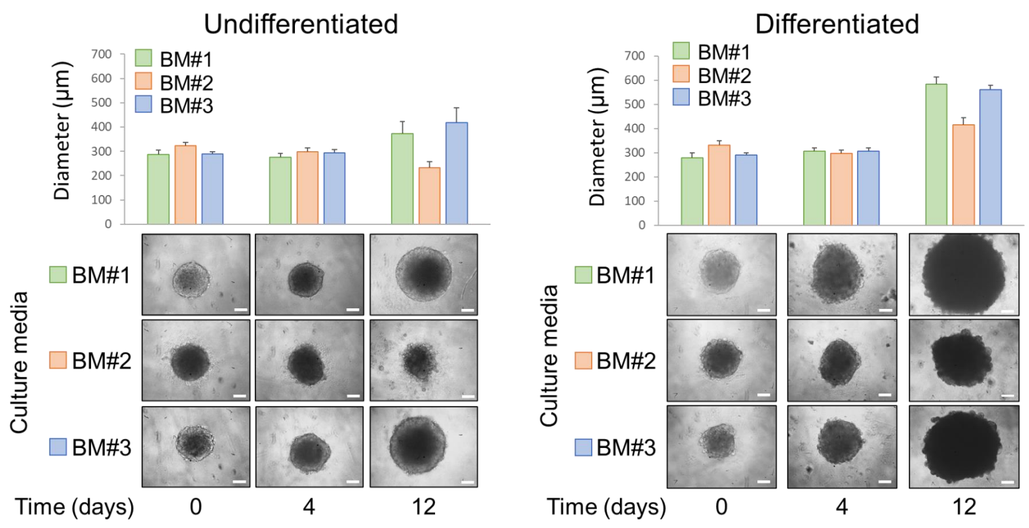
METHODS

Model: Primary melanoma cells and human preadipocytes were used to generate 3D multicellular spheroid. Methods: Scaffold free methods (ultra-low attachment plates, ULA) and 3 different culture medium were used for spheroid formation. Spheroids were cultured for 9 additional days in differentiation media and then maintained 10 days to evaluate dedifferentiation. Standard cell biology techniques were used to characterize spheroids.



