Does Schwann cell dedifferentiation originate dermal neurofibromas?

Abstract
Dermal neurofibromas are characteristic of neurofibromatosis type one (NF1), and their developmental origin still unsolved. Although NF1 loss is required for neurofibroma initiation, some features of these benign tumors resemble a skin injury state and cutaneous trauma or other insults might support tumor development. Since adult terminal Schwann cells ensheathing nerve endings are able to dedifferentiate into a progenitor-like state in response to nerve crushing, we hypothesized that dedifferentiation of NF1−/− Schwann cells could be at the origin of human dermal neurofibromas. In support of this, here we show that CDH19 (a marker specific of Schwann cell precursors) and Schwann cell dedifferentiation marker SOX2 are significantly upregulated in NF1 tumors. We posit that onset of nerve regeneration might have a role in dermal neurofibroma initiation via dedifferentiation of NF1−/− Schwann cells.

1 | BACKGROUND
Neurofibromas are benign peripheral nerve sheath tumors known to contain a subpopulation of NF1−/− Schwann cells (SCs) and a variety of NF1−/+ (haploinsufficient) cell types.6 Dermal neurofibromas (dNF) occur in virtually all adults with neurofibromatosis type 1 (NF1) and unlike plexiform neurofibromas, present a restricted growth and no malignant transformation potential. This phenotype may reflect the developmentally late occurrence of the NF1 second-hit mutation.5 In fact, it has been suggested that adult multipotent stem/progenitor cells could originate dNFs.2–4 Nevertheless, dedifferentiation of a terminal SC that has suffered a second-hit mutation remains an enticing possibility.5

2 | PREMISES
1. NF1−/− SCs originate dNFs (reviewed by ref. 4).52–56
2. Trauma to the skin and cutaneous nerves induces nerve regeneration and hypertrophy, activating the nearby progenitors to transform into a dNF.3 Neurofibromas resemble an injured state in their complex mixture of cells and in that SCs are dedifferentiated and mostly dissociated from axons. In NF1-deficient mice, myelinating SCs (mSCs) give rise to neurofibromas distal to the wound site, suggesting that adult mSCs can be the cell of origin for neurofibromas5 and highlighting the importance of tissue injury. Local trauma can also be a factor in the development of dNFs in NF1 patients.57 Neurofibromas have been described as unrepaired wounds.5,6
3. Although loss of NF1 is required for neurofibroma development, it is not sufficient and tumor microenvironment plays a key role in this process. Poorly regulated wound healing has been described in NF1 haploinsufficient tissues,58 again highlighting a possible relationship between wounding and tumor initiation.

3 | HYPOTHESIS
In the skin, terminal SCs ensheathe peripheral nerve endings.59 These SCs are able to dedifferentiate into a progenitor-like state in response to trauma or other insults.6–8 Furthermore, recent data from our group suggest terminal SCs may behave as bona fide dermal stem cells.9 Based on all of these premises, we hypothesize that dedifferentiation of SCs could originate human dNFs.

4 | EVIDENCE SUPPORTING THIS HYPOTHESIS
We analysed the differentiation stage of dNFs of 8 patients diagnosed with NF1 (Data S1), looking at the expression levels of genes characteristic of the different dermal stem cell stages by qRT-PCR of isolated tumor tissue (Fig. 1a,b).9 We found that SC markers SOX10 and S100B were upregulated in 100% and 75% of tumors, respectively (Fig. 1c). Interestingly, NGFR, NCAM1 and SOX2, all of them dermal stem cell markers were present in tumors although in variable numbers (Fig. 1d). In contrast, MCAM and SOX9 which mark more differentiated stages were mostly non-expressed in tumors (Fig. 1e). CDH19, a marker of Schwann cell precursors and adult dermal stem cells, was upregulated in 100% (8/8) of the cases (Fig. 1f). Levels of neurofibromin (NF1) were unaltered (75%) or slightly upregulated (25%; data not shown).

To confirm CDH19 expression at the protein level, we analysed dNF and unaffected skin sections of NF1 patients as well as healthy donor skin by immunofluorescence. We confirmed upregulation of CDH19 in dNFs as compared to controls (Fig. S1). The difference was especially clear in the lower dermis, where most lesional NGFR+, S100B+, and SOX10+ cells are present (Figs S2–S4). We also observed that S100B+/NGFR+ early stage microneurofibromas were present in “unaffected” NF1 patient skin and that, interestingly, CDH19 was also
Finally, we pursued characterization of CDH19 expression by immunofluorescence in dermal spheres isolated from control foreskin and dNFs. As expected, CDH19+ cells were detected in both normal and dNF-derived spheres (Fig. S6).

