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Epidermal Stem Cells in Regenerative Medicine

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Abstract

Stem cells present in the epidermis, and hair follicle, guarantee the conservation of adult skin maintenance and hair renewal, but they also play a pivotal role in wound repair and tissue regeneration. Adult stem cells present in the epidermis are also responsible for epidermis different layers' regeneration.

We here summarize the epidermal stem cells information in term of their central features in stem cells niche, their signalling pathways and their maintenance, and activation.

Keywords

Regenerative medicine · Stem cell · Wound repair

Abbreviations

BMP-4	bone morphogenetic protein-4
CEA	cultured epithelial autografts
ECM	extra-cellular matrix
iPSCs	induced pluripotent stem cells
JEB	junctional epidermolysis bullosa
MCSP	chondroitin sulfate proteoglycan
SCID	Severe Combined Immunodeficiency
TACs	transient amplifying cells

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1 Biology of the Skin

The skin is the large body organ as well as one of the most vital due to its pivotal role as a protecting structure beside numerous exterior agents and working also as a regulator of the body temperature (Kanitakis 2002).

Skin is constituted of three layers: hypodermis, the deepest one, dermis and the most superficial, the epidermis (Martinotti and Ranzato 2020). The most abundant cells present in the epidermis are the keratinocytes, while other cell types present are melanocytes, Merkel cells and Langerhans cells (Rognoni and Watt 2018).

The epidermis is normally constituted of some sheets that are classified agreeing to keratinocytes level and expression. Keratinocytes originate from stem cells present at basal level migrating from the lower layers as metabolic active cells and transforming into dead cells as they move towards the upper surface (Candi et al. 2005). On the contrary, the dermis structure derived from the extracellular matrix (ECM), formed by fibroblasts. In the dermis, there are also

endothelial cells of the vessels, nerve ending, hair follicles, and cells of the adipose tissue (Ranzato et al. 2013). The hypodermis is the deepest skin layer containing loose connective tissue, blood vessels, nerves and cells that stores fat.

2 Wound Repair Physiology

Wound repair is a well-orchestrated biological phenomenon occurring in all tissues and organs (Martinotti et al. 2012a). The ultimate aim of wound repair is to restore tissue integrity and homeostasis. The wound repair process encompasses a well-adjusted activity of epithelial cells and connective tissue as well as the involvement of vascular cells and inflammatory mediators. These mechanisms strongly require an ECM to assist the repair event. The process of wound repair is normally summarized as four, partially overlapping, parts: haemostasis, inflammation, proliferation, and maturation (Martinotti et al. 2012b).

Tissue repair is a normal process occurring in all tissues, but in difficult conditions, such as different types of burns or during diabetes, this orchestrated event is not sufficient to allow real management.

The skin displays remarkable repair potential thanks to the existence of several stem cells types, present in the skin and its appendages. Some authors have already demonstrated that skin stem cells, localized in the lower epidermis layer as well as in the bulge of follicle hair, are vital sources of new cells for renewal and skin repair (Yang et al. 2019).

3 Epidermal Stem Cell and Wound Repair

Stem cell can be divided in two groups: somatic stem cells and embryonic stem cells. Embryonic stem cells derived from the inner cell mass of blastocyst. They are pluripotent, so with the potential to originate progeny cells of the three germ layers (i.e. endoderm, mesoderm and ectoderm). Somatic stem cells are normally present in tissues or organs. These cells are typically multipotent but the majority of somatic stem cells are one-lineage limited.

In the idea to use potential regenerative abilities of embryonic stem cells, Guenou and co-workers (Guenou et al. 2009) demonstrated that human embryonic stem cells maintained in a medium with ascorbic acid and BMP4 (bone morphogenetic protein-4) could originate keratinocytes of the epidermal basal level, utilized to reconstitute epidermis.

The epidermis is a tissue that undergoes continuous renewal, considering keratinization and exfoliation as natural features of epidermis. This process relies on stem cells resident in the epidermis (Staniszewska et al. 2011).

Skin stem cells are somatic stem cells, but for the several types of skin cells have already been described (Shi et al. 2006).

Subgroups of skin stem cells are:

- *epidermal stem cells*, normally present in the lower layer of the epidermis. They can be divided into transient amplifying cells (TACs) and terminal-differentiated epidermal cells. Specific cell markers are CD71 and chondroitin sulfate proteoglycan (MCSP) (Suzuki and Senoo 2012). TACs divide fast, and they differentiate after some cell division rounds (Hsu et al. 2014). The TAC, or cells that enter the transit stage, are able to generate some differentiated cells, also during wound repair and tissue repair process (Rangel-Huerta and Maldonado 2017).
- *follicular stem cells*. The bulge of the hair follicle is a source of keratinocytes and hair follicles cells. These stem cells are capable of regenerating a number of skin structures including the hair sebaceous glands and follicles (Cha and Falanga 2007). Specific cell markers are CD34, Lgf5, K15, Sox9 (Jaks et al. 2008).
- *melanocyte stem cells*. Melanocyte stem cells originate TACs and differentiated melanocytes (Lang et al. 2013).

In the past decade, important advancement has been made in the identification of stem cells/

progenitors markers for their isolation and enrichment (Barrandon and Green 1987). Through different cell culture approaches, we know that epidermal keratinocytes represent a heterogeneous population for their clonogenicity (Barrandon and Green 1987). In fact, it is possible to recognize different colonies types, and in particular three types such as holoclones, paraclones, and meroclones. These types originate from single keratinocytes due on their proliferative potential.

