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Review Article

The Critical Role of Epidermal Stem Cell in Diabetic Foot Ulcer

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Abstract

The diabetic chronic wound healing especially diabetic foot ulcer is the commonest problem faced by both physicians and surgeons. The applications of stem cells opened a new way to heal diabetic wound. In this regard, we focus on the important role of Epidermal Stem Cell (ESC) in diabetic foot ulcer. There were four parts in this paper. First, the special characteristics of diabetic wound healing were summarized. Second, the ESC was introduced briefly. Thirdly, the application of ESC on diabetic foot in lab research and clinical practice was introduced. At last, we combine our own results to outlook the research direction and clinical application of ESC on diabetic foot.

Keywords: Diabetic Foot; Epidermal Stem Cell; Wound Healing

Introduction

The number of diabetes mellitus, especially type 2 diabetic patients is increasing sharply worldwide due to urbanization, lack of physical activity, and population aging, as well as growing rapidly due to central obesity. The delayed wound healing even non-healing is one of the diabetes complications. Particularly the diabetic foot (Figure 1).

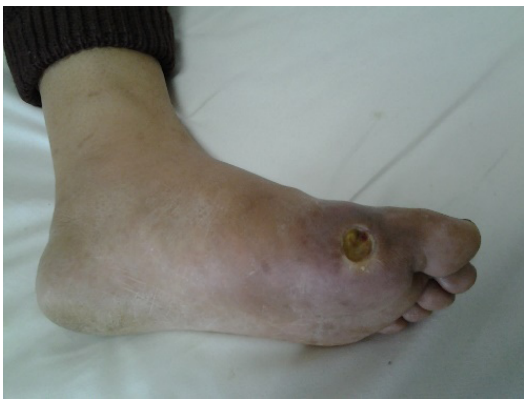

Figure 1: A male patient with diabetic foot ulcer, the wound is deep and difficult to heal.

Become the main course of amputation gradually these years. So, the diabetic foot is also a basic subject in the field of orthopedics and traumatology. This problem not only affects the personal life of the individual but also has a huge economic effect on society [1].

The common therapies that effect on diabetic wound repair have been proposed including adequate surgical debridement, effective antibiotic therapy, correction of metabolic abnormalities, proper moist dressings are essential for healing the chronic diabetic wounds. In addition, the hyperbaric oxygen therapy, electronic stimulation, negative-pressure wound therapy [2] are also for timely and complete healing, presenting a need to improve the diabetic wound healing outcome. Many new medical mechanisms and methods have been utilized in the researching field of diabetic foot recent years. The stem cell technology is one of *de novo* approaches in the pathological mechanism researching. In particular, the critic role of Epidermal Stem Cell (ESC) in wound healing attractive many surgical researchers eye balls. When a new method was applied in basic or clinical medicine, the diabetic specialists often think if it can be used in themselves' studies. So, a systematic evaluation of ESC in diabetic wounds including diabetic foot ulcer will be summarized in this review article.

The Special Characteristics of Diabetic Wound Healing

Wound healing is a complex biological process to restore

the integrity of skin after injury. Normal cutaneous wound repair is characterized by four overlapping phases of healing termed the coagulation or hemostasis inflammation, proliferation or re-epithelialization, and remodeling phases. Hopeful wound healing should include the re-establishment of both skin anatomic structure and physiological function. The ultimate goal for wound healing is a speedy recovery with minimal scarring and maximal function [3].

But the diabetic wound healing is more complex. Especially diabetic foot is particularly prolonged and incomplete, resulting in poor anatomical and functional outcomes. The high level of blood glucose can affect so many tissues and organs of the whole body. It can affect almost every step of the wound healing. The diabetic foot becomes the problem faced by both physicians and surgeons. The diabetic skin wound had its special characteristics: (1) Actually the original structure of diabetic skin is in pathological state related to the high blood glucose condition. The gross skin specimen of diabetic rat was thinner than that of normal rat in our previous observation. The skin of diabetic mouse was also thinner than that of normal one under microscope (Figure 2).

