Development of an effective method to obtain functional dermal papilla cells for hair regeneration.

開發獲得用於毛髮再生的功能性真皮乳頭細胞的有效方法

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Research Background

As an essential organ in mammals, hair plays various important functions such as protection, thermal regulation, sexual and social interaction. Therefore, alopecia syndrome, an autoimmune disorder causing hair loss, has been considered a significant social burden. Despite its clinical significance, there have been only two medications clinically available to treat hair loss patients, including finasteride, a selective inhibitor of type II and III isoforms of 5ɑreductase, and minoxidil. Nonetheless, these drugs have limitations and cause several critical side effects. Hair transplantation has been considered as the last option, but transplanted hair is not maintained long term. Therefore, there is a need to explore alternative therapeutic solutions to regenerate hairs.

It has been well known that hair is generated in the hair follicle (HF), which is comprised of two main cell types: hair follicle stem cells (HFSCs) and dermal papilla cells (DPCs). The HFSCs are epithelial cells, and DPCSs are derived from the mesenchyme cells. The mutual interaction between HFSCs and DPCs named the epithelial-mesenchymal interactions (EMI) has been known to regulate hair development. The DPCs secret several signals such as Wnt/ -catenin and BMP to the surrounding matrix cells (epithelial stem cells) and HFSCs, maintaining its proliferation and differentiation into different cell types to eventually form a hair shaft. Thus, efficient crosstalk between these two compartments is considered for functional HF formation, regeneration and cycling, mainly through paracrine mechanisms, and has become the theoretical basis of HF regeneration.

DPCs, the differentiated dermal cell at the base of HFs, are originated from fibroblasts. DPCs have the capability to stimulate epithelial HFSCs and are considered to be a master regulator of HF cycling. Several previous studies reported that DPCs isolated from rat and guinea pig vibrissae as well as humans could also induce HF formation when implanted into recipient non-hairy skin, which indicates that DPCs could reprogramme non-hairy epidermis to a follicular fate. Subsequently, DPCs, either fresh or after tissue culture expansion, could also reproduce new HFs if placed in proximity to the epithelium.

Based on their strong HF-inducive ability, many attempts to coculture DPCs with other cell types to regenerate HFs have been studied, such as the two-dimensional juxtaposition of other epithelia, cultured epithelial cells, keratinocytes, corneal epithelium and amnion epithelium. DPCs can secrete various factors to initiate HF formation by activating skin epithelial stem cells (Epi-SCs), so the mixture of DPCs and Epi-SCs also promotes functional HF regeneration in vivo.

DPCs with specific marker molecules possess HF-inducive capacity, including CD133+ DPCs and Versican+ DPCs. CD133+ DPCs have been shown to be a specific subpopulation of cells in DPCs, and they can produce Wnt ligands and mediate signaling crosstalk between the mesenchyme and the epithelial compartment, further promoting adult HF growth and regeneration. In addition, Versican+ DPCs exhibit the typical characteristics of aggregation growth, on which HF formation is highly dependent. In addition, many functional molecules are involved in the positive regulation of DPCs HF-inducive capacities such as endothelin-1 and stem cell growth factor, insulin-like growth factor-1 (IGF-1) and histidine decarboxylase, but matricellular protein connective tissue growth factor (CCN2) negatively regulates HF regeneration, physiologically curbing HF formation by the destabilization of β-catenin. In addition, hedgehog gene activation could shift the dermal fibroblast fate towards DPCs and result in extensive HF neogenesis. Hoxc genes are able to reprogramme DPCs, and a single Hoxc gene is sufficient to activate dormant DP niches and promote regional HF regeneration through canonical Wnt signaling. Monoterpenoid loliolide regulates the HF inductivity of human DPCs by activating the Akt/β-catenin signaling pathway.

Although DPCs possess the potential to regenerate HFs, freshly isolated human DPCs are not efficient in regenerating new HFs when they are directly transplanted. To restore their intrinsic properties, three-dimensional (3D) spheres offer a more physiologically relevant system where cell-cell communication, as well as microenvironments, have been studied. It has been reported that sphere formation increases the ability of cultured human DPCs to induce HF from mouse epidermal cells, in which glucose metabolism and epigenetics may be important regulators. The natural vitamin E form tocotrienol acts upstream of DP formation to induce HF anagen, dependent on E-cadherin loss and activation of β-catenin. Pretreatment with 1α,25-dihydroxy vitamin D3 (VitD3) could significantly improve DPCs functionality and hair folliculogenesis, which is mediated by

the activation of Wnt10b, alkaline phosphatase (ALP) and transforming growth factor β2 (TGF-β2). Consistent with VitD3, platelet-rich plasma has been shown to function in HF regeneration by enhancing DPCs proliferation, which may result from the downregulation of MYC, CCAAT/enhancer-binding protein beta and E2F transcription factor-1 gene. Icariin promotes mouse HF growth by increasing IGF-1secreted by DPCs. Utilization of keratinocyte-conditioned medium, coculture with keratinocytes or the addition of BMP642 and basic fibroblast growth factor (FGF) to DPCS expansion cultures could preserve their HF-inducive capacity. JAK inhibitor regulates the activation of key HF populations, such as the hair germ, and improves the inductive potential of DPCSs by controlling a molecular signature enriched in intact, fully inducive DPCs.

Research Objectives

Accordingly, the objective of the current proposal is to develop of efficient protocol to obtain a sufficient number of highly functional DPCs. To achieve this aim, we will perform the experiments 1) to verify whether CD133 and/or Versican are critical surface functional markers for identifying functional DPCS and thus, 2) to develop protocols to increase functional DPCSs.