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REVIEW

Skin Stem Cells; Definition, Function, Importance and Methods of Isolation

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ABSTRACT

Stem cells (SCs) are a population of undifferentiated cells with high self-renewing and differentiation potency. On the basis of origin, SCs are divided into four main groups: embryonic stem cells (ESCs), fetal stem cells (FSCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs). Interestingly, in different literatures, ASCs are considered as unipotent progenitor cells, multipotent stem cells or even pluripotent stem cells with variety of differentiation potential. ASCs reside in many adult tissues such as liver, bone marrow, adipose tissue, neural tissues, skin and etc. Among adult tissues, skin is considered as a fast self-renewing tissue which is capable to reconstruct itself during skin homeostasis and injuries. In fact, skin is mentioned as a pool of different types of SCs including keratinocyte stem cells (KSCs), hair follicle stem cells (HFSCs) and sebaceous gland stem cells (SGSCs). During skin regeneration, cooperation between these stem cells is essential for reconstruction of skin. Among these SCs, KSCs are most common cells in epidermis layer (mostly in basal layer) which are the important population of SCs for regeneration of epidermis. Herein, we reviewed different methods for skin stem cells isolation and characterization, and their potential for clinical application.

Key words: Epidermal stem cells, Keratinocytes, Hair follicle, Characterization, Clonal conversion, Holoclone, Paraclone, Meroclone, Clinical application.

Introduction

Stem cells (SCs) are a population of unspecialized cells with self-renewal ability and differentiation potential [1, 2]. SCs are able to proliferate and differentiate into many cell lineages of tissue of origin that they are derived from [2]. Therefore, applying of SCs has been considering for addressing issues like tissue regeneration, drug screening and organogenesis [3]. Typically, SCs are divided into four main groups based on their tissue of origin: embryonic stem cells (ESCs), fetal stem cells (FSCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs) [2–4]. ESCs are totipotent cells which are derived from the inner cell mass (ICM) of blastocyst and capable to make an entire organism and various cells of three main germ layers (mesoderm, endoderm and ectoderm) [5]. In contrast, ASCs have been considered as unipotent, multipotent and even pluripotent cells which are

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limited in their differentiation potential rather than ESCs, FSCs, and iPSCs [2, 3, 6–8]. In spite of high differentiation potential of ESCs, their applications have been limited due to ethical concerns, tumorigenic potential and difficulties in controlling of their rate of differentiation [2, 9]. Therefore, using of ASCs, as a most promising source for clinical practice and trials, has been developed because represent evidence of safety [9–11]. Generally, ASCs are multipotent cells which are derived from many adult tissues such as liver, bone marrow, adipose, neural tissue, and skin which are essential for regeneration of tissues/organs during various damages [12, 13]. Epidermal SCs, endothelial stem cells (EPCs), hematopoietic stem cells (HSCs), bone marrow stem cells (BMSCs) and neural stem cells (NSCs) are most common ASCs have been employed for therapeutic applications in tissue regeneration [9, 14]. Among different types of ASCs, epidermal or skin SCs are most common cells which are employed for skin repair [15, 16]. As a result, development of scientific knowledge about nature, biological function and importance of epidermal SCs in skin regeneration are required for development of call-based approaches in skin tissue engineering.

