A Snapshot of Direct Cell–Cell Communications in Wound Healing and Scarring

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Significance: The repair of wounds usually terminates with a scar. The healing from a severe tissue loss can create a new clinical problem, excessive scarring. Approaches to prevent excessive scarring will optimize the repair process. Controlling gap-junction communications between cells and/or the transport of the proteins that form gap junctions offers new approaches for controlling this problem.

Recent Advances: Gap-junctional intercellular communication (GJIC) requires hemichannels, connexon structures, embedded in the plasma membrane of coupled cells. The connexon is composed of six proteins from the connexin (Cx) family. The docking of connexons between the neighboring cells forms a gated channel, where small molecules can pass directly between the cytoplasm of cells. In wound repair, GJIC between fibroblasts in granulation tissue advances wound repair. Also, the GJIC between mast cells and fibroblasts during the remodeling phase of repair may explain how mast cells promote excessive scarring. In addition, Cx can affect transforming growth factor beta (TGF- β) intracellular signaling through its shared binding site on microtubules within fibroblasts.

Critical Issues: Can excessive scarring be controlled through limiting the local amassing of mast cells or preventing their interactions with wound fibroblasts through GJIC?

Future Directions: The prevention of the accumulation of mast cells in granulation tissue or interfering with their communications via GJIC with fibroblasts offers new approaches for preventing excess scarring. The association of Cx with microtubules altering TGF- β signaling presents a new target for improving the quality of repair as well as the deposition of unnecessary fibrosis.

SCOPE

THE REPAIR PROCESS proceeds through an overlapping sequence of phases: lag, proliferative, and remodeling. Experimental evidence obtained from studying discharged pediatric burn patients, who went on to develop hypertrophic scar, found a disruption in the remodeling phase of repair that preceded the onset of excess scarring.¹ Unlike granulation tissue, which contains mostly myofibroblasts, normal scar contains only fibroblasts and is devoid of myofi-

broblasts. On the other hand, hypertrophic scar is populated with both fibroblasts and myofibroblasts.² The myofibroblast is the prominent cell phenotype, during the proliferative phase of repair. In the development of normal scar, myofibroblasts either undergo apoptosis or revert back into fibroblasts.³ Inflammation is essential for the progression of fibrosis, but in developing hypertrophic scars, neither neutrophils nor macrophages, the major inflammatory cells in wound repair, are

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Submitted for publication February 4, 2013.

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Abbreviations and Acronyms

ALK = activin receptor-like kinase

cDNA = DNA copy of mRNA

Cx = connexin

GJIC = gap-junctional intercellular communication

H&E = hematoxylin & eosin

LiCl = lithium chloride

MC = mast cell

PCLs = populated collagen lattices

PVA = polyvinyl alcohol

RMC-1 = rat mast cell-1

siRNA = small interfering RNA

SMA = smooth muscle actin

Smad = a latent gene regulatory protein, where the name is from Sma in *C. elegans* and Mad in *Drosophila*

TGF- β = transforming growth factor beta

present. The mast cell is the inflammatory cell identified in developing hypertrophic scar.⁴ What is the mechanism for mast cells directing the fibrosis associated with hypertrophic scarring? As demonstrated in scarless fetal repair, eliminating inflammation minimizes scarring.^{5,6} Transforming growth factor beta (TGF- β) is a potent instigator of fibrosis,⁷ increasing fibroblast proliferation and collagen synthesis, as well as the transformation of fibroblasts into myofibroblasts.^{8,9} The required controls of the repair process will benefit from direct cell–cell intercellular communications via gap-junctional intercellular communication (GJIC). Gap-junction channels are composed of proteins from the connexin (Cx) protein family. Cx-43 is the major Cx protein found in fibroblasts and keratinocytes, the major cells in wound repair. Research into intercellular communication via gap junctions in wound healing has introduced new possible approaches in improving the quality of repair.¹⁰

CLINICAL RELEVANCE

Great strides have been made in achieving the survival of the severely burned, but advancements have been limited in preventing the excessive scarring as an expected outcome from the tissue loss of severe burn trauma. The morbidity of hypertrophic scar generates both cosmetic and functional problems that often require return hospital visits for corrective surgeries. The investigation of excess fibrosis associated with hypertrophic scarring has been hampered by the lack of reliable animal models. However, things are looking up with recent reports of hypertrophic scar models in both pigs and mice.^{11,12} Better understanding of the fibrotic process as the hypertrophic scar develops is a reasonable approach for identifying the techniques or procedures for the possible control of the excessive fibrosis associated with excessive scarring. The mast cell, the major inflammatory cell identified in developing hypertrophic scar, appears critical for promoting the fibrosis associated with hypertrophic scar. Gap-junction channels between fibroblasts and myofibroblasts were documented in granulation tissue 40 years ago by Gabbiani and coworkers.¹³ GJIC between mast cells with fibroblasts and/or myofibroblasts within granulation tissue was documented.¹⁴ A more comprehensive understanding of the role mast cells directing profibrotic activities in fibroblasts should generate new approaches for controlling the development of hypertrophic scar. If mast cells through GJIC with fibroblasts are critical for directing excessive fibrosis, then either preventing the amassing of mast cells in

the remodeling phase of repair or blocking GJIC between mast cells and fibroblasts is a suggested approach for preventing excessive scarring.