Holoclones possess the highest growth potential, as well as self-renewing capabilities, giving origin to both meroclones and paraclones (Senoo et al. 2007). Meroclones contain a transitional cell type considered as reservoir of TACs and paraclones (Staniszewska et al. 2011). The passage from holoclone to meroclone to paraclone is described as "clonal conversion" and is usually not reversible (Ojeh et al. 2015).

Despite works regarding the phenotypic features of skin stem cells, the specific control differentiation and proliferation activities are still not completely described. The "stem cell niches," i.e. the microenvironment of stem cells, are fundamental in controlling the propagation, differentiation and migration of stem cells (Spradling et al. 2001). Some signalling pathways are involved and among them Wnt (wingless/integrated) and Notch signalling pathways play a pivotal role for the stem cell "niches" (Kretzschmar and Clevers 2017).

When the skin is wounded, the stem cell niche varies in term of cytokines production, cell signalling involved, ECM alterations and other stimuli, resulting in the stimulation of the regulatory network, including Wnt and Notch signalling pathways in the wound tissue (Zhang et al. 2018).

Generally, more are the remaining skin stem cells on the tissue damaged area, the faster the healing speed, and the less the development of the scar. However, how and which signalling pathways modulate the differentiation and the growth of skin stem cells remain still unclear as well as their relationship with tissue restoration processes and scar development.

4 Skin Stem Cell for Epithelial Repair

In intact skin, keratinocytes do not move. They migrate only vertically in the epidermis, being passively pushed by cells growing at the *stratum basale* and gradually differentiating to keratinocytes.

In response to wound, stem cells represent an important reservoir for wound re-epithelialization. They exhibit lateral migration and cells migrate laterally to cover the wound surface (Staniszewska et al. 2011).

Some studies have already established the epidermal stem cells survival in *in vitro* cell culture system (Ojeh et al. 2015). Dunnwald and co-workers (2001) used *in vitro* cell culture approach to discriminate in mouse three groups of epidermal cells: stem cells, TACs, and not-proliferative basal cells. In fact, only the stem cell population, when seeded with dermal fibroblasts on a collagen type I gel, can induce the formation and maintenance of a normal epidermis for up to 6 months.

Further indications derived from *in vitro* keratinocyte layers use, also known as cultured epithelial autografts (CEA), resulting from skin. In the best conditions, keratinocytes obtained from a 3-cm^2 skin biopsy, are maintained in *in vitro* conditions to produce large, multi-layered CEA after few weeks (usually 3 or 4) in culture (Green et al. 1979).

Epidermal stem cells show the ability to renew epidermis, and they represent a suitable instrument for genetic manipulation, demonstrating a novel treatment option. The group of prof. De Luca utilized genetic modified keratinocytes from patients with junctional epidermolysis bullosa (JEB) on a SCID (Severe Combined Immunodeficiency) mouse model for efficient skin propagation and renewal (Mavilio et al. 2006). The same group showed also that cultures from autologous transgenic keratinocyte are able to regenerate a whole functional epidermis on a seven-year-old child JEB (Hirsch et al. 2017). In this work, the authors by clonal tracing demonstrated that the growth of epidermis is not maintained by equipotent progenitors, but it relies

on a limited number of long-lived stem cells, noticed as holoclones. These holoclones were *in vitro* and *in vivo* able to widely self-renew and to induce precursors that refill terminally differentiated keratinocytes.

5 Induced Pluripotent Stem Cells

Adult differentiated somatic cells (e.g., fibroblasts, skin keratinocytes, as well as other cells) can be organized to produce induced pluripotent stem cells (iPSCs) with comparable features to embryonic stem cells (Aasen et al. 2008). The genetic reprogramming of adult somatic cells could be achieved using retroviral transduction of four transcription factors (c-Myc, Sox2, Klf4 and Oct-3/4) (Takahashi and Yamanaka 2006).

iPSCs have already been utilized to produce a varied range of differentiated cell varieties including melanocytes and keratinocytes (Aguiar et al. 2016; Ohta et al. 2013).

Some results suggest the possible utilization in iPSC-based therapy of skin stem cells (Bilousova and Roop 2014; Dinella et al. 2014). These cells may be successfully incorporated into skin scaffolds to create all cell varieties, components, and skin appendages for the handling of long-lasting wounds and other integumentary system disorders. However, notwithstanding experimental data supporting the positive effects of iPSCs, some safety issues are still unresolved, and they need to be addressed before any large use in a clinical setting. These unresolved issues comprise inefficient cell re-programming, related tumour risk expansion through retroviral vectors utilization, genetic instability, epigenetic memory taken from parent cells, and potential immunogenicity (Okano et al. 2013). However, iPSCs utilization overcomes any ethical and moral issues related to the manipulation of embryonic stem cells.

6 Conclusions

The research strongly needs to elucidate the biology and possible application of skin adult stem cells, to dissect related cellular pathways, and associated extracellular matrix constituents, in order to re-establish troubled skin homeostasis, thus helping in the future progress of more effective therapeutic approaches. Moreover, other basic knowledge as well as clinical trials are essential to promote iPSC-based therapy long-term effects and to offer safer and more effective opportunities for upcoming clinical uses.

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