Skin stem cells

Basically, skin, as a mechanical barrier against infection and electrolyte loss, contains SCs in order to renew itself in response to external signal (e.ge injury) and also during lifespan of the animal [13, 17]. During skin damages, skin SCs are divided asymmetrically in order to maintain a daughter cell as a SC while producing a differentiated cell which termed transit amplification (TA) cells [18, 19]. Similar to other SCs, skin SCs reside their niches in order to keep balance between SCs and differentiated cell population within skin tissue. Totally, the SC niches refer to a supportive microenvironment in which SCs are quiescent until their equilibrium to be disrupted by an external signal (e.g. tissue damage)[20–22]. As a result, epidermal skin SCs niches appeasers to represent a pool of SCs within skin in order to provide the maintenance of skin homeostasis and hair regeneration during adult life [23]. Skin SCs were categorized into three main groups, including: Interfollicular (IF) epidermal SCs (also mentioned as epidermal SCs or keratinocyte SCs (KSCs)), hair follicle SCs (HFSCs) and sebaceous gland SCs [24, 25] (Figure 1). During continues renewal of adult skin, appropriate function of skin SCs lead to the maintenance of skin homeostasis [26, 27]. Among skin SCs, KSCs are most common population, which are located in most inner layer of epidermis which is called basal layer [24]. KSCs are mentioned as slow-cycling and long-standing cells reside in their niches in the skin [18, 19]. With respect to importance of KSCs in skin regeneration, expansion of knowledge about characterization, importance and function of KSCs as the most common cell population within epidermis layer, will be helpful for future development of cell-based therapy.

KSCs; isolation and characterization

As discussed above, KSCs are main cell populations (95% of cells) within epidermis [28]. Additionally, these cell populations are considered as unipotent SCs (differentiate into one cell type) [8] which can proliferate and differentiate into adult keratinocytes in order to regenerate epidermis [8, 28]. With respect to key role of KSCs in regeneration of epidermal layer, characterization and isolation of KSCs as predominant SCs in epidermis layer is essential for development of therapeutic approaches for skin regeneration in burns and diabetic foot ulcers [29, 30]. As are mentioned before, KSCs in IF epidermis area, an area between hair follicle units, along with HFSCs which are located in bulge area are recognized as high proliferative cells which contribute to skin regeneration during skin wounds [8, 31]. In spite of crucial role of HFSCs in reconstruction of epidermis, KSCs within IF epidermis, but not HFSCs are the principal SCs population which contribute in long-time wound healing [31, 32]. As a result, development of different KSCs isolation methods can help to improve the cell-based therapeutic applications for skin regeneration [33]. For this purpose in mind, first effort for development of a method for more rapidly isolating of KSCs was reported by Jones et al. in 1993 year [34]. Furthermore, application of FACS strategy on basis expression of α_6 integrin and CD71 surface markers to isolate KSCs from skin tissue has been reported by Li and Schlüter et al. respectively [19, 35].

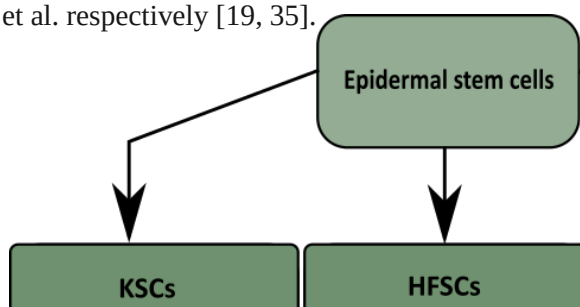

Figure 1: Different types of skin stem cells. Skin is a complicated structure which acts as a stem cell niche and contains different types of stem cells which have distinct differentiated potential, from unipotent stem cells (e.g. KSCs) to pluripotent stem cells (e.g. HFSCs). KSCs are most important cells for epidermis regeneration. Additionally, activation of HFSCs and sebaceous gland stem cells are crucial for regeneration of hair or hair follicle and sebaceous gland cells respectively. Cooperation between these cells is crucial for wound healing during skin regeneration process. HFSCs: hair follicle stem cells, KSCs: keratinocyte stem cells; SCs: stem cells.

Table 1: Different methods for cell isolation. Keratinocyte stem Cells can be isolated basis on, surface markers (MACS method); physical priorities (serial filtration system); rapid adhesion on collagen or any extracellular matrix proteins (adherence to collagen type IV) and floating cells in monoculture (Pop Up isolation method). MACS: magnetic-activated cell sorting), KSCs: keratinocyte stem cells), EVPOME: ex vivo product mucosa equivalent.