Another role for GJIC in affecting excessive scarring such as fibrosis applies to TGF- β and Smad signaling. There is experimental evidence showing that transport of Cx-43 from its site of synthesis to its incorporation within a connexon or hemichannel on the cell's plasma membrane involves its finding to a specific site on tubulin within microtubules. The Smad signaling protein, Smad 2/3, binds to the same site on tubulin as does Cx-43 (40). The binding of CX-43 releases Smads 2/3 from tubulin into the cytosol of the cell work dissipates in the Smad signaling pathway.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Gap-junction communications

Here, the exchange of small molecules between keratinocytes and fibroblasts through gap-junction channels is proposed to influence the repair process. Examples of the small molecules that pass between coupled cells include ions, metabolites, and secondary messengers. In addition, the interactions between Cx-43 and microtubules can affect the progression of wound repair. Research in the role of GJIC in open-wound re-epithelialization by keratinocyte migration is better understood than that of GJIC between fibroblasts in the deposition of granulation tissue. Ongoing research on GJIC in fibroblasts opens up new approaches for improving the quality of wound repair and scarring.¹⁰

By the electron microscope, Gabbiani and his coworkers in 1978 were the first to identify the gap-junction channels between myofibroblasts in healing wounds.¹³ Gap-junction channels facilitate the direct exchange of small molecules, having a molecular weight of <1,000 Da, between the cytoplasm of coupled cells. The gap-junction channel is made up of a pair of plasma membrane-embedded hemichannels, connexons, from neighboring cells (Fig. 1A). The hemichannel or connexon is composed of six proteins from the Cx gene family. Humans have 20 Cx family gene members. They are designated with numerical suffixes, referring to their molecular mass, where Cx-43 has a molecular weight of 43,000.¹⁵ The amino acid sequence of Cx-43 is presented in Fig. 1A. Connexons are localized in clusters referred to as plaques on the cell's plasma membrane. Gap junctions are gated channels that rapidly open or close. Cx-43, the major Cx making up the gap-junction channels in fibroblasts and keratinocytes, is the most studied Cx in wound

Figure 1. Gap-junction structure from connexin (Cx) protein to gap junction. (A) The Cx protein passes through the cell's plasma membrane four times, and it has both its N-terminus and C-terminus within the cell's cytoplasm. (B) The connexon is composed of six Cxs embedded in the cell plasma membrane. A gap-junction channel forms by the linking of connexon pairs from neighboring cells 1 and 2.

repair. The role of GJIC is a dynamic one, where both the expression and disappearance of Cx-43 in keratinocytes occur during their migration for re-epithelialization of open wounds.¹⁶

The repair of tissue loss is a key survival element for living organisms that involves the coordinated migration and synthetic activities of different cell phenotypes. A major focus in the study of wound repair has been on the influence on cell activities by released cytokines and growth factors into the extracellular compartment and their eventual binding to specific sites on local cell populations. There are major changes in the GJIC between keratinocytes, during re-epithelialization. Keratinocyte migration was enhanced by treating open wounds with this a topical Cx-43 antisense RNA gel that reduced Cx-43 expression.¹⁷ In that study, both a reduction in inflammation along with minimal

scarring of excisional wounds were noted. The application of a Cx-43 antisense gel, which caused the knockdown of Cx-43, led to a dramatic increase in the rate of wound closure through enhanced keratinocyte migration and a significant decrease in the number of neutrophils surrounding the wound site. This approach of changing the expression of Cx-43 at the wound site opens up new avenues of research, leading to new ways for improving wound repair.

Mast cells

What characterizes the inflammatory response that occurs during the disruption of the remodeling phase repair, which leads to the fibrotic response of excess scarring? Neutrophils and macrophages, the inflammatory cells orchestrating the proliferative phase of repair, are absent in the normal remodeling phase repair as well as in the developing hypertrophic scar. It is assumed that an inflammatory cell would be required to promote a secondary proliferative phase of repair, during the remodeling phase of repair. The inflammatory cell that replaces neutrophils and macrophages is the mast cell.¹² More importantly, mast cell populations are not a feature in normal scars. Because of their presence in hypertrophic scar and their absence in normal scar, the mast cell is the most likely the inflammatory cell candidate in guiding fibroblasts in generating hypertrophic scars. The mast cell, a tissue-dwelling inflammatory cell, has traditionally been associated with allergic responses.¹⁸ There are two subsets of mast cells, MC-T and MC-TC, which are based upon their tissue localization and protein makeup within their cytoplasmic granules. The MC-T mast cell subset is localized in mucosal tissues, and it contains only the protease, tryptase. The MC-TC mast cell subset is localized in connective tissues, and it contains Tryptase as well as the proteases Chymase and Cathepsin G. The MC-TC mast cell subset is assumed important for the development of hypertrophic scar. The most likely mechanism for mast cells promoting profibrotic activities of hypertrophic scarring is through their release of cytoplasmic granules. The speculation is that the release of Tryptase and histamine from mast cell cytoplasmic granules promotes hypertrophic scar.^{19,20} Another mechanism is the mast cells engaging with fibroblasts through GJIC promote hypertrophic scarring.²¹

