REVIEW

Fetal Skin Possesses the Ability to Regenerate Completely: Complete Regeneration of Skin

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Until a certain developmental stage, cutaneous wounds in mammalian fetuses heal rapidly without scars with complete regeneration of the skin. In the process of fetal wound healing, inflammatory responses, granulation proliferation, and scar formation that are observed in adults are not seen. Numerous studies have reported the causes of fetal scarless cutaneous regeneration, including reduced expression of TGF-β1 and higher levels of hyaluronan in the extracellular matrix, from the viewpoints of molecular biology and cellular biology, but the mechanisms are not completely understood. Although a variety of substances that inhibit scar formation have been investigated, currently it is almost impossible for adult cutaneous wounds to heal completely without scars. Except for a few animal species, perfect regeneration after wounding can occur only during the gestation period. By strictly comparing the stages before and after the transition from the regeneration of skin to scarring, it will be possible to investigate the mechanisms of cutaneous regeneration. (doi: 10.2302/kjm.2011-0002-IR; Keio J Med 61 (4) : 101–108, December 2012)

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Introduction

Adult mammals are not capable of regenerating defective tissue to the original state, except for a limited number of organs such as the liver. Most wounded organs, once they are injured, can heal with scar formation, but they are not regenerated to their original state. Similarly, in the case of the skin, the dermal structure in wounded areas is disturbed and forms scar tissues that can be definitely distinguished as scars. Unlike normal skin, scar tissue of the skin exhibits characteristic features such as disturbance of the alignment of collagen fibers, excessive dermal fibrosis, disappearance of elastic fiber and appendages, and disruption of skin texture (**Fig. 1**). In contrast, if mammalian fetal skin is experimentally injured during early developmental stages, the wounds heal rapidly without forming scars and the skin is regenerated completely (**Fig. 2**).

In 1954, Hess[1] was the first to show that experimentally wounded fetal skin healed very rapidly with absolutely no scar formation. The major goals of the studies of fetal wound healing are to compare the differences between wound healing in fetuses and adults, to investigate the mechanism of fetal cutaneous regeneration, and to develop a method of regenerating wounded adult skin by utilizing this mechanism. Analysis of fetal wound healing is complicated because development and wound healing progress together. The complex factors of wound healing are added to the active processes of cell migration, cell proliferation, cell differentiation, and apoptosis, so it is important to proceed with analysis of fetal wound healing while always making comparisons with skin at the same developmental stage. In this review, we introduce studies of fetal wound healing that have been carried out on the basis of molecular and cellular biology to give an overview of fetal wound healing and to discuss future prospects in the field.

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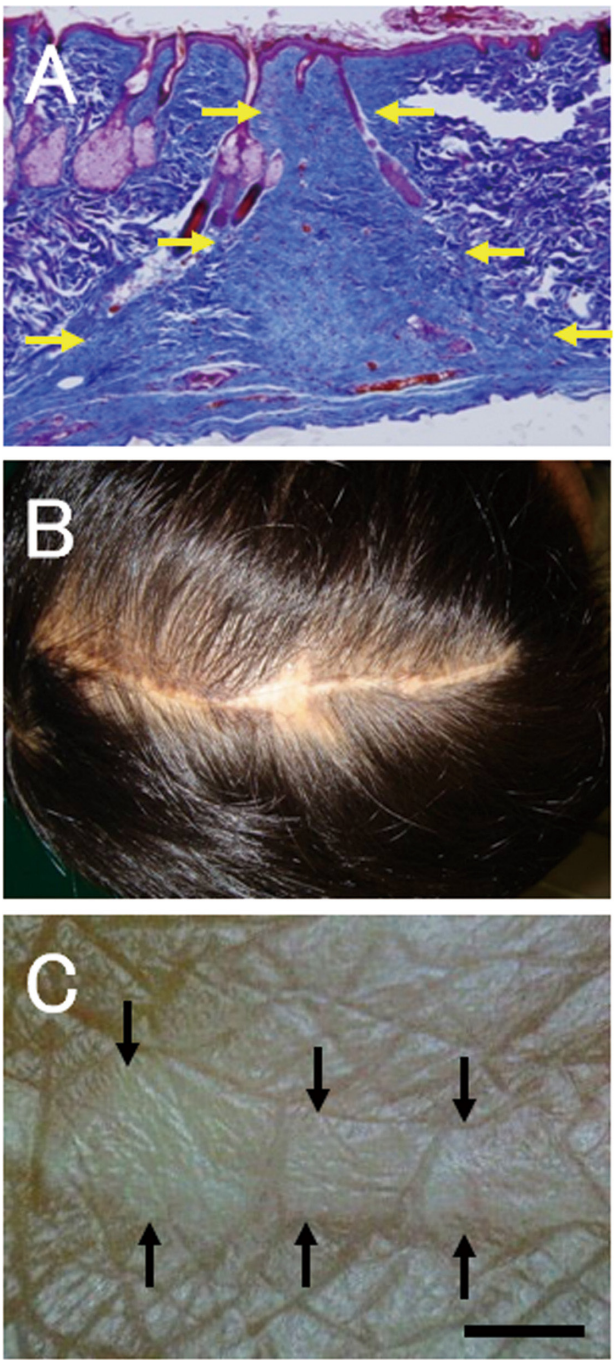

