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# *Research on the Direct Conversion of Human Skin Cells into Nerve Cells*

**Nenghan Zhang**[1,a,*]

[1]*Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, 710061, China*
*a. zhangnh0826@stu.xjtu.edu.cn*
**corresponding author*

***Abstract:*** The direct conversion of human skin cells into nerve cells is an emerging field with potential applications in regenerative medicine. This study investigates the conversion of Human Skin-Derived Progenitor Cells (hSKPs) and Human Dermal Fibroblasts (hDFs) into neural cells using specific transcription factors or molecular cocktails. A review of the literature was conducted to inform the design of a research study employing a combination of in vitro and in vivo experiments and molecular biology techniques to induce neural differentiation in skin-derived cells. The findings demonstrate that both hSKPs and hDFs can successfully differentiate into neural cells, displaying neuronal morphology and electrophysiological functionality. These studies conclude that skin cells possess significant potential for direct reprogramming into functional neurons, providing a direct approach for studying neurological diseases and developing therapeutic strategies. However, challenges regarding the efficiency and safety of the reprogramming process persist, necessitating further optimization prior to clinical application.

***Keywords:*** Direct conversion, hSKPs, hDFs, Nerve cells

## 1. Introduction

The transformation of skin cells into nerve cells has a potential theoretical basis, given that both cell types originate from the same germ layer during early embryonic development. Furthermore, this process has a convenient practical use in research, as the cells are easily accessible with minimal invasion, less pain, and quick recovery. Experimental evidence has confirmed the direct transformation of skin cells into nerve cells. By reviewing a series of literature, this paper focuses on two types of cells commonly used in research: Human Skin-Derived Progenitor Cells (hSKPs) and Human Dermal Fibroblasts (hDFs). It introduces the characteristics of these two types of cells and outlines the processes of transforming them into nerve cells. The specific methods employed in the transformation processes can be classified into two categories: the introduction of specific transcription factors, such as Neurogenin, or the exposure of cells to different combinations of molecules, including purmorphamine, CHIR99021, BMP4, GDNF and NGF. The discovery that hSKPs and hDFs can differentiate into neural and mesodermal cells in both in vivo and in vitro environments, without the need for any genetic alterations, establishes these cells as a reliable, safe and readily available option for therapies involving cell transplants. Furthermore, it illustrates the potential for their use in the creation of bespoke cell models, thereby underscoring the untapped

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therapeutic potential of these cells in the treatment of neurological disorders and providing insights into the mechanisms underlying cellular transformation.

## 2. Direct conversion of Human Skin-Derived Progenitor Cells (hSKPs) into nerve cells

### 2.1. Definition and characteristics of hSKPs

Human Skin-Derived Precursors (hSKPs) are a population of multipotent adult stem cells isolated from the dermis of human skin. They have demonstrated the potential to self-renew and differentiate into neural cells, including neurons with a peripheral neural phenotype and glia in previous researches [1].

Note that hSKPs are not the same as other stem cells, such as dermal mesenchymal stem cells. First off, dermal mesenchymal stem cells are more widely linked to skin conditions like skin atrophy or skin repair, while hSKPs have been shown to express neural-related proteins and produce neural progenies, suggesting a closer association with neural crest cells and an accessible, abundant multipotent source for disorders of the nervous system like spinal cord injury [2,3]. Second, DMSCs are normally cultivated in an adherent condition with serum, whereas hSKPs mostly grow as floating spheres in suspension serum-free medium supplemented with certain growth factors including bFGF, EGF, and B27, which is comparable to neurosphere culture.

Furthermore, the hSKP floating spheres showed heterogeneity, containing a variety of cell types. A subpopulation of p75 neurotrophin receptor (NTR)+ CD56+dermospheres produced from hSKPs that maintained neural competence were identified by Etxaniz et al. as belonging to the Schwann cell lineage in the adult human dermis. Instead of resident precursor cells, these neurogenic cells are produced by the dedifferentiation of specialized Schwann cells at the peripheral nerve ending in the skin. It is also possible for terminal Schwann cells to act as true dermal stem cells. Additionally, Gresset et al. and Iribar et al. have shown that mature neural crest cells in the skin include populations that resemble stem cells and may be cultivated as floating spheres.[4]

The results demonstrate that floating culture-derived spheres consist of a diverse population encompassing stem cells, progenitor cells, and differentiated cell types. The effect of this cellular diversity on the biotechnological uses of hSKPs is still unknown, though. Moreover, there aren't any trustworthy indicators to differentiate purified hSKPs from other cells at the moment.