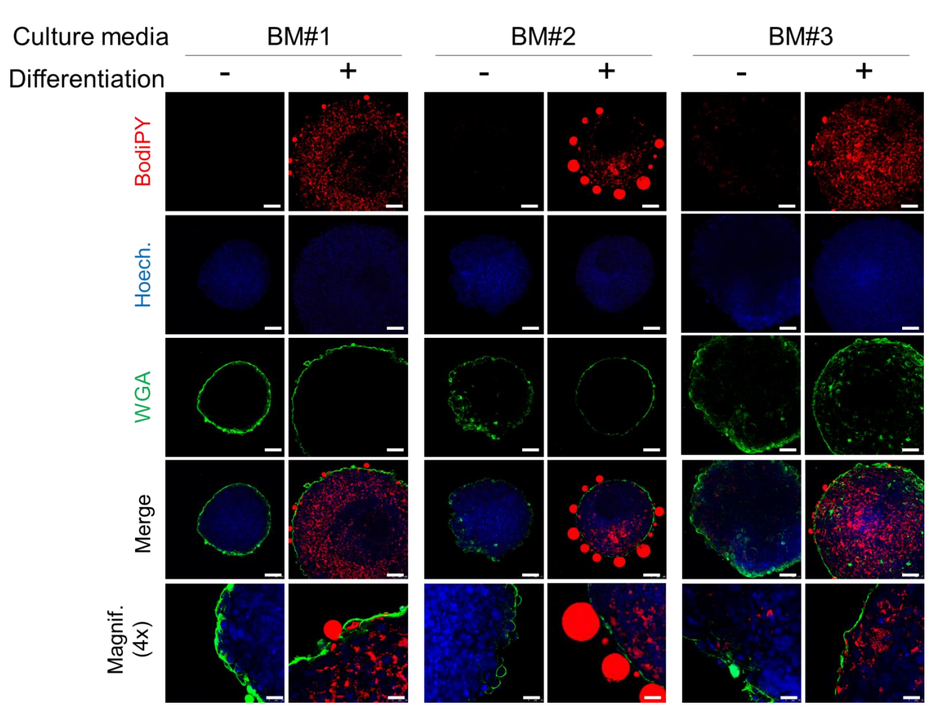
RESULTS

1. Mixed cell types spheroids generated by scaffold-free methods expand in culture.



Mixed cell types spheroid formation. Melanoma cells and preadipocytes were seeded in p96 ultra-low attachment plates (ULA) and cultured for 3 days in 3 different basal medium (BM#1, BM#2 o BM#3) for spheroid formation. Spheroids were cultured for 9 additional days in either basal medium **(A)** or in differentiation media **(B)** to monitor growth. Phase-contrast image of spheroids over 12 days and statistical analysis of spheroids diameters (µm) are shown, scale bar 100µm; n≥8 spheroids were quantified per condition; bars are mean±SEM. Basal medium BM#2 did not allow spheroid growth nor maintenance. Basal media BM#1 and BM#3 allowed spheroid growth.

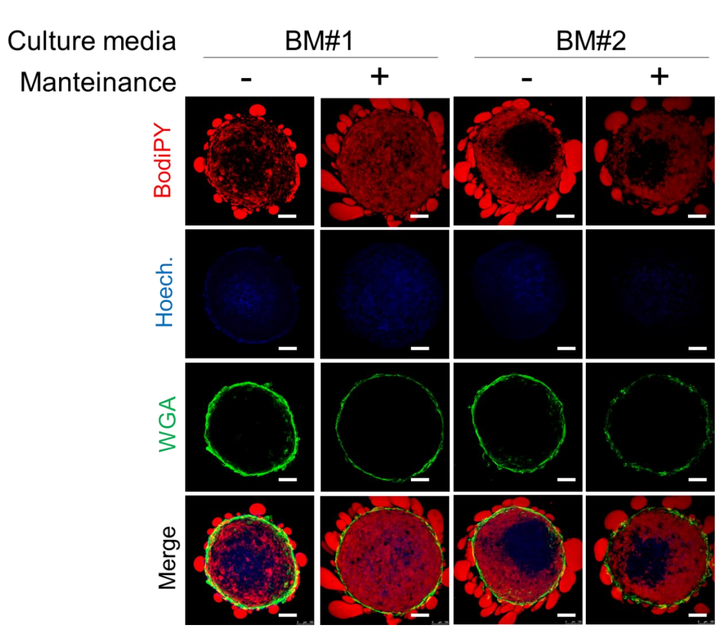
2. Induction of adipocyte differentiation during formation of mixed preadipocyte/melanoma spheroids.



Mixed cell types spheroid differentiation. Melanoma cells and preadipocytes were seeded in p96 ULA plates, cultured for 3 days in 3 different basal medium (BM#1, BM#2 o BM#3) for multicellular spheroid formation. Spheroids were cultured for 9 additional days in either basal medium **(-)** or in differentiation media **(+).**