Fig. 1 The characteristics of cutaneous scar tissue.
Disturbance of the alignment of collagen fibers (arrows) (A), disappearance of appendages (B), and disruption of skin texture (arrows) (C) were observed. Bar=1mm.

Differences between Fetal Wound Healing and Adult Wound Healing

Differences between the fetal environment and the adult environment

The mechanism of cutaneous regeneration has been studied based on either the environment surrounding the fetus or the fetal tissue itself. Longaker and co-workers showed that when human fetal skin was implanted subcutaneously in nude mice and wounds were made, the layer was closed by human fibroblasts only, resulting in scarless regeneration.[2,3] Another study demonstrated that when adult sheep skin was grafted on the back of a fetal sheep and wounds were made on the implanted skin after grafting, grafts healed with scarring.[4] These results indicate that adult cutaneous wounds heal with scarring even in a fetal environment, whereas fetal cutaneous wounds in an adult environment undergo regeneration without scar formation. It has thus been shown that the fetal environment is not the main determinant of regeneration, but the fetal tissue itself is the key to regeneration.

Inflammatory responses

It has been reported that fetal wound healing does not cause inflammatory responses. However, when an inflammatory substance was administered in fetuses during the gestation period when scarring does not occur, macrophages were reported to be recruited to the injection sites.[5,6] In addition, in E16 mouse fetuses in which histological cutaneous regeneration is known to occur, macrophages were recruited to the wound surface, but Mac-1-positive activated macrophages were reported to be limited in number.[7] These findings indicate that even at the stage when fetal cutaneous regeneration occurs, inflammatory cells exist and may respond to inflammation.

Nerve dependency

Limb regeneration in urodele amphibians is nerve dependent, and denervated limbs are not regenerated; therefore, nerve-driven factors are related to limb regeneration in amphibians.[8] The nerve dependence of fetal wound healing has also been reported. In lamb fetal limb experiments, the presence of scarring was reported on the denervated side, whereas the skin was regenerated without scarring on the opposite enervated side.[9] From comparisons among cases of wound healing at various gestational stages in mice, we suggested correlations between cutaneous regeneration and nerve regeneration;[10,11] however, it is not clearly understood what nerve factors are involved.