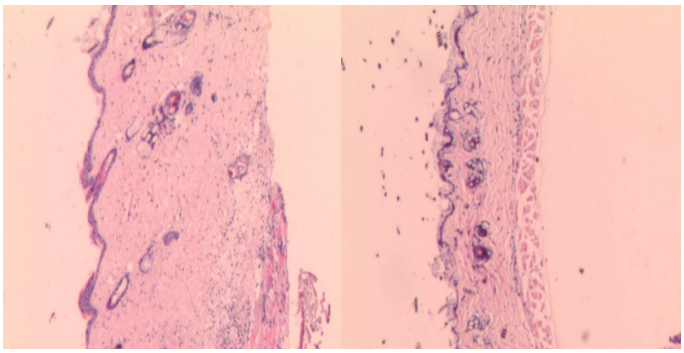

Figure 2: The left was normal mouse skin and the right was diabetic mouse skin under light microscope, HE staining ×40.

In addition, capillary density, collagen fibers and content in the diabetes were significantly reduced compared with the normal ones [4,5]. So, the diabetic skin is abnormal in fact even before wound. (2) The diabetic subjects tolerate infection poorly and infection adversely affects blood glucose control. This repetitive cycle leads to uncontrolled hyperglycemia, further affecting the host's response to infection [6]. The inflammatory mediators, cytokines and chemokines can be changed in the hyperglycemia environment, too [7-9]. The inflammatory mediators including interferon-γ, tumor necrosis factor-α, interleukin-1, C reactive protein, and some cytokines including Hypoxia-inducible Factor (HIF)-1[10], Cycle Oxygenase (COX)-2, Prostaglandin (PGE)-2, β-Fibroblast Growth Factor (β-FGF), Vascular Endothelial Growth Factor (VEGF)-α, nerve growth factor [11], the chemokines including Matrix Metallo Proteinase (MMP)s, stromal derived factor-1 α(SDF-1α) [11], methylglyoxal, osteopontin, syndecans [12,13] will be changed in the hyperglycemia environment. All these factors can affect the diabetic wound proliferation phase. (3) The re-epidermal function of diabetic skin was defect. Both the quality and quantity of ESC decreased in the skin of diabetic subjects. The high level of glucose could affect keratinocytes in epidermis and fibroblasts in dermis directly [14,15].

In addition, the stem cells including mesenchymal stem cell, adipose-derived stem cell endothelial progenitor cells and so on also affected in both structure and biological functions [16,17] followed by abnormal proliferation phase and remodeling process during wound healing. These cells usually played important role in skin wound healing. (4) The impaired neovascularization of diabetes often caused malnutrition of the local wound skin. Especially high glucose related neopathology often induced nerves sensory defect. This can lead to second trauma on lower limbs as a result of sensory impairment. It is the common reason of diabetic foot ulcer. Diabetes also causes structural and functional variations within the artery and capillary systems, notably with thickening of the basement membrane. This thickened membrane impairs leukocytes migration and hampers the normal hyperemic or vasodilatory response to injury and following anti-inflammatory responses, thus simultaneously increasing the susceptibility to injury while also blunting the typical manifestations of such an injury. Due to this blunted neuro-vascular response, diabetic patients were inevitably lack of a crucial component of the body's natural first line of defense against pathogens and wound [13]. (5) Furthermore, fasting hyperglycemia and the presence of an open wound created a catabolic state. Negative nitrogen balance ensued secondary to insulin deprivation, caused by gluconeogenesis from protein breakdown. This metabolic dysfunction impaired the synthesis of proteins, fibroblasts and collagen, and further systemic deficiencies were propagated, leading to nutritional compromise [6]. All these above reasons lead to the difficult healing of diabetic wound.

General Introduction of Epidermal Stem Cell

The skin includes the stratified epidermal and the thick layer of collagen-rich dermal. The epidermis provides the surface barrier, which is enfolded to form various structures including the hair follicle, sebaceous glands, and sweat glands. These places are formed by a stratified epithelium where the position of the ESC within the tissue relates to its state of differentiation [18]. The epidermis, consisting of keratinocytes with variable degrees of differentiation is constantly maintained by the population of self-renewing ESC [19]. These cells can divide to repopulate many cutaneous structures including the epidermis, hair follicles, and sebaceous glands [20]. ESC located not only in epidermal layer, but also in the bulb or matrix of hair follicles and in the bulge or upper outer root sheath [21] (Figure 3).

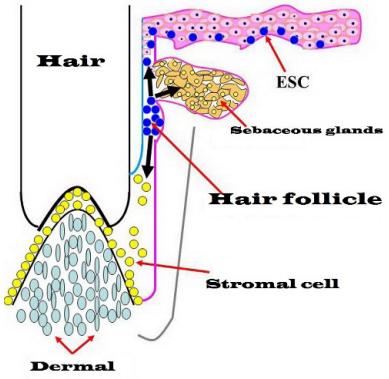

Figure 3: The diagram demonstrates location of ESC in epidermal. The blue particles stand for ESC.

