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Abbreviations and Acronyms

α -SMA = alpha-smooth muscle actin

bFGF = basic fibroblast growth factor

HA = hyaluronic acid

MEK/ERK = mitogen-activated protein kinase/extracellular signal-regulated kinase

NMWA = normalized mucosal wave amplitude

PDGF = platelet-derived growth factor

PI3K/Akt = phosphoinositide-3 kinase/protein kinase B

PLGA = poly(D,L-lactic-coglycolic acid)

PTP = phonation threshold pressure

VEGF = vascular endothelial growth factor

Basic Fibroblast Growth Factor in Scarless Wound Healing

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Significance: The benefit and role of basic fibroblast growth factor (bFGF) in scarless wound healing in clinical application and basic mechanism are discussed. bFGF is a glycoprotein which is widely used in treating wounds and ulcers. bFGF is easily applicable to any type of wound and leads to a better outcome in color, texture, and firmness.

Recent Advances: The amniotic fluids from human mid-gestational trimester may play an important role in scarless wound healing. To investigate cell properties, we used amniotic fluid to augment both adult and fetal fibroblast mitogenic activities, including DNA synthesis and cell proliferation. Preincubation by both bFGF receptor blocker and anti-bFGF antibody significantly decreases proliferative activity in both adult and fetal skin fibroblasts.

Critical Issues: Adult wound healing, to some extent, demonstrates scar formation, leading to unfavorable clinical mismatch of tissue texture and color and causing stiffness. bFGF may improve the outcome of wound healing by normalizing the tissue texture and color to the adjacent intact skin and optimally enhancing wound healing.

Future Directions: bFGF activates ERK and Akt phosphorylation in a dose-dependent manner in both adult and fetal skin fibroblasts, which suggests that bFGF in amniotic fluid plays the most major role in cell proliferation. Application of bFGF from an early wounding stage may lead to better fibroblast proliferation and DNA synthesis through the process of ERK/Akt phosphorylation.

SCOPE

ADULT WOUND HEALING GENERALLY produces scars in the final process after several months or years. The skin repair process is distinct between fetuses and adults. By the second trimester, a fetal wound is healed in a scarless manner. Mammalian experiments demonstrate that intrinsic properties of the fetal tissues are playing a major role. In an effort to understand the fetal environment, human amniotic fluids from the second trimester were tested. Testing revealed the presence of basic fibroblast growth factor (bFGF) along with platelet-derived growth

factor (PDGF), both of which stimulate the DNA synthesis and proliferation of cells. These mitogenic effects are mediated through mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK) and phosphoinositide-3 kinase/protein kinase B (PI3K/Akt) signaling pathways. Intriguingly, there is no difference between fetal and adult fibroblasts to amniotic fluids. This suggests that the cells equally respond to regulatory molecules such as bFGF and PDGF.

bFGF is clinically widely accepted and used in accelerating wound healing, thereby improving scar

quality and regeneration with exogenously administered somatic stem cells. A recent finding is that postoperative color uniformity in split-thickness skin grafting leads to better scar quality of wound healing. The practical use and action of bFGF in scar management is highly important both clinically and in understanding the mechanism of this molecule toward scarless wound healing.

TARGET ARTICLES

1. Chrissouli S, Pratsinis H, Velissariou V, Anastasiou A, and Kletsas D: Human amniotic fluid stimulates the proliferation of human fetal and adult skin fibroblasts: the roles of bFGF and PDGF and of the ERK and Akt signaling pathways. *Wound Repair Regen* 2010; **18**: 643.

2. Akita S, Akino K, Yakabe A, Tanaka K, Anraku K, Yano H, and Hirano A: Basic fibroblast growth factor is beneficial for postoperative color uniformity in split-thickness skin grafting. *Wound Repair Regen* 2010; **18**: 560.

TRANSLATIONAL RELEVANCE

To investigate cell properties, the amniotic fluids from human mid-gestational trimester were tested for both adult and fetal fibroblast mitogenic activities including DNA synthesis, cell proliferation, and possible signaling pathways. Preincubation by both bFGF receptor blocker and anti-bFGF antibody significantly reduced the proliferative activity in both adult and fetal skin fibroblast assays. bFGF activates ERK and Akt phosphorylation in a dose-dependent manner in both adult and fetal skin fibroblasts, and these phosphorylations are significantly regulated by the bFGF receptor blocker, which suggests that bFGF in amniotic fluid plays the most major role in cell proliferation.