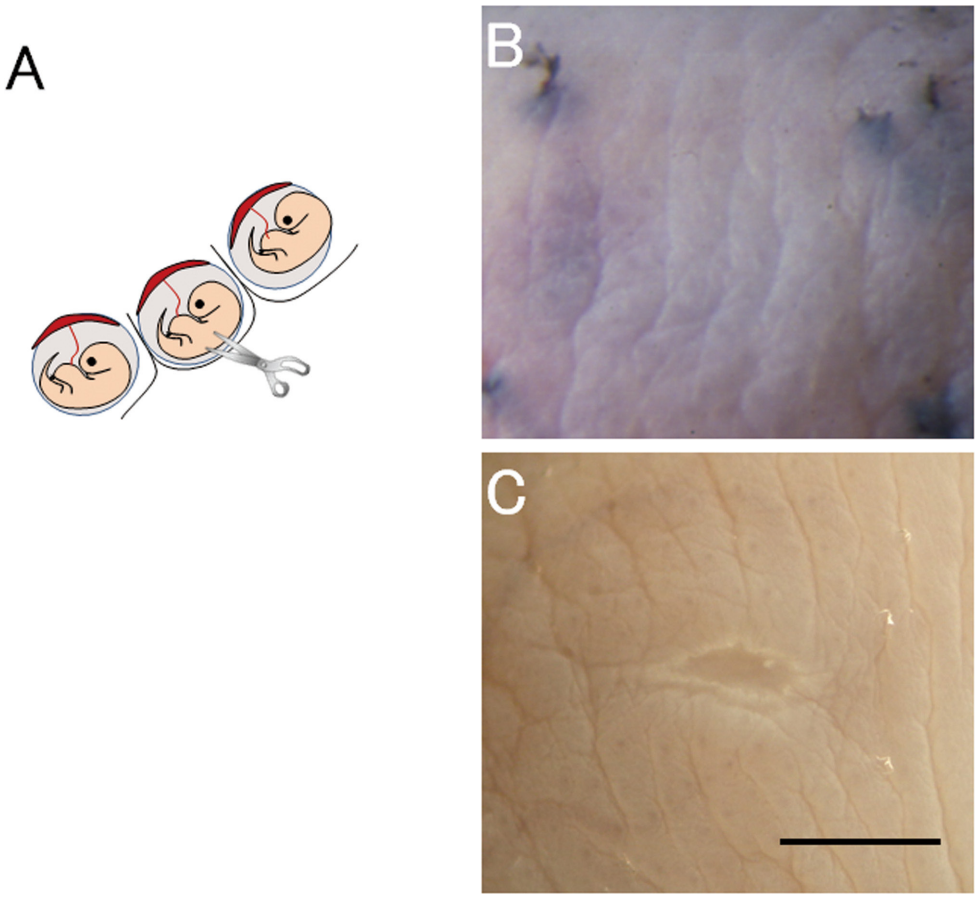

Fig. 2 Mice embryonic day 13 cutaneous wound regenerates completely.
Under a microscope, the flank skin of fetal mice was wounded in utero, and was harvested 72 h later (A). Wounds made on embryonic day 13 healed completely, including the skin texture (B, the wound was made within the area marked by dots). The skin texture did not regenerated when wounds were made after embryonic day 14 (C). Bar=1 mm.

Molecular differences

(1) Growth factors

Many studies have been conducted on the transforming growth factor-β (TGF-β) family in relation to fibrosis. In particular, TFG-β1 is considered to play the central role in scarring. TGF-β1 induces fibrosis in various organs, and injection of recombinant human (rh) TGF-β1 protein in adult skin induces fibrosis. In the adult wound healing process, TGF-β1 (primarily secreted by macrophages) is expressed excessively in wounds.[12,13] When rhTGF-β1 protein was administered in rat wounds, scar fibrosis was intensified, but when TGF-β1 and β2 expressed around rat wounds were neutralized using neutral TGF-β1 and β2 antibodies, scars were reportedly reduced.[14,15] There is no agreement on whether TGF-β1 in the fetal wound healing process is expressed transiently at a very early stage or whether it is not expressed at all. Whitby and Ferguson stated that TGF-β1 was not expressed excessively throughout the process of fetal wound healing,[12,13,16,17] whereas Martin *et al.* reported that it was expressed for a short time at a very early stage in the fetal wound healing process and subsequently it disappeared rapidly.[18] In any case, in fetal wound healing, TGF-β1 is not expressed during most of the cutaneous regeneration process. When rhTGF-β1 was administered to fetal wounds, fibrosis was induced and wound contraction was intensified.[12,17,19,20]

Among other members of the TGF-β superfamily, TGF-β3 was shown to reduce scarring, which is the opposite effect to that of TGF-β1.[21,22] When a TGF-β3(−/−) mouse fetus is wounded, wounds heal with scarring. When rhTGF-β3 was administered in adult wounds, scars were reduced.[23–25]

