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Concepts and Challenges in Hair Follicle Cloning

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Introduction

It was shortly after World War II that Lille and Wang first demonstrated that feather follicle development is dependent on mesenchymal-epithelial interactions.[1] Mechanisms underlying follicle development are reprised in the development of other appendages, and so these observations by Lille and Wang paved the way for advances in the field, in particular regarding the recognition that all hair follicle development and adult activities are regulated by interactions between the mesenchyme and the epithelium. Later on, Oliver was the first person to demonstrate that rodent mesenchyme–derived papilla, when isolated from the follicle, can initiate these interactions and induce new hair follicle growth in adult skin.[2] Since this, a multitude of experiments have demonstrated that both intact papilla and also cultured papilla cells are capable of inducing de novo hair growth not only in skin, but several other types of epithelia.[3] Interestingly, one other striking behaviour of cultured rodent whisker papilla is their propensity to aggregate, both *in vitro* and after subdermal injection.[4] Cultured rat dermal papilla cells are capable of self-aggregating to form condensate-like clumps, while we have never observed this aggregation phenomenon after injection of human cells into the skin.

Dichotomy of Activity Between Hair Follicle Dermis and Interfollicular Skin

We have previously proposed that dermal papilla, sheath, and fibroblasts are not in a steady state within the skin.[5] Moreover, there is experimental evidence supporting the lack of a steady state between the papilla and sheath cells during the follicle cycle.[6] We believe that hair follicle dermal cells may have an additional role in skin, acting as wound healing fibroblasts in the context of skin injury or trauma.[7] This idea is supported largely by the observation that hair follicle dermal cells assume different roles after cell culture. Once in culture, hair follicle dermal cells can act as mesenchymal stem cells and differentiate down a variety of mesenchymal lineages.[8] This raises the question of whether cultured hair follicle dermal cells will act as hair follicle cells, or in another capacity, when transplanted back into the skin for the purpose of hair follicle regeneration.

Strategies for Targeting Follicle Regeneration

For several years, researchers have been trying to exploit the inductive potential of the dermal papilla and demonstrate that human dermal papilla cells hold the same inductive properties as rodent cells.[9] To this effect, there are currently two experimental strategies that utilise hair-associated dermal cells for follicle regeneration. The first of these involves injecting cultured dermal cells into the dermis, where it is hypothesized that they will augment existing follicles, and transform a vellus follicle to a terminal fate by contributing to, and enlarging the size of the dermal papilla. This is supported by observations that the size of the dermal papilla is directly related to the size of the hair fibre produced.[10] The second strategy involves injecting or grafting hair follicle dermal cells so they are in contact with skin epithelium, where it is proposed they will initiate mesenchymal-epithelial interactions to instruct new follicle growth. By and large, these experiments have been unsuccessful, and to understand this, we have to go back and look at the behaviour of human hair follicle dermal cells when compared to their rodent counterparts (Figure 1). In the absence of spontaneous aggregation by human papilla cells, they may be behaving as fibroblasts in what is essentially a wound environment after their injection or grafting. Coupled with their loss of specificity by culture, hair follicle dermal cells will not necessarily incorporate into a hair follicle, but rather will contribute to the surrounding interfollicular tissue.

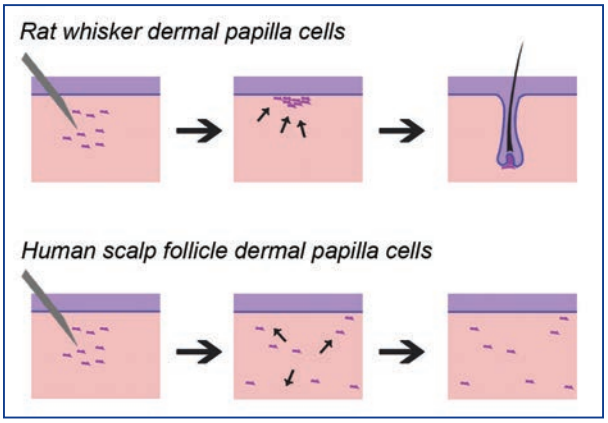

Figure 1. *Cartoon illustrating differences between rat and human dermal papilla cells.* After injection into skin dermis, cultured rat whisker dermal papilla cells have a propensity to aggregate, while human cells often act in an opposite manner and disperse. The aggregation of rat dermal papilla cells enables them to initiate epithelial-mesenchymal interactions, resulting in the growth of de novo follicles in specific sites.

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Hair Follicle Cloning *from front page*

New Approaches for Follicle Regeneration

By expansion of dermal papilla cells by growth in culture, you are essentially taking them from a three-dimensional environment where they are surrounded by other cells, to a two-dimensional environment where they have plastic on one side and culture medium on the other. This results in a decrease in communication between the dermal cells, which likely contributes to their loss in specificity or identity in culture. Recently, we demonstrated that growth of cultured human dermal papilla cells in hanging drop cultures results in formation of three-dimensional dermal spheroids. We were able to show that dermal spheroids maintain their specificity after transplantation into human skin, where they are capable of inducing growth of de novo hair follicles, rather than contributing to the interfollicular dermis.[11] Moreover, 22% of genes expressed in intact papillae, whose expression was deregulated by normal culture growth, were restored by growth of dermal papilla cells in spheroids. This indicates that the microenvironment within dermal spheroids results in increased communication between cells, and a partial restoration of dermal papilla identity—enough to initiate the cascade of events leading to new follicle development. This being said, the molecular contribution of the epidermal cells to the interactive process has still to be elucidated.

Conclusions

Thirty years ago, we first demonstrated that cultured rodent dermal papilla cells could be used to induce new hair follicle growth.[12] We now know that hair follicle cloning is possible using human hair follicle cells. However, the hairs we have produced are quite small, directionally non-uniform, and it remains to be seen how long they will grow for and whether the follicles will cycle. Therefore, many reproducibility and engineering challenges still remain before conventional hair transplantation procedures will be replaced; however, we will continue to take lessons from biology, and by developing a better understanding of the properties of hair follicle cells we will, in time, be able to improve on this important proof of principle study.

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