Chapter 1

Introduction to Hair-Follicle-Associated Pluripotent Stem Cells

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Abstract

Nestin-expressing stem cells of the hair follicle, discovered by our laboratory, have been shown to be able to form outer-root sheaths of the follicle as well as neurons and many other non-follicle cell types. We have termed the nestin-expressing stem cells of the hair follicle as hair-follicle-associated pluripotent (HAP) stem cells. We have shown that the HAP stem cells from the hair follicle can effect the repair of peripheral nerve and spinal cord injury. The hair follicle stem cells differentiate into neuronal and glial cells after transplantation to the injured peripheral nerve and spinal cord, and enhance injury repair and locomotor recovery. When the excised hair follicle with its nerve stump was placed in Gelfoam® 3D histoculture, HAP stem cells grew and extended the hair follicle nerve which consisted of βIII-tubulin-positive fibers with F-actin expression at the tip. These findings indicate that βIII-tubulin-positive fibers elongating from the whisker follicle sensory nerve stump were growing axons. The growing whisker sensory nerve was highly enriched in HAP stem cells, which appeared to play a major role in its elongation and interaction with other nerves in 3D Gelfoam® histoculture, including the sciatic nerve, the trigeminal nerve, and the trigeminal nerve ganglion. These results suggest that a major function of the HAP stem cells in the hair follicle is for growth of the follicle sensory nerve. Recently, we have shown that HAP stem cells can differentiate into beating cardiac muscle cells. HAP stem cells have critical advantages for regenerative medicine over embryonic stem (ES) cells and induced pluripotent stem (iPS) cells in that they are highly accessible from each patient, thereby eliminating immunological issues since they are autologous, require no genetic manipulation, are non-tumorigenic, and do not present ethical issues.

Key words Hair follicle, Bulge, Nestin, Pluripotent, Stem cells, GFP, Neurons, Schwann cells, Sciatic nerve, Spinal cord repair, Cardiac muscle cells

1 Introduction

1.1 Hair-Follicle-Associated Pluripotent (HAP) Stem Cells

Our laboratory discovered nestin-expressing hair-follicle-associated pluripotent (HAP) stem cells. This discovery resulted in a new era of adult stem cells [1].

The totally unexpected serendipitous discovery of nestin-expressing HAP stem cells was with the use of transgenic mice in which the regulatory elements of the neural stem cell marker, nestin, drive the expression of green fluorescent protein (GFP)

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(ND-GFP). We visualized the HAP stem cells by their bright GFP expression in the hair follicle. The HAP stem cells are relatively small, oval-shaped, surrounding the hair shaft and interconnected by short dendrites. In mid- and late-anagen, the HAP stem cells were located in the upper outer-root sheath as well as in the bulge area but not in the hair matrix bulb. Mignone et al. [2] have confirmed these results in ND-GFP mice. Yu et al. [3] showed that nestin was present in human hair follicle stem cells confirming our original observation in mice [1]. Yu et al. suggested that the nestin-expressing stem cells isolated from the human hair follicle are different from keratinocyte stem cells and melanocyte stem cells which were also present in the hair follicle. These observations indicate that the hair follicle probably contains several distinct populations of stem cells [4–6].

1.2 HAP Stem Cells Differentiate to Many Types of Cells

After our discovery of HAP stem cells, we originally predicted HAP stem cells could differentiate to neurons and possibly other cell types [1]. Subsequently, it was demonstrated that nestin-expressing HAP stem cells could differentiate to neurons, glia, keratinocytes, smooth muscle cells, melanocytes, and heart muscle cells [7–13]. The existence of HAP stem cells has been confirmed by at least four laboratories [2, 3, 7, 8, 12, 14–19].

1.3 HAP Stem Cells Can Repair Severed Nerves

Mouse HAP stem cells were injected in the region of a severed sciatic nerve of nude mice which subsequently rejoined. The regenerated nerve recovered function and contracted the gastrocnemius muscle upon electrical stimulation [20]. HAP stem cells can be readily isolated from the human scalp and can be used to regenerate the injured mouse sciatic nerve [16].

1.4 Human HAP Stem Cells

Yu et al. [3, 19] characterized human nestin-expressing HAP stem cells. These cells expressed Nanog and Oct4 which are neural-crest and neuron stem-cell markers as well as embryonic stem-cell transcription factors. The human HAP cells formed spheres in vitro and differentiated into myogenic, melanocytic, and neuronal cell lineages in single-cell culture. The human nestin-expressing HAP stem cells also differentiated into adipocyte, chondrocyte, and osteocyte lineages [8].