(2) Extracellular matrix

Hyaluronic acid is a major component of the fetal skin matrix,[26,27] and the increase in the ratio of hyaluronic acid in fetal wounds to that in the surrounding skin was higher than in adult wounds.[27–29] When procedures for decomposing hyaluronic acid in fetal wounds were car-

ried out using *Streptomyces* hyaluronidase, fibrosis proceeded with scarring.[30] In vitro wound healing experiments using a mouse fetal limb tissue culture model at the later gestation period (i.e., when scarring can occur) showed that formation of scars was reduced if the hyaluronic acid concentration in the culture was elevated.[31] In addition, it was reported that hyaluronic acid depresses proliferation of fetal fibroblasts and accelerates migration of accelerates their migration.[32,33] Hyaluronic acid acts advantageously for cellular migration and seems to inhibit inflammation, thus it regulates the microenvironment advantageously for fetal cutaneous regeneration.[34,35] Hyaluronic acid up-regulates type III collagen and TGF-β3 expression. These results suggest that TGF-β3 may promote a fetal-like cell environment that favors scarless healing.[36]

Collagen, which is a major component of extracellular matrix in adult skin, is present at lower levels in fetal skin than in adult skin, but it is more quickly deposited in wounds during fetal wound healing than it is in adult healing. The collagen amino-acid sequence in wounds in fetal wound healing is the same as in normal fetal skin.[37] The quantity of collagen in fetal skin is lower than that in adult skin, but type I and III collagen contents in wounds increased compared to those in peripheral skin in the wound healing process.[38–42] The major reason for increases in the quantity of collagen may be that more cells migrate to wounds rather than that cells synthesize more collagen. The ratio of type III to type I collagen in skin is higher in the fetus than in the adult.[42]

In addition, the relationships of syndecan-1 and -4 and tenascin with fetal wound healing have been reported. Syndecan-1 and -4, which are transmembrane proteoglycans and have important roles as cell surface receptors during cell–cell and/or cell–matrix interactions, are expressed strongly in the adult wound healing process, whereas they are not expressed at all in fetal wound healing.[43] Tenascins, which are extracellular matrix glycoproteins, are expressed strongly in the locations to which cells migrate. Tenascins are expressed in the periphery of wounds both in the adult and fetus; expression occurs early in wound healing in the fetus, but this quickly disappears. Expression is delayed in adults.[44–46]

When human fetal and adult skins are compared, elastin is found not to be present in the fetal dermis, whereas it is present in adult dermis. Most of the differences between fetal and adult skin are found at the level of the expression of dermal extracellular matrix molecules, and the differences in the dermis may be the cause of fetal cutaneous regeneration.[47]

(3) Other molecules

Both PRX-2 and HOXB13 are homeobox genes, and their expression is enhanced in fetal fibroblasts and in fetal skin. In addition, expression of PRX-2 is enhanced in fetal wound healing compared to that in normal fetal skin. In contrast, the expression of HOXB13 in fetal wounds is lower than in normal fetal skin.[48] Recently, it was shown that excision wounds healed completely without scarring in *Xenopus* froglets, and that mononuclear cells expressing prx1 accumulated under the new epidermis of skin wounds. These results suggest that scarless skin-wound healing may require activation of prx1.[49] Hoxd8 expression is also increased in mid-gestational wounds, suggesting that it may play a role in scarless wound repair.[50]

Msx-1 and Msx-2 are other homeobox genes and are expressed in the fingertips in mice. The regenerative ability of mouse fingertips is restricted to areas in which the amputation plane is within the region of Msx1 expression.[51] Msx2-null mice exhibited faster wound closure with accelerated reepithelialization. These results suggest that Msx2 regulates the cellular competence of keratinocytes and fibroblasts in skin injury repair.[52] Increased canonical Wnt signaling is observed during postnatal cutaneous wound repair but not during fetal cutaneous wound repair.[53] However, Wnt-4 expression is increased during fetal and postnatal wound repair (**Fig. 3**).[54]

Cellular differences

Fetal cells actually synthesize growth factors and extracellular matrix to allow tissue to be regenerated. Many studies have explained the mechanism of cutaneous regeneration in the fetus based on the differences between fetal cells and adult cells.