### 2.2. Origin and niche of hSKPs

The prevailing concept posits that hSKPs stem from endogenous neural crest precursors that are first present during embryogenesis and continue into adulthood. This hypothesis is supported by multiple lines of evidence: Firstly, direct plating and culture of dissociated single cells from foreskin samples have demonstrated the ability of these dermal cells to differentiate into neurons, glial cells, and smooth muscle cells without the need for passaging or expansion. This capacity for multipotent differentiation is a hallmark of neural crest cells. Secondly, gene expression analysis of hSKPs has revealed the expression of various transcription factors associated with neural crest cells. These include Pax3, Snail, Slug, and the p75 neurotrophin receptor [5,6], which are characteristic markers of embryonic neural crest stem cells and suggest a common developmental program. Thirdly, studies utilizing rodent models have provided further support for the neural crest origin of hSKPs. Research by Fernandes et al. has shown that transplanted SKPs can follow the migratory pathways of the host's neural crest. Additionally, SKPs derived from Wnt1-Cre/Rosa26R transgenic mice, which selectively express reporter genes in cells of neural crest origin, have been found to express these transgenes, indicating that hSKPs share a similar developmental origin with neural crest cells [7].

Fernandes revealed that rodent SKPs were found in the dermal papillae of hair and whisker follicles in 2004. This led to the hypothesis that hSKPs might also reside in a similar niche as rodent

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SKPs.[8] Expanding on this hypothesis, Hunt et al conducted microdissection specifically targeting the dermal papilla from adult human skin and successfully isolated sphere-forming cells.[9] They observed that these microdissected samples exhibited a higher number of spheres and demonstrated superior expansion capabilities compared to whole skin samples. By employing fate-mapping techniques in conjunction with enriched papillasphere generation, they conclusively established the presence of hSKPs within the dermal papillae of hair follicles, thus confirming their role as a niche.[7][10]

### 2.3. Experimental summary of hSKPs' differentiation

The directed differentiation of hSKPs into neurons has been achieved by introducing specific transcription factors like Neurogenin. For example, hSKPs expressed the neural marker ßIII-tubulin and experienced a sequential transition and revealed a neuronal-like shape when cultivated in neurobasal media supplemented with neurotrophic agents such as nerve growth factor, brain-derived neurotrophic factor, and NT-3.[1] Since they expressed p75NTR, a hallmark for peripheral neurons, these neurons were primarily peripheral in nature. A mature central neuronal marker of the K*-Cl cotransporter KCC2 was even expressed by certain neurons produced from hSKPs.Furthermore, it was discovered that neurons produced from hSKPs exhibited synaptic activity, as evidenced by the existence of spontaneous small excitatory postsynaptic currents, and expressed the presynaptic marker synaptophysin. Neurons produced from hSKPs co-cultured with mouse glia were able to produce action potentials and voltage-dependent calcium transients in response to signals from hippocampus astrocytes.[11]

Besides, hSKPs induced by molecules such as purmorphamine,CHIR99021, BMP4, GDNF and NGF can be differentiated into sensory neurons. At the end of differentiation,some genes were significantly up-regulated: the content of PRDM12 was 1000 times higher and the content of BRN3A was 2500 times higher than that in undifferentiated SKPs. By using immunostaining,65% of cells expressed sensory neuronal marker BRN3A and 80% of cells expressed peripheral neuron marker PERIPHERIN.[12]

Neurobasal medium supplemented with N2, forskolin, and heregulin ß was shown to produce a bipolar morphological population expressing S100ß and/or p75NTR and/or CNPase and/or glial fibrillary acidic protein (GFAP) in terms of glial cell differentiation [1]. In vitro co-cultivation of these Schwann cells produced from hSKPs with embryonic rat dorsal root ganglion neurons revealed the ability to induce myelination and express myelin-related proteins.[13]

In conclusion, these researches demonstrated that routine in vitro production of functional SNs-like directly from in vivo adult precursor cells is possible.

## 3. Direct conversion of Human Dermal Fibroblasts into Neurons(hDFs)

### 3.1. Definition and characteristics of Human Dermal Fibroblasts

Fibroblasts are elongated and spindle-shaped cells with high proliferative and migratory abilities. They constitute the main cellular component of connective tissue and serve various biological functions.[14] Fibroblasts are primarily located in the dermis of human skin, which is divided into two layers: the upper papillary layer and the lower reticular layer. The dermis serves as a protective barrier of the organism and dermal fibroblasts play a vital role in maintaining skin function, including processes of wound healing, production and maintenance of the extracellular matrix, participation in the aging process of the skin.[15]

Human foreskin fibroblasts (hFFs) are a kind of Human Dermal Fibroblasts, which can be isolated from neonatal and adult foreskin tissues. They express intermediate filament proteins such as vimentin and nestin, which are characteristic of mesenchymal cells.In addition, they not only have

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the ability to produce extracellular matrix molecules and various bioactive factors that contribute to immune regulation and wound healing, the tissue is also easily accessible, with less ethical controversy and rejection problems. The use of hFFs as a feeder layer in the culture of pluripotent stem cells, such as human embryonic stem cells (hESCs), has been widely adopted to reduce the risk of transmitting pathogens across different species. Additionally, hFFs have demonstrated their versatility in stem cell research and regenerative medicine by successfully generating induced pluripotent stem cells (iPSCs).[16]