Isolation methods	Basis of isolation	Advantages	References
magnetic separation method (MACS)	Immune labeling of 34 CD marker	• Reproducible and profitable method • Acquiring great numbers of viable KSCs • Cost-effective and quick method	[36, 37]
Serial filtration system	Cell size	• Development of a suitable EVPOME for intra and exraoral grafting	[38]
Cell adherence on collagen type IV	Adherence to collagen type IV	• Simplified and effective method for epidermal stem cell • Long-term maintenance of epidermal stem cell culture	[39]
Pop Up isolation method	Pop Up cell in cell suspension	• Generation of large number of KSCs without using trypsin or any enzyme for passaging • Production of small cells which have basal cell progenitor/stem cell characterization	[40]

According to findings of their studies, three distinct subsets of KSCs are recognized within IF epidermis, such as $\alpha_6^{bri}CD71^{dim}$ (termed as quiescent), α_6^{bri} $CD71^{bri}$ (termed as actively cycling or transient amplifying) and α_6^{dim} (termed as early differentiating cells). Since 1993 till now, several

methods for isolating KSCs have been proposed by researcher around the world (Table 1). Generally, development of isolation KSCs methods can facilitate KSCs characterization which in turn development of Stem cell-based therapeutic strategies for treatment of skin disorders such as full-thickness burns and diabetic foot ulcers [29, 41]. With this purpose in mind, Barrandon et al. reported a method for analysis of colony-forming epidermal cells [42]. According the finding of their study, extensive cultivation of epidermal keratinocytes forms three different types of colonies including, holoclones (holo means entire), paraclones (para means beyond) and meroclones

(mero means partial) (Figure 2). Moreover, the finding of Nanba's et al. revealed that these colonies have different growth potential and also holoclone and paraclones are distinguishable by actin filament organization (radial distribution of actin filament in holoclones and peripherally distribution in paraclones) [43]. Additionally, during a molecular mechanism, termed as clonal conversion process, transition from holoclones to meroclones and paraclones result in progression towards cell differentiation [43, 44]. As a result, clonal conversion is an irreversible phenomenon in normal condition [43] which leads to restriction on proliferative potential of KSCs [45]. Therefore, it's essential that gain an insight into the molecular mechanism that control clonal conversion in order to regenerate epidermis by development of cell-based therapeutic approaches for treatment of skin disorders [46]. Moreover, the finding of Barrandon's et al. and Vollmers's et al. Studies revealed that, the IF epidermis contains a mixture of three colonies [42, 47]. Furthermore, Vollmers et al. reported that isolated holoclones are able to create more percentage of holoclones in contrast to isolated meroclones [47]. As a result, it seems holoclones have higher proliferative potential in contrast to meroclones, even though, both of keratin 15 (K15) (as a epidermal stem cells biomarker)[48] and P63 (as a KSCs marker) [49, 50] are expressed in holo- and meroclones [47].