CLINICAL RELEVANCE

In adult wound healing, increased inflammation and scar formation are common, whereas the fetal environment up to second trimester is characterized by decreased angiogenesis, absence of scars, and contraction. In adult wound healing, it is best to avoid excessive scars and facilitate wound healing by an exogenous factor. In addition, it is highly advocated to apply the fetal wound healing environment such as amniotic fluid to adult wound healing. In human amniotic fluids, cytokines such as PDGF and bFGF seem to stimulate the cutaneous fibroblast proliferation.¹ Thus, bFGF, a

glycoprotein of approximately 17 Kd in human recombinant form and activated through a distinct seven-spanning membranous receptor, is beneficial in both clinical and objective scar assessment, thus shortening healing time and promoting postoperative color uniformity in split-thickness skin grafting.² bFGF is a potent mitogen, and was first named for its ability to induce fibroblast proliferation. It promotes angiogenesis, and has been shown to downregulate collagen I and tropoelastin mRNA transcription in periodontal ligament fibroblasts and to upregulate hyaluronic acid (HA) synthesis in skin fibroblasts *in vitro*. bFGF has also been shown to potentiate leukocyte recruitment to inflammation sites in skin and to improve dermal wound healing outcomes when directly delivered, either through a targeted peptide delivery system, or alongside tissue engineering scaffold.³ bFGF recovers vocal fold scar deposition in a rat model³ and results in improved phonation threshold pressure (PTP), normalized mucosal wave amplitude (NMWA), and less contraction of the lamina propria in injured larynges in a canine model.⁴ In a mini-swine model, during 6 weeks postoperatively, the combined application of poly(D,L-lactic-coglycolic acid) (PLGA) and bFGF ensured sustained release of growth factor in the target region, and the bFGF-incorporating PLGA scaffold can promote a neovascular formation, enhance blood-flow perfusion, and improve myocardial function, although the original scaffold lumina were eventually occluded by inflammatory cells and scar tissue.⁵

EXPERIMENTAL MODEL OF MATERIAL: ADVANTAGES AND LIMITATIONS

In the skin fibroblast, cells are determined by cell proliferation assay by a Coulter counter, DNA synthesis assay by thymidine incorporation, and cell cycle by fluorescent-activated cell sorting. The G0/G1 arrest decreased in both adult and fetal cells with amniotic fluids but induction of S phase in both cells. DNA synthesis increased two-fold in both adult and fetal cells. The adult age did not seem similar to an influential factor. The cell proliferation assay and regulation by preincubation of bFGF-receptor blocker and anti-bFGF antibody in amniotic fluids demonstrate that bFGF is the most significant factor in this condition in both adult and fetal skin fibroblasts. In the signal pathway, important factors such as phosphorylated ERK and Akt related to cell proliferation demonstrate a time-dependent increase up to several hours in amniotic fluids in both adult and fetal skin cells,

and this pathway is dose-dependently augmented by bFGF. This experimental model is very persuading in cell proliferation with regard to the signaling pathway; however, there are no distinctive data or other cell type than fibroblasts in skin, which is also important in wound healing, including scarless healing but endothelial cells, keratinocytes smooth muscle cells should be discussed.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The bFGF is a multipotential glycoprotein that promotes many cells such as dermal fibroblasts, keratinocytes, endothelial cells, and melanocytes. Due to its mitogenic and angiogenic characteristics, the bFGF can induce tissue remodeling, wound healing and neovascularization. The bFGF efficiently inhibits terminal differentiation to myofibroblast, which is a key mediator when it is activated in keloids and hypertrophic scars, and this is confirmed by a significant decrease of α -smooth muscle actin (α -SMA)-positive cells in the dermal cell culture supplemented with bFGF. The decreased myofibroblasts may also inhibit endothelial/epithelial to mesenchymal transition, because *de novo* activation of quiescent dermal fibroblasts to α -SMA-positive myofibroblast is the most important mechanism in scarring.⁶ In combined use of an artificial dermis, which composes 90% fibrillar atelocollagen and 10% heat-denatured atelocollagen and then cross-linked by heat, the bFGF may be sustained in site with the atelocollagen by a reservoir action of the artificial dermis.⁷ In the study using amniotic fluids, both adult and fetal skin fibroblasts exert a mitogenic action, DNA synthesis, cell cycle, and cell proliferation. This is mainly explained by a strong component of bFGF in the amniotic fluids, as the cell proliferation is significantly inhibited by either bFGF-blocker or anti-bFGF antibody in both adult and fetal fibroblasts. In addition, the signaling pathway is elucidated by MEK/ERK and PI3K/Akt, which are known to mediate the mitogenic action of bFGF. The bFGF as well as PDGF significantly induces both MEK/ERK and PI3K/Akt pathway in both adult and fetal fibroblasts and attenuate by each specific receptor blocker. Additionally, MEK inhibitor and PI3K inhibitor attenuate both cell proliferation and percentage S-phase in both adult and fetal fibroblasts, which explains that bFGF is one of the most remarkable factors in the human amniotic fluids in cell proliferation, which directly mediated via MEK/ERK and PI3K/Akt pathway.¹

