Tissue-Engineered Skin Model Derived from Type 1 Neurofibromatosis (NF1) Patients to Study Tumor Genesis and to Predict Response to Therapy

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Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant multisystemic disorder caused by aberrations in the neurofibromin gene. Typically, patients develop multiple cutaneous tumours that grown from axon of peripheral nerve, called neurofibromas and schwannomas. These benign tumours are generally composed of Schwann cells (SC) and fibroblasts, but others cells type can also be found. Highly variable clinical manifestations between NF1 patients are observed. Actually, there is no specific treatment for this stigmatizing disease. The purpose of this study is to develop a tissue-engineered human skin model derived from NF1 patients to characterized and understand the formation of neurofibromas and schwannomas.

Methods

We used a spheroid suspension culture method to generated neurofibroma-like and schwannoma-like tumours. Schwannomas are composed of immortalized SC isolated from an NF1 tumor and neurofibromas are composed of an equal number of the same SC lineage and fibroblasts isolated from skin biopsies. The auto-assembly model was used to generate tissue-engineered skin (TES) in vitro with fibroblasts and keratinocytes isolated from NF1 patients (n=3). Spheroids were added within the skin between the dermis and the epidermis.

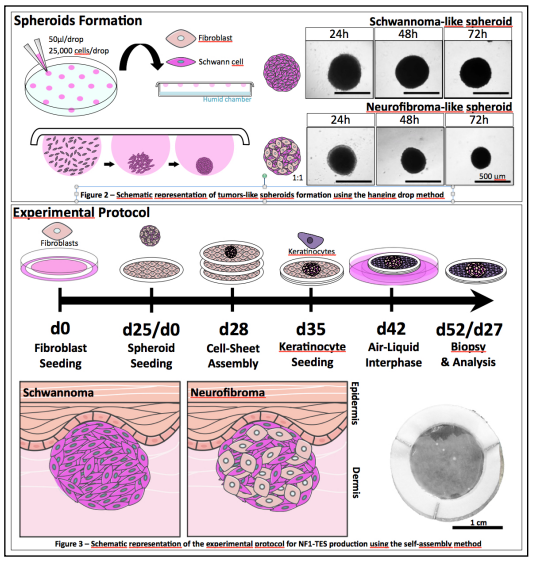

Figure 2 – Schematic representation of tumors-like spheroids formation using the hanging drop method

Figure 3 – Schematic representation of the experimental protocol for NF1-TES production using the self-assembly method

Results

We first determined the best conditions for the formation of spheroids. Surface area of spheroids was significantly increased already at day 3 and continued until day 10 after seeding. Spheroids growth was significantly faster than control cells. Immunofluorescence revealed that spheroids/tumors-like, seeded with NF1-TES, are in a proliferative state. Furthermore, non-apparent activation of apoptosis within spheroid is detected. Histological analysis revealed similar patterns normally found in schwannomas and neurofibromas.

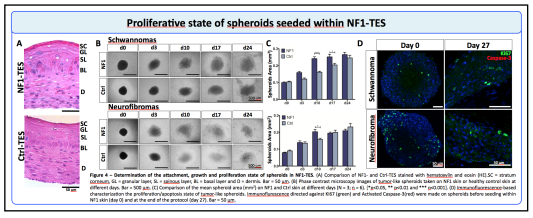

Figure 4 – Determination of the attachment, growth and proliferation state of spheroids in NF1-TES. (A) Comparison of NF1- and Ctrl-TES stained with hematoxylin and eosin (HE).SC = stratum corneum, GL = granular layer, SL = spinous layer, BL = basal layer and D = dermis. Bar = 50 µm. (B) Phase contrast microscope images of tumor-like spheroids taken on NF1 skin or healthy control skin at different days. Bar = 500 µm. (C) Comparison of the mean spheroid area (mm²) on NF1 and Ctrl skin at different days (N = 3; n = 6). *p<0.05, ** p<0.01 and *** p<0.0001. (D) Immunofluorescence-based characterization the proliferative/apoptosis state of tumor-like spheroids. Immunofluorescence directed against KI67 (green) and Activated Caspase 3(red) were made on spheroids before seeding within NF1 skin (day 0) and at the end of the protocol (day 27). Bar = 50 µm.