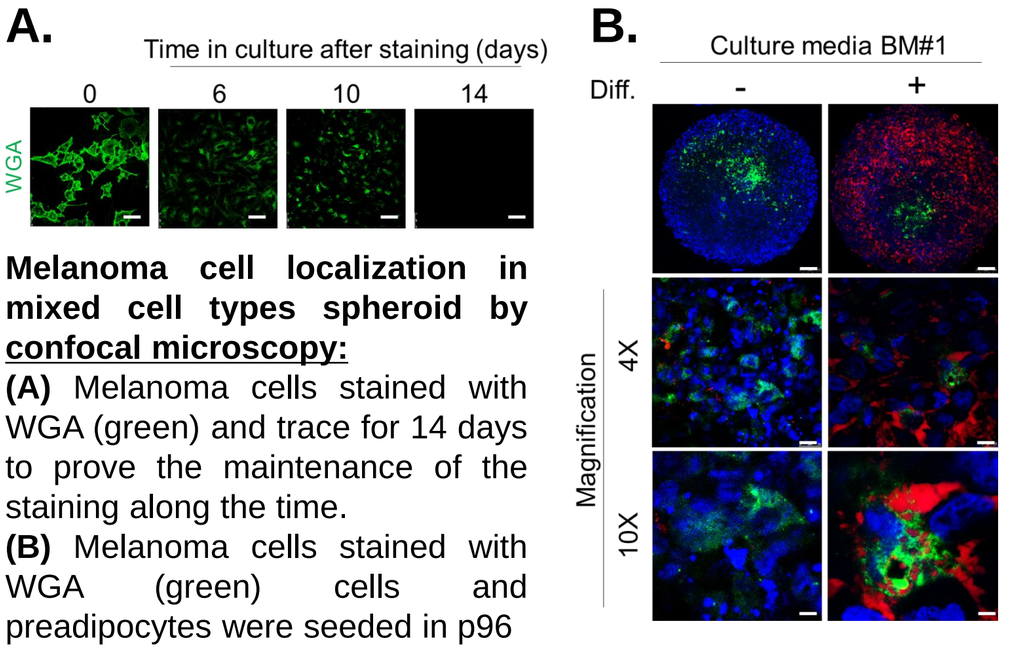
Spheroids were analysed by: **(A) Confocal microscopy:** Lipid accumulation stained with BodiPY (red), nuclei with Hoechst (blue) and spheroid perimeter with WGA (green). Scale bar 100µm and 25µm for magnificated images (4x). **(B) Western blot:** whole cell extracts of undifferentiated or differentiated adipocytes in monolayer (2D) or spheroids (3D). Level of Perilipin was used as adipocyte differentiation marker. GAPDH used as loading control.

3. Adipocytes remain differentiated in cultured spheroids for long time.



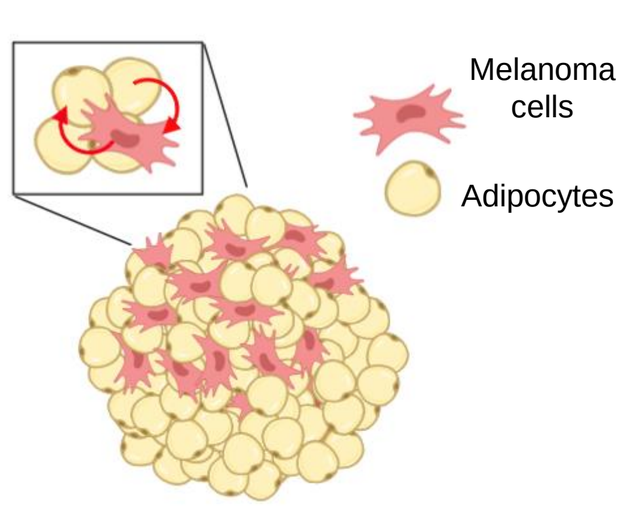
Adipocyte spheroid maintenance. Preadipocytes were seeded in p96 ULA plates, cultured for 3 days in different basal medium (BM#1, BM#2) for spheroid formation, cultured for 9 additional days in differentiation media and then cultured in maintenance medium to evaluate dedifferentiation and lipid content after 10 days. **Confocal microscopy:** Lipid accumulation stained with BodiPY (red), nuclei with Hoechst (blue) and spheroid perimeter with WGA (green).

4. Melanoma cells uptake lipids from adipocyte in mature preadipocyte/melanoma spheroids.



Melanoma cell localization in mixed cell types spheroid by confocal microscopy:
(A) Melanoma cells stained with WGA (green) and trace for 14 days to prove the maintenance of the staining along the time.
(B) Melanoma cells stained with WGA (green) cells and preadipocytes were seeded in p96 ULA plates, cultured for 3 days in basal medium (BM#1) for mixed cell types spheroid formation and differentiated for 9 additional days. Preadipocyte/melanoma spheroid were analysed by immunofluorescence. Lipid accumulation was stained with BodiPY (red) and nuclei with Hoechst (blue). Magnification show lipid uptake from the differentiated adipocytes. Scale bar 100µm, 25µm and 10x for magnifications 4x and 10x, respectively.

CONCLUSION



Preadipocyte/melanoma spheroid model offers an improved culture system to study tumour-adipocyte interactions in melanoma progression.

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