Some reports suggested that ESC is located at the top of the ridges, whereas others suggested a location at the base of the ridge. From a cell migration viewpoint, the latter implied a slightly simpler migratory sequence [20].

Current studies identify at least three distinct populations of ESC in epidermis. These include the Inter Follicular (IF) ESC in the epidermal basal layer, the Hair Follicle (HF) ESC of the bulge and the Sebaceous Gland (SG) ESC located immediately above the hair bulge [22]. The hair bulge is a very distinct structure in the follicle and is a primary source of ESC in the mouse. While in humans the hair bulge is less distinct and IF ESC appears to be the more abundant [23]. The central basal ESC under the so-called 'epidermal proliferative unit' is known to be slow-cycling, and similar units have been shown to occur in reconstituted epidermis consisting of retrovirally tagged epidermal cells [21].

ESC presents in total keratinocyte population at 4-8%. ESC of the epidermis undergoes asymmetric divisions. The effect is self-renewal of ESC and the development of sister cells named Transient Amplifying Cells (TACs), which undergo a limited number of mitotic divisions and ultimately differentiate. The final differentiation into keratinocyte begins after loss of contact with the basement membrane and is related to renewal of the epidermis [24]. The proliferation of ESC depends on growth factors including fibroblast growth factor (FGF)-4, Keratinocyte Growth Factor (KGF), Epidermal Growth Factor (EGF), Hepatocyte Growth Factor (HGF) [25], IL-6, onkostatin M [24], and so on. The morphology of ESC is small with a high nuclear to cytoplasmic ratio [20]. ESC has strong adhesion to basal lamina extra cellular matrix, type IV collagen, or fibronectin. ESC is classically characterized as normally slow-cycling and long-lived in discrete niches in the skin. Slow cell cycle of these resemble somatic ESC prevents the accumulation of mutations. It is important biologically because it conserves the cell's proliferation potential and minimizes DNA replication-related errors. Long life span and proliferation ability provides maintenance and repair of the tissue they reside. ESC can self-renew and is responsible for the long-term maintenance of the tissue. It has a higher proliferative potential capacity than the epidermis [21]. So, ESC can be activated also by wound or by *in vitro* culture conditions to proliferate and to regenerate the tissue. Gene expression studies of niche-resident cells have revealed a number of stem cell makers and regulators, including the Wnt/β-catenin, Notch, p63, c-Myc, Bmp5 [26], Rac-1[27] and Hedgehog pathways. It was generally regard that Wnt/β-catenin regulated the development and regeneration of hair follicles in large wounds [1]. Development of a probabilistic model to cluster genomic sequences based on the similarity of temporal changes of multiple epigenomic marks reveals a variety of rules of dynamic gene regulation during ESC differentiation and proliferation [28].

There is not a clear and confirmed marker of ESC yet mainly because the ESC is lineage-negative cell. The lack of markers for bulge cells hindered the study of this area. Only a few proteins are expressed on both mouse and human bulge cells, including Tenascin C, CD200, Keratin 15, and Keratin 19. The human bulge expresses Bmi-1 and Zfp145, which although not detected on mouse or rat bulge cells, are expressed in cultured rat ESC. The mouse bulge marker CD34, often used for isolating murine bulge cells, is expressed below the bulge region in human hair follicles [18,27]. In the epidermis, typically keratin 5 and keratin14 are expressed in the basal layer, with keratin 1 and keratin10 being found in the suprabasal layer. The hair follicles can express these keratins but also keratins 6, 16, and 17 [20]. We used keratin15 and CD 34 as union markers of ESC in our research on wound healing (Figure 4).

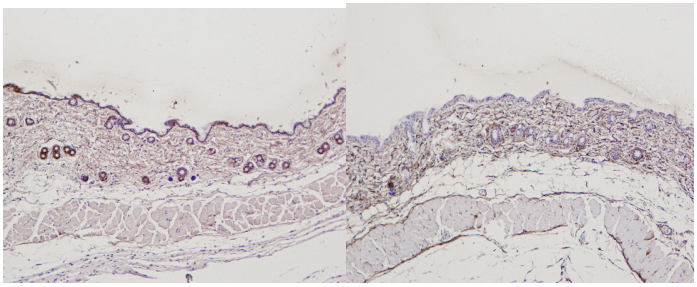

Figure 4: The mouse dorsal skin immunohistochemistry staining (DAB staining, PFA fixed paraffin-embedded sections, × 100). The left is Keratin 15 and the right is CD 34. The deep brown color is the location of ESC.