When transplanted to a severed sciatic nerve in mice, human HAP stem cells differentiated into glial fibrillary-acidic-protein (GFAP)-positive Schwann cells and promoted the recovery of pre-existing axons, leading to nerve generation upon transplantation to the severed sciatic nerve [16].

1.5 HAP Stem Cells Can Effect Spinal Cord Repair

Mouse HAP stem cells were injected into the injured spinal cord of nude mice. The HAP stem cells promoted the recovery of the

injured spinal cord, by differentiating into glial-like cells with subsequent locomotor improvement [15, 21].

1.6 HAP Stem Cells Originate in the Bulge and Can Traffic to the Dermal Papilla

HAP stem cells are present in the BA throughout the hair cycle, but in the DP only in early anagen where they have apparently migrated from the bulge. HAP stem cells from both regions have very long processes extending from them [21–24].

HAP stem cells from the BA trafficked to the DP as well as into the epidermis, including during wound healing, indicating that the bulge is the source of HAP stem cells [22].

HAP stem cells from both the BA and the DP could differentiate into neurons and other cell types in vitro. HAP stem cells from both the BA and the DP had equal capabilities for functional spinal cord repair into Schwann and neural type cells [21].

1.7 HAP Stem Cells Can Differentiate to Motor Neurons and Reduce Muscle Due to Atrophy Nerve Injury

HAP stem cells expressing RFP were induced by retinoic acid and fetal bovine serum to differentiate, and when transplanted together with Matrigel into the transected distal sciatic or tibial nerve stump of nude mice, differentiated into neurons with large round nuclei and long extensions expressing the neuron marker Tuji1 as well as motor neuron markers Isl 1/2 and EN1. Muscle fiber areas in the HAP stem cell-transplanted animals were much larger than those in control animals. HAP stem cells can thus differentiate into motor neurons and reduce muscle atrophy after peripheral nerve transection [25]. These results suggest HAP stem cells may have regenerative potential for ALS as well.

1.8 HAP Stem Cells Produce Neurons in 3D Gelfoam Histoculture

It was observed over a 2-week period of Gelfoam® histoculture of whiskers that HAP stem cells trafficked from the BA toward the DP area and extensively grew out onto the Gelfoam® forming nerve-like structures [24].

In a subsequent study, mouse vibrissa hair follicles, including their sensory nerve stump, were excised from ND-GFP mice and were placed in Gelfoam® histoculture. HAP stem cells in the nerve stump produced β-III tubulin-positive fibers, extending up to 500 mm from the whisker nerve stump, with their tips expressing F-actin indicating they were growing axons [26].

1.9 HAP Stem Cells Can Differentiate to Beating Heart Muscle Cells

Mouse vibrissa hair follicle were separated into three parts (upper, middle, and lower), and each part was suspended separately in DMEM containing 10% FBS. All three parts of hair follicle differentiated to beating cardiac muscle cells as well as neurons, glial cells, keratinocytes, and smooth muscle cells. The differentiation potential to cardiac muscle is greatest in the upper part of the hair follicle. The beat rate of the cardiac muscle cells was stimulated by isoproterenol and inhibited by propanolol [10].

2 Conclusions

HAP stem cells originate in the BA [1] and migrate to the DP [22, 24] and hair-follicle-associated nerve [26].

Mouse HAP stem cells can differentiate into many cell types including neurons, glial cells, and heart muscle cells [7, 11, 12]. HAP stem cells can effect peripheral nerve [7, 16] and spinal cord repair [15, 21] by differentiating into neural and glial type cells. Human HAP stem cells have also been shown to be multipotent and can effect repair of peripheral nerves [7, 16].

Compared to embryonic stem cells or iPS cells [27–29], HAP stem cells are superior in that they can be used autologously, they are non-oncogenic, and do not have ethical issues.

Recently, Sakaue and Sieber-Blum [30] have shown that highly pure populations of human Schwann cells can be formed from HAP stem cells from the BA of hair follicles. Ex vivo expansion of the HAP stem cells isolated from hair bulge explants and manipulation of WNT, sonic hedgehog and TGFβ expression as well as incubation with growth factors resulted in differentiation to Schwann cells expressing SOX10, KROX20 (EGR2), p75NTR (NGFR), MBP, and S100B by day 4 in virtually all cells. The Schwann cells matured by 2 weeks. In co-culture of the HAP stem cells-derived human Schwann cells with rodent dorsal root ganglia, the Schwann cells interacted with axons. These results confirm our earlier conclusions and results [1, 7, 20, 21, 25, 26, 31].

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