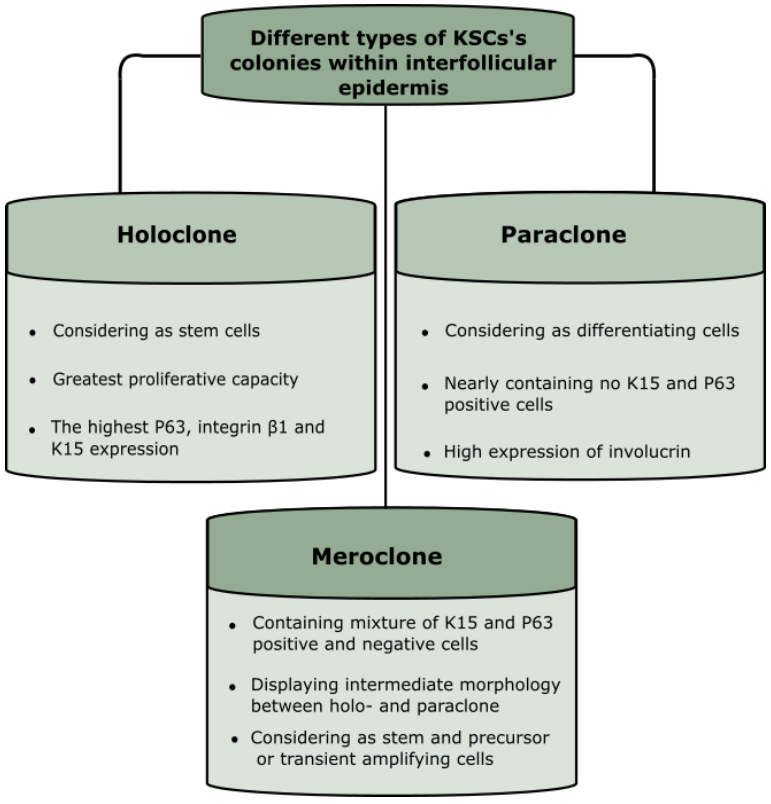

Figure 2: Different types of KSCs's colonies within interfollicular epidermis. According to colony-forming analysis of epidermal cells, KSCs within interfollicular epidermis create three distinct colonies: holoclones (large colonies contain the cells with highest reproductive capacity), meroclones (the colonies which displaying intermediate stage between holo- and paraclones and contain combination of cells with different growth potential), paraclones (small colonies with terminal differentiated cells which have short reproductive capacity). KSCs: keratinocyte stem cells), K15: keratin 15.

KSCs; function and clinical application

It is now very good understood that KSCs are most common cell population within basal layer of epidermis which participate in skin regeneration both in normal and damaged skin [24, 50]. With respect to importance of KSCs in skin regenerative, first efforts for isolation and serial cultivation of KSCs were done more than three decades ago by Rheinwald and Green (R&G) [51] which were applicable clinically [52]. In this method R&G recruited some xenogeneic cells (mouse 3T3 fibroblast cells) as a feeder layer [51]. As a result, KSCs or keratinocyte sheets are prepared by this method are not an appropriate autograft source and also are considered as xenograft source [53]. As a result, application of cultured autologous keratinocytes for clinical application is more desirable [52, 54]. However, application of cultured autologous keratinocytes faces with some difficulties, such as: Taking long time culturing (almost 3 weeks) of keratinocytes in order to preparation of a suitable autograft sheet for grafting, the lack of suitable stability, difficulties in handling and also this autograft sheets have low functional due to lack of dermis [41]. With respect to these limitation, application of KSCs [55] or other SCs types such as mesenchymal stem cells (MSCs) [56, 57] and adipose-derived stem cells (ASCs) [58] in combination with skin substitute is considered as most promising approach for treatment of severe injuries specially extensive burns [59]. Generally, all of these methods focus on development of new strategies for rapidly isolation and cultivation of KSCs without using feeder layers or any xenogeneic materials which are safe and cost effective for clinical applications.

Conclusion

Generally, adult tissues for example liver, intestine and blood undergo rapid self-renewal during life time of animals. In common with these tissues, the human skin is a self-renewing tissue which reconstructs itself during skin injuries. As a matter of fact, the skin is considered as a stem cell pool which variety of SC niches is embedded in it. Among different types of SCs within skin, KSCs play crucial role in epidermis permanent regeneration. As a result, development of isolation, characterization and cultivation methods of KSCs can accelerate clinical applications of KSCs for treatment of skin disorders and injuries. In spite of key role of KSCs in skin repair, application of these cells face with some difficulties and limitations, such as long culturing time, high cost, the probably of highly significant scaring in deep burn injuries which are treated by cultured keratinocytes. With these limitations in mind, application of some strategies such as: recruitment of KSCs along with epidermis layer in order to prevention of high significant scaring, combination of KSCs with scaffolds or skin substitutes as a cell delivery system and integration of melanocytes, endothelial cells even different types of ASCs like mesenchymal SCs and adipose derived-SCs into keratinocyte culture are most promising approaches for development of cell-based therapeutic methods in an improved treatment of burns, chronic wounds and hereditary skin disorders.

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