### 3.2. Experimental summary of aHDFs in Parkinson's disease population

The research team selected 10 different reprogramming factors, including Ascl1, Lmx1a, Lmx1b, Foxa2, Otx2, Nr4a2 (Nurr1), SMARCA1, CNPY1, EN1, and PAX8,based on their critical roles in normal DA neurogenesis, their expression patterns in the human fetal ventral midbrain, their predictive value for the functional DA differentiation of hPSCs, and their impact on midbrain-specific chromatin modeling. aHDF cells were generated from donors with Parkinson's disease. The reprogramming process was achieved through a specific combination of reprogramming factors with the knockdown of the RE1-silencing transcription factor (REST). To support the maturation and functional expression of the reprogrammed cells, researchers optimized the culture conditions, including the use of specific media and growth factors. Finally researchers detected the expression of neural marker TAU and the DA neuron-specific marker TH in fibroblasts to compare which combination effectively induced the differentiation.

Functional validation through patch-clamp technology also showed that these reprogrammed neurons were not only capable of generating action potentials but also exhibited sodium and potassium ion currents, demonstrating their electrophysiological functionality. By applying these methods, researchers have successfully transformed aHDFs into functional iDA neurons expressing DA neuronal markers . This provides a new cellular model for studying idiopathic Parkinson's disease and demonstrates the potential of future therapeutic strategies.[17]

### 3.3. Experimental summary of Human foreskin fibroblasts(hFFs) in direct conversion

hFFs can be directly reprogrammed into glutamatergic neuron-like cells serially by exposed to a combination of small molecules. The selection of these small molecules was based on their functions in neural fate patterning such as epigenetic modification, metabolic activity, and signaling pathways involved.

The research chose human newborn foreskin fibroblasts, using immunofluorescence analysis to avoid contamination. The reprogram procedure began by applying a combination of small molecules called 16SM, which included compounds like CHIR99021, LDN193189, SB431542 and others. Through a series of experiments involving the sequential removal of individual compounds, the researchers identified an optimal combination of 12 small molecules (12SM) that significantly enhanced the efficiency of neuronal conversion.

The conversion of fibroblasts into neurons was confirmed by the upregulation of neuronal markers, including TUJ1, MAP2, NeuN, and TAU through immunocytochemistry and quantitative PCR analysis. The reprogrammed neurons exhibited distinct morphological changes that are characteristic of neurons. Furthermore, they expressed neurotransmitter-related markers indicative of a glutamatergic phenotype. Importantly, there were no indications suggesting pluripotency or neural stem cell characteristics in these converted cells. These findings strongly supported that a direct conversion process occurred.

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Functional validation through calcium imaging and patch-clamp electrophysiology demonstrated that the reprogrammed cells responded to specific neuronal agonists and exhibited action potentials and ion currents, confirming their electrophysiological functionality.

By applying these methods, the research introduces a novel and effective non-genetic method to chemically stimulate the differentiation of human fibroblasts into cells resembling neurons. These induced neuronal cells (iNs) exhibit typical transcriptional networks associated with neurons, display mature firing patterns, and establish functional connections between synapses. Significantly, when transplanted into the brains of newborn mice, iNs successfully integrate into local circuits.[18]

## 4. Conclusion

This research provides supporting evidence for the conclusion that hSKPs and hDFs can differentiate into neural and mesodermal cells both in vivo and in vitro by introducing specific transcription factors like Neurogenin or being exposed to different combination of molecules rather than genetic alterations. These two types of cells exhibit multipotency, self-renewal capabilities, and can be isolated with minimal invasion from various skin types. They have been shown to differentiate into a range of cell types, including neurons and glial cells and have been found to be poorly immunogenic and possess immunosuppressive properties, which could be beneficial for allogeneic transplantation. While the potential of hSKPs and hDFs for reprogramming into nerve cells is significant, challenges remain. These include ensuring the efficiency and safety of the reprogramming process, achieving the full neuronal functionality of the reprogrammed cells, and addressing ethical considerations associated with cellular manipulation. These researchers not only offer a promising therapeutic avenue but also enhance the fundamental understanding of the nerve system. As the technology matures and our understanding of the underlying mechanisms deepens, the direct conversion of skin cells into nerve cells is expected to make significant contributions to the treatment of neurological disorders including constructing disease modeling and promoting drug discovery in central nervous system disorders. Further research is required to gain a full understanding of the long-term behaviour of hSKPs or hDFs in vivo, to optimize isolation methods, and to explore their mechanisms of action before clinical translation can be achieved. In addition, there is a need to focus on refining the conversion protocols to increase the yield of functional neurons and to explore the long-term integration and safety of these cells in vivo.

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