5 HOW TO TEST THIS HYPOTHESIS

1. To test that human NF1−/− dedifferentiated SCs originate dNFs, a xenograft model should be tested in which:
   a. Human NF1−/− dermal spheres isolated from unaffected areas of NF1 patient skin may be used to isolate NGFR+ NCAM1+ (which are CDH19+ dedifferentiated SCs) and NGFR− NCAM1− cell fractions (which are non-Schwann, non-perivascular cells).9
   b. NF1 may be silenced in these cell fractions and then NF1+/− and NF1−/− cells of each fraction transplanted to immunocompromised, pregnant NF1+/− female mice to test for dNF development.356 We predict that only NF1−/− NGFR+ NCAM1+ cells would form neurofibromas.
2. To further demonstrate that SC dedifferentiation, which is mediated by SOX2 expression levels, plays a role, we propose to treat NGFR+ NCAM1+ cells with pLKO.shSox29 which should abolish neurofibroma formation.
3. To discriminate between the possibility of resident dermal precursor or dedifferentiated SCs being responsible for dNFs, we propose to cross two transgenic lines. One in which SOX2+ dermal stem cells may be ablated by DTA expression under Sox2-CreERT. The other line would be CMV-CreERT; NF1lox−/−. In the crossed animals, subcutaneous tamoxifen injections would promote ablation of resident dermal stem cells and deletion of NF1 in dermal SCs. In these animals, we predict that dedifferentiation of SCs would produce dNFs even in the absence of resident dermal stem cells.

6 RELEVANCE AND PERSPECTIVES

Our preliminary results are consistent with dermal regeneration via SC dedifferentiation playing a role in dNF initiation. Further work is needed in order to confirm these preliminary results in a higher number of patients and to elucidate the molecular mechanisms behind the SC dedifferentiation process, to further illuminate our understanding of human dNF development.

ACKNOWLEDGEMENTS

This study was supported by grants of Ministerio de Economía y Competitividad (ISCIII—PI10/02871) and Departamento de Desarrollo Económico y Competitividad of the Basque Government (SAIO10-PE10BF01), Spain, to AI.

AUTHOR CONTRIBUTION

HI, AJ, NO and AGR performed the research. AGR, AI and AT designed the study. HI, NO, AJ and AT contributed to essential reagents or tools. HI, AJ, NO and AGR contributed to data acquisition. AGR and AI analysed the data. AGR and AI wrote the manuscript.

ETHICS APPROVAL

This research was approved by the Clinical Research Ethics Committee of Hospital Donostia on 23 of March of 2011 (certificate no. 3/11).

CONFLICT OF INTERESTS

The authors have declared no conflicting interests.

Keywords
CDH19, dermal neurofibroma, dermal stem cell, neurofibromatosis type one, Schwann cell
Establishment of a new three-dimensional human epidermal model reconstructed from plucked hair follicle-derived keratinocytes

1 | BACKGROUND

For decades, many in vitro three-dimensional (3D) skin models have been established and widely used in lieu of animals for skin research. Models that mimic human skin in as many physiological aspects as possible are suitable for academic and industrial research. Such models have been increasingly required because of worldwide expansions of bans and limitations on animal experimentation. Several models of reconstructed human epidermis (RHE) are currently available. These models have been used for various studies, such as metabolic studies of pharmaceutical products (s1–s3), determination of absorption properties (s4–s6), assessment of cutaneous corrosivity (s7–s9) and epidermal responses to irritants and sensitizers (s10–s12). However, the major drawback of these models is that they are derived from unknown donors, hampering studies on differences among individuals. Limat et al. first reported a hair follicle-derived keratinocyte RHE model as a less invasive treatment for chronic leg ulcers, and it was recently used to evaluate sunscreens. They used keratinocytes derived from plucked hair follicles with feeder cells in addition to foetal calf serum or autologous serum for primary and 3D cultures. A safer and simpler RHE model is needed.

2 | QUESTIONS ADDRESSED

We established a RHE model from plucked hair follicle-derived keratinocytes using safer (without serum), simpler (without fibroblasts) and less invasive (without biopsy) techniques and compared this model with the skin-derived RHE model.

3 | EXPERIMENTAL DESIGN

Primary keratinocytes derived from plucked hair follicles containing the outer root sheath (ORS) or skin (for controls) were cultured with serum-free medium. To reconstruct 3D epidermis, harvested keratinocytes