(1) Fibroblasts

Fibroblasts are assumed to play major roles in fetal cutaneous regeneration. Fibroblasts from fetal and adult skin are different in many respects, such as the ability to migrate in response to TGF-β signaling, collagen synthesis in response to TGF-β,[55,56] responses to insulin-like growth factors,[57] and synthesis of hyaluronic acid.[58] Fetal fibroblasts demonstrate the following characteristics that are advantageous for regeneration. Fibroblasts from fetal and adult skin demonstrate a difference in ability to migrate in response to migration stimulating factor (MSF) on three-dimensional collagen.[59] Adult fibroblasts show higher expression of TGF-β1 than fetal fibroblasts do.[60] Collagen gel contraction by IL-1β stimulation is significantly lower in fetal fibroblasts.[61] The tyrosine kinase Shc is expressed in fetal fibroblasts, whereas it is not expressed in adult fibroblasts.[62–64] Fetal fibroblasts inhibit inflammation and wound contraction and have characteristics that are advantageous for fibrosis inhibition. Adult gingiva has an ability to regenerate. Comparative studies of fibroblasts from the gum with fibroblasts from fetal skin have been carried out. Fibroblasts from the gum and fetal fibroblasts have many common features such as the ability to migrate in response to cytokines. Several stud

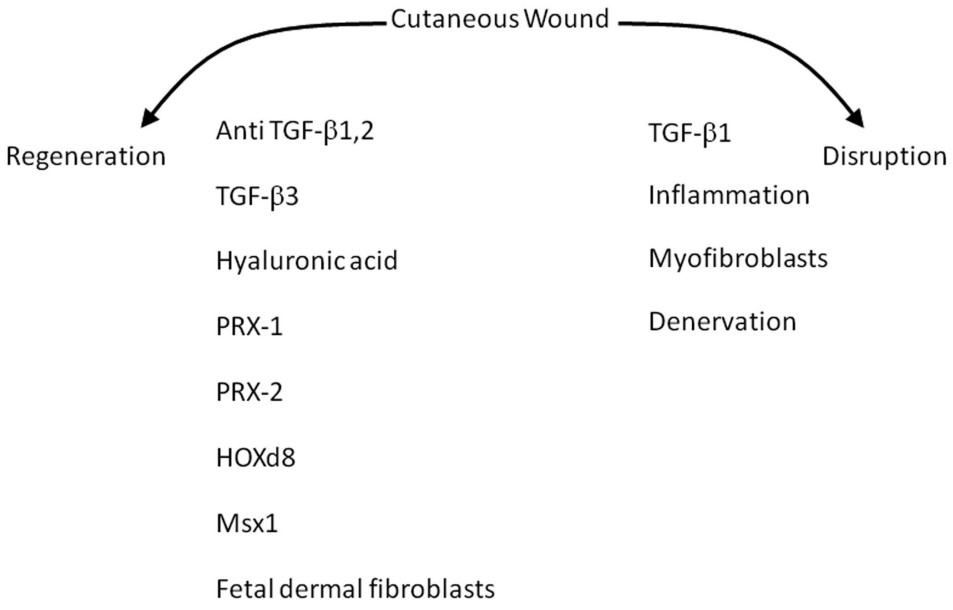

Fig. 3 Summary of fetal cutaneous regeneration.

ies have suggested that features of fetal fibroblasts are related to the inhibition of scar formation. [59,65,66]

As evidence for the fact that fetal cutaneous cells have cutaneous regeneration ability, enzymatically suspended murine fetal mesenchymal cells and epidermal cells were mixed together and transplanted onto skin defects in immune-deficient mice. When epidermal cells and deep fascial cells were mixed together, they did not regenerate skin. However, when dermal cells and epidermal cells were mixed together and transplanted on the skin defects, they regenerated complete skin, including skin appendages.[67] By adding cultured human fetal cutaneous cells to second- to third-degree burn-injured surfaces essentially requiring skin grafting, wounds prematurely close naturally, and clinical reports have described reduced constriction.[68]

(2) Myofibroblasts

Myofibroblasts are assumed to play a major role in wound contraction. Myofibroblasts were not found in wounds created in the mid-gestation period, but they were found in wounds created after the latter portion of the gestation period.[69] In addition, fetal wound healing depends on when wounds are created and the sizes of wounds. As the size of a wound increase, myofibroblasts start appearing in fetal wound healing, suggesting a possible relationship to scar formation.[70] Also, the scar formation in fetal skin derived by denervation was accompanied by a-smooth muscle actin-positive cells.[10]