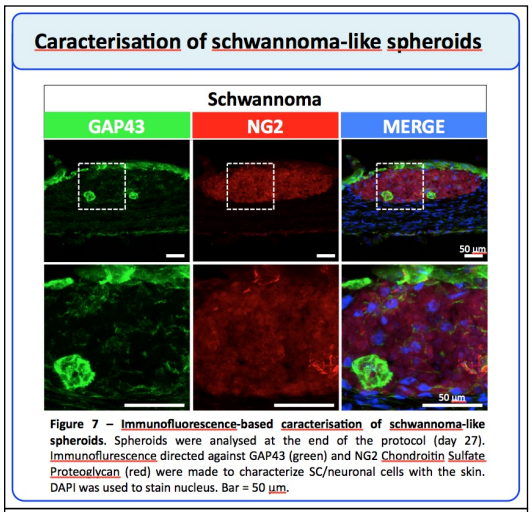

Figure 7 – Immunofluorescence-based caracterisation of schwannoma-like spheroids. Spheroids were analysed at the end of the protocol (day 27). Immunofluorescence directed against GAP43 (green) and NG2 Chondroitin Sulfate Proteoglycan (red) were made to characterize SC/neuronal cells with the skin. DAPI was used to stain nucleus. Bar = 50 µm.

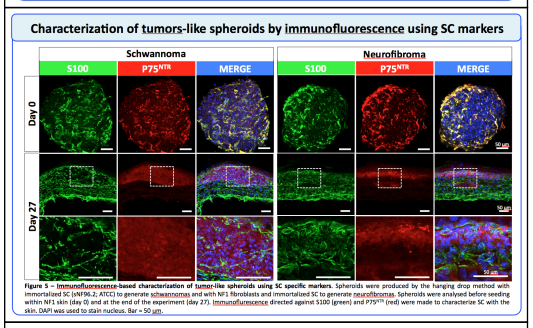

Figure 5 – Immunofluorescence-based characterization of tumor-like spheroids using SC specific markers. Spheroids were produced by the hanging drop method with immortalized SC (sNF96.2; ATCC) to generate schwannomas and with NF1 fibroblasts and immortalized SC to generate neurofibromas. Spheroids were analysed before seeding within NF1 skin (day 0) and at the end of the experiment (day 27). Immunofluorescence directed against S100 (green) and P75NTR (red) were made to characterize SC with the skin. DAPI was used to stain nucleus. Bar = 50 µm.

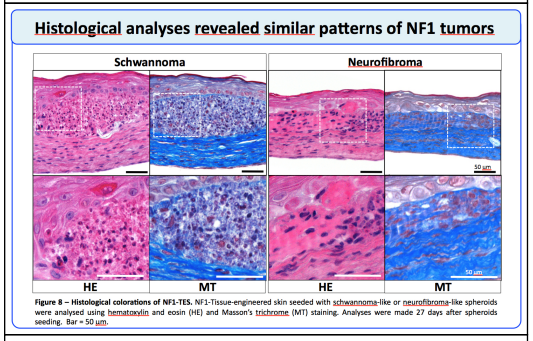

Figure 8 – Histological colorations of NF1-TES. NF1-Tissue-engineered skin seeded with schwannoma-like or neurofibroma-like spheroids were analysed using hematoxylin and eosin (HE) and Masson's trichrome (MT) staining. Analyses were made 27 days after spheroids seeding. Bar = 50 µm.

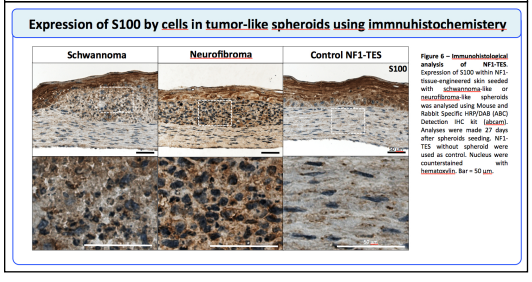

Figure 6 – Immunohistological analysis of NF1-TES. Expression of S100 within NF1-tissue-engineered skin seeded with schwannoma-like or neurofibroma-like spheroids was analysed using Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (abcam). Analyses were made 27 days after spheroids seeding. NF1-TES without spheroid were used as control. Nucleus were counterstained with hematoxylin. Bar = 50 µm.

Conclusions

Our NF1 skin model could become a unique tool to better characterize the mechanism of action of a new drug on NF1 tumor shape and growth as well as to assess tumorigenic properties of each of the tested NF1 gene mutation, and ultimately provide better tools to develop new therapies for patients through development of precision/personalized medicine strategies.

References

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