The Role of ESC in Diabetic Foot Ulcer Therapy

In healthy skin, ESC divided infrequently but upon skin injury, ESC rapidly divided to repair the wound [18]. It was reported by different researchers that the epidermal equivalent prepared from autologous keratinocyte and applied to the surface of the recurrent leg ulcer. The formation of proliferating cell islands was observed. The larger the area of implantation, the faster the re-epithelialization occurred. Patients reported that both pain and wound secretion were reduced significantly and no side effects.

These results suggested that multifocal skin grafting, as well as the transplantation of single-cell suspension, can be very effective treatments for long-lasting leg ulcers. Skin regeneration in chronic non-healing wounds is similar [24].

Zhong et al. reported that the reduction of β-catenin and its downstream target, cyclin D1 in ESCs could lead to poor wound healing in diabetes mellitus rat. Their results suggested that might be one of the important mechanisms of delayed wound healing in DM [29]. So, it may be the therapeutic target for diabetic foot ulcer in the future. Furthermore, their group used Human Amniotic Membrane (HAM) loading labeled BrdU ESC to treat the wound models of diabetic SD rats. They found the wound healing rate of ESC group was significantly higher than those of single HAM or blank control groups. The HAM could supply nutrition to ESC and auto reduce after ESC become epidermal mature. That confirmed ESC had a direct correlation with epidermal migration of wound margin and wound epithelialization in diabetic rats, may contribute to healing of diabetic skin wound. Tissue engineered skins with ESC and HAM may contribute to healing of diabetic impaired wound [30,31]. observed that in diabetic animals, only injected ESC or unsorted epidermal cells could accelerate the restoration of the blood flow. These data indicated that ESC could adopt non-skin phenotypes and functions, and the apparent pluripotency was not lost by differentiation of ESC into transient amplifying cells [5]. In addition, as we know, the substance P was often lower than normal at diabetic situation. observed the effect of sensory neuropeptide substance P combined with ESC on wound healing and nerve regeneration in diabetic rats. They found that joint application of substance P and ESC could effectively promote healing of wound and nerve regeneration in diabetic rats [32]. So, from the opinion of highlighting neovascular pathology of diabetic foot, ESC will be the potential therapy because of its improvement on both blood flow and nerve sensitivity.

But there are some limitations to these studies. First, the wound model of these studies was acute but not chronic diabetic wound. The diabetic animal was very weak. It usually could not be tolerance the second trauma injury. It is difficult to establish the chronic trauma model on diabetic animal. Nevertheless, studies using acute wound healing in diabetic models currently remain the most important tool in expanding scientific knowledge and testing new strategies for repair of non-healing diabetic ulcers, owing to their reasonable cost and maintenance. Second, there is no multi -centers random control trial in clinic yet. So, the data of ESC on diabetic skin wound was not so many.

Outlook at The Research of ESC on The Diabetic Foot

Our research group in laboratory investigated wound healing in db/db diabetic mice. In this model, silver nanoparticles could accelerate dorsal skin wound healing relative to the control group [3]. So, our hypothesis is nano silver particles can promote the proliferate and differentiation of ESC in diabetic foot healing. The details will be published. Studies on diabetic mouse model of chronic wounds have shown that an engineered form of recombinant fibronectin greatly enhances the regenerative effects of administered growth factors [33]. The others reported that mesenchymal stem cells could enhance diabetic rat wound healing through recruitment of tissue regeneration [34]. So, we can union the ESC, the others stem cells and cytokines, as well as combine the prior technique, for example the 3D bio-printing [35,36] or 3D tissue culture technique [19] to repair the deep diabetic foot in dimensions.

The technology of gene transfer to ESC has been reviewed already. The gene therapy and tissue engineering for treatment chronic wound and systemic disorders such as diabetes are also discussed [37]. But the gene therapy focus on diabetic foot is not researched deeply enough. This is the direction of researching diabetic foot furthermore. Due to the superficial location, the skin is so easy to access, and ESC can be expanded in culture acutely. Compare with the other stem cells including embryonic stem cell, hematopoietic stem cell, mesenchymal stem cell and induced pluripotent stem cell, ESC has limited tumor formation and less immune rejection response, especially no ethnics arguments. So, we hope that future studies can lead to a better understanding of the nature and growth regulation of ESC. This exciting laboratory research on diabetic foot care will be successfully translated to the enhancing management in clinic.

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