(3) Inflammatory Cells

As mentioned previously, there are no inflammatory responses in fetal wound healing at an early gestation stage. It was reported that F4/80-positive cells (monocyte-derived macrophages) were found in wounds created after gestation day 14.5 in mice, whereas they did not appear in wounds created before and up to gestation day 14.5.[71] Mac-1 positive cells, which are markers for activated macrophages, do not appear in wounds created before gestation day 16, whereas they start appearing in the latter gestation period.[7] Considering that activated macrophages secrete many cytokines and growth factors that are involved in scar formation in adult wound heating, there is a high possibility that the presence of activated macrophages is significantly related to scar formation.

(4) Platelets

Once wounds are made on the adult skin, platelets are brought into contact with collagen exposed at the wound surface, they become activated, aggregate, and then undergo degranulation to release many cytokines and growth factors to start wound healing. Fetal platelets aggregate, but they aggregate poorly to collagen.[72] Despite this phenomenon, when platelets are in contact with collagen, cytokines are actually released.[73] Reduced aggregation does not explain the fact that there is less inflammation in fetal wound healing; however, the amounts of TGF-β1, TGF-β2, and PDGF-AB released by fetal platelets are much lower than those in adult healing.[74] It was also reported that hyaluronic acid, which is the major component of extracellular matrix in fetal tissue, inhibits aggregation of fetal platelets.[34]

Transition from Regeneration to Scar Formation

It is important to determine when during the fetal gestation period fetal cutaneous regeneration changes to adult wound healing with scar formation and to identify the phenomena that change immediately before and immediately after the transition point. However, in most studies of fetal wound healing, the mechanism of regeneration is explored by comparing the wound healing process at one point during the gestation period with that during the adult wound healing process. There are numerous differences in cell and tissue differentiation between fetal and adult wound healing, thus it is difficult to identify one factor as the direct cause of regeneration.

In several studies, the changes from fetal wound healing to adult wound healing were investigated in several species to determine the transition point during the gestation period. Wounds made on the 16th day of gestation in rats (gestation period: 21 days) were histologically regenerated, but wounds made on the 18th day of gestation were associated with scarring.[75] In Rhesus monkeys (*Macaca mulatta*, gestation period: 165 days) wounds created on the 75th day of gestation on the lips resulted in complete regeneration, and the collagen sequence of fetal derma was regenerated on the 85th to 100th day of gestation, but hair root and sebaceous gland were not regenerated. When wounds created on the 107th day of gestation healed, histological scarring was evident.[76] Therefore, the transition point in many species of animals was found to be in the middle stage of gestation. *Monodelphis domestica*, the gray, short-tailed opossum, was selected because it gives birth when the fetus is at an early stage of development and the fetus then grows in the mother's pouch. Wounds were made in *Monodelphis domestica* at various stages of development after birth, and the transition point from regeneration to healing with scarring was investigated; the transition was observed at about the 9th day after birth. In addition, inflammatory responses of the wounds became prominent at the same developmental stage.[77,78]

Based on the studies mentioned above, the following changes were observed. If wounds are made at an early stage of gestation, the skin, including appendages and panniculus carnosus muscle (PCM), is completely regenerated. If wounds are made at a slightly later stage, the hair bulb and skin texture are no longer regenerated and visible scarring (visible marks) remains.[77,78] If wounds are made at an even later stage, histological scarring remains. Based on the studies mentioned above, it is clear that fetal wound healing changes to adult wound healing, and this process is accompanied by a series of changes.

Transition from complete regeneration to disruption

The timings of regeneration of skin texture and regeneration of the dermal pattern of extracellular matrices have been investigated separately to clarify the transition points, and distinct transition points were found in mice. Until embryonic day (E) 13, murine fetuses have an ability to regenerate injured skin completely. However, when the fetuses were injured after E14, they no longer regenerate skin texture[73] (**Fig. 2**). The causes of regeneration can be elucidated by investigating physical and cellular characteristics that change rapidly before and after the transition point while comparing wound healing immediately before and after the transition point.

Conclusions

Despite the complex nature of fetal wound healing, if comparisons can be made of the relevant processes before and after the transition from regeneration to healing without regeneration, the mechanism of cutaneous regeneration will be elucidated in the future.

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