The color and texture mismatch of the grafted skin and adjacent recipient is often concerned with patients and social needs, especially exposed areas of the body such as face, upper and lower extremities. In a clinical randomized trial for evaluating postoperative color uniformity in split-thickness skin grafting, the bFGF demonstrates the significantly greater wound healing rate and the superiority in pigmentation, pliability, scar height and vascularity, Vancouver Scar Scale, and the objective color analysis of clarity, red and yellow. The bFGF-treated scar histologically resulted in more organized dermal arrangement with a thick rete ridge and intact basal layer of the epidermis and more arrayed collagen bundle formation by Masson's trichrome staining. The augmented phosphorylated 125^FAK on melanocytes may explain for bFGF action, but further *in vivo* and clinical efficacy should be obtained.²

On the contrary, in a rat study using oral poly-enzyme therapy after laparotomy and abrasion of the visceral and parietal peritoneum reduces the extent of postoperative intestinal adhesions, and this favorable effect can be explained by the lower levels of angiogenic factors such as vascular endothelial growth factor and bFGF or laminin after the administration of hydrolytic enzymes.⁸ Another study in a basal lamina gel incorporated in a neuro-inflammation cell culture model and in a glial scarring around electrodes, serum and inflammatory factors to induce the neural progenitor cell activation and migration to the site of injury, where growth factors such as bFGF and PDGF induce proliferation of cells that eventually form the glial scar formation.^{9,10} These scarring effects via bFGF may be due to the visceral and brain cells to external skin environmental differences, but even in the vocal fold scar models of a rat and in a canine, bFGF decreases the scar formation and improves clinical manifestations. In a rat chronic model, 100 ng of bFGF improves glottal efficiency, vocal fold vibratory amplitude, and area symmetry,³ and in a canine acute model, excised larynx measurements reveals significantly lower PTP and increases NMWA.⁴ In very intractable chronic radiation wounds, the bFGF with autologous adipose-derived stem cells and artificial dermis successfully leads to complete healing in a patient with continuous sacro-coccygeal radiation ulcer for four decades.¹¹

INNOVATION

In an extensive full-thickness burn, immediate resurfacing is highly expected for life saving.

Efficient reconstruction is obtained by applying bFGF and artificial dermis after complete debridement of full-thickness skin to the level of fat. In case of extensive burns, it is often encountered with a limited donor site. In this regard, resurfacing of wide skin defect with bFGF and artificial dermis is clinically useful and important. In 2 years postoperatively, the range of motion of the shoulder is minimally limited with a full stretching of the arm, and, thus, functionally favorable results are obtained.

The bFGF together with artificial dermis and autologous adipose-derived stem cells are used in intractable wounds deep to the bone, tendon, and fascia for several decades. The combined use of cells, bFGF, and artificial dermis are of future use in wound management both for accelerating wound and for improving the quality of the scar. The minimally invasive technique is appreciated in the case of elderly patients with severe basic illness.⁷

SUMMARY ILLUSTRATION

- (A) An 18-year-old man was accidentally burned when his clothes caught fire during his work in the factory. The decompression incisions were performed to assist the respiration. The total body surface area of mainly deep burns (III-degree) was 50% on his chest, abdomen, back, and bilateral upper extremities. The initial surgery was undertaken on the third day in the chest, abdomen, and bilateral upper extremities with split-thickness skin grafting from his thighs, buttocks, and leg.
- (B) On day 6, extensive debridement of his entire back and remaining upper arm tissue was performed, followed by covering drainage-slit artificial dermis (Terudermis®, Olympus Terumo Biomaterials Corp, Tokyo, Japan) with bFGF spraying. Over the slit on the surgical ward and daily dressing changes for 7 days and then good re-epithelialization was observed.
- (C) At 10 weeks, the majority of the back was healed with Spotted well-vascularized raw surface.

TAKE-HOME MESSAGE

Basic science

To investigate the cell properties, the amniotic fluids from human mid-gestational trimester tested both adult and fetal fibroblast mitogenic activities including DNA synthesis, cell proliferation through a signaling pathway. In the amniotic fluids, bFGF is the most powerful factor to increase adult and fetal cell proliferation. Preincubation by both bFGF receptor blocker and anti-bFGF antibody significantly reduces the proliferative activity in both adult and fetal skin fibroblast assays. The bFGF activates the ERK and Akt phosphorylation in a dose-dependent manner in both adult and fetal skin fibroblasts, and these phosphorylations are significantly regulated by bFGF receptor blocker, which suggests that bFGF in amniotic fluid plays the most major role in cell proliferation.

Clinical science

In adult wound healing, it is best to avoid the excessive scars that may be introduced by an exogenous factor. In addition, it is highly advocated to imitate the fetal wound healing environment such as amniotic fluid to adult wound healing. In the human amniotic fluids, cytokines such as PDGF and bFGF seem to stimulate the cutaneous fibroblast proliferation. Thus, bFGF, a glycoprotein of approximately 17 Kd in human recombinant form and activated through a distinct seven-spanning membranous receptor, is beneficial in both clinical and objective scar assessment and shortening healing time as well as postoperative color uniformity in split-thickness skin grafting. Since bFGF promotes angiogenesis, is a potent mitogen, and was first named for its ability to induce fibroblast proliferation, it has been shown to downregulate collagen I and tropoelastin mRNA transcription in periodontal ligament fibroblasts, and upregulate HA synthesis in skin fibroblasts *in vitro*. The bFGF has also been shown to potentiate leukocyte recruitment to inflammation sites in skin, and improve dermal wound healing outcomes when directly delivered, a targeted peptide delivery system, or alongside tissue engineering scaffold. In extensive burns, it is recommended to use both bFGF and artificial dermis in the wide and stable area of the defect to resurface as soon as possible.

Relevance to clinical care

In clinical wound healing, bFGF accelerates any kind of wounds such as pressure ulcer, burns, leg ulcers, and diabetic ulcers. If applied immediately after debridement of ulcer or second-degree burns, the scar quality is remarkably improved by scar assessment, and color match is better in split-thickness skin grafting. Even in chronic and intractable wound treatment, there is appropriate use of either artificial dermis or combined artificial dermis and the cells. As observed in the chronic radiation wound treatment with bFGF, artificial dermis and autologous adipose-derived stem cells are very easy and useful in better scar quality.

- (D) The movement of shoulder flexion is of minimal limitation, and tissue pliability is obtained.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

The bFGF should not be used in patients with cancer. For mucous membranes including eye balls, nose mouse should be protected by bFGF spraying. For any kind of wound, bFGF is recommended and used daily; however, sharp debridement and

sufficient hemostasis just before bFGF use is highly important for successful treatment. After being dissolved in the media solution, it has to be finished within 2 weeks with preservation in the 4°C refrigerator. The combined use of the artificial dermis and stem or progenitor cells is recommended when it is available in the full-thickness skin defects for better wound healing and reduced scar formation, whereas the partial-thickness skin defect such as second-degree burn, bFGF should be started as soon as possible after such an injury.

FUTURE DEVELOPMENT OF INTEREST

Sustained and gradually releasing of the bFGF in a more effective way is expected. Currently, once the bFGF is dissolved in the solution, then it has to be stored at 4-degree Celsius and finished approximately within 2 weeks. It is more stable at room temperature for storage, and prolonged expiration dates of use are clinically favorable. Forms other

than liquid and spraying directly to the wound surface should be developed.

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