In vivo gap junctions and Cx-43 expression

The repair of the tissue lost through trauma is essential for survival. With few exceptions, skin loss in adults does not get replaced by the regeneration of new skin, but instead repaired with a

Figure 2. (A, B) Rat polyvinyl alcohol (PVA) sponge implants treated with and without an uncoupler, showing increases in the cell density by hematoxylin & eosin staining and birefringence differences in the organization of collagen fibers: (A) representative of H&E-stained sections from an 8-day control, untreated PVA sponge implant with typical granulation tissue; (B) an uncoupler-treated implant showing a higher density of cells and reduced connective tissue deposition. S = PVA sponge material. (C, D) Representative polarized light microscopy-viewed Sirius red-stained sections of the PVA sponge implants: (C) from a control-untreated implant showing robust birefringence from aligned collagen fibers, typical of maturing granulation tissue; (D) an uncoupler treated implant showing weak birefringence from fine collagen fibers, showing an immature granulation tissue.

patch, a scar. A major focus of wound healing and scar research has been identifying the cellular responses to released cytokines on the resident cells within the repair site. Another approach is to identify the role of GJIC between cells in healing wounds. GJIC influences on wound repair include inflammation and keratinocyte migration.^{22–24} Changes in keratinocyte activities occur through altered GJIC between these cells in the re-epithelialization of open wounds.^{16,25} Soon after injury, alterations in GJIC between keratinocytes occur. Keratinocytes located near the periphery of the open wound lose their ability to express Cx-43. The absence of Cx-43 leads to the loss of GJIC between keratinocytes, which promotes their migration and the eventual resurfacing of the open wound through re-epithelialization.¹⁶ In a mouse study, limiting the expression of Cx-43 in keratinocytes reduces the time needed to close open wounds by re-epithelialization.²⁶

The participation of wound fibroblasts in repair requires their coordination in profibrotic activities that include cell migration, proliferation, protein synthesis, the transformation of fibroblasts into myofibroblasts, and organization of scar collagen fibers into mature connective tissue. In wound repair, GJIC between wound fibroblasts was examined histologically in newly deposited granulation

tissue, using rat polyvinyl alcohol (PVA) sponge implants. Daily injections for 6 days of endosulfan, a potent gap-junction inhibitor that eliminates GJIC between cells, were given, and implants harvested and studied histologically on day 7.²⁷ Treated implants showed an increased density of fibroblasts at the periphery and a diminished cell density within the central portions of sponge implants (Fig. 2A, B). The numbers of myofibroblasts, identified by α -smooth muscle actin (α -SMA) immunofluorescent staining, were reduced within treated implants. Polarized light microscopy of treated implants showed reduced connective tissue birefringence intensity, indicating a lack of organization of collagen fibers (Fig. 2C, D). The histological differences between the untreated controls and endosulfan-treated implants support the notion that GJIC between wound fibroblasts is critical for their transformation into myofibroblasts and the organization of the newly synthesized connective tissue into a scar. The effects of increasing GJIC between wound fibroblasts in granulation tissue were examined. One of the activities of lithium chloride (LiCl) is the promotion of GJIC. LiCl was injected daily for 10 days into PVA sponge implants in rats. Control implants received the same volume of saline injections over the same period.²⁸ All implants were harvested for

histological evaluation on day 11. Connective tissue at the periphery of the treated implants showed a decreased cell density as compared to the saline controls. In addition, Sirius red-stained implants, histological sections that were viewed with polarized light microscopy, showed an intense red birefringence as a result of the collagen fibers arranged in parallel arrays. In control implants, a less intensity of birefringence appeared due to the arrangement of more collagen fibers in random arrays. The more uniform organized newly deposited collagen fibers within the LiCl-treated sponge implants show a more rapid maturation of granulation tissue, which is in agreement with enhanced wound repair. It appears that the promotion of GJIC between wound fibroblasts improves the quality of the deposited scar.

In vitro studies

Experiments with cultured keratinocytes demonstrate the significance of the changing expression of Cx-43 in promoting the locomotion of keratinocytes. Evaluating cell migration in scratch wounds made in confluent keratinocyte cultures was studied. The elimination of Cx-43 expression resulted in the loss of GJIC between keratinocytes, which produced a more rapid migration of keratinocytes and the quicker filling of the gap within the cultured cell scratch wounds.¹⁷

In another experiment, the specific phosphorylation of a single serine residue, S368, in Cx-43 by protein kinase C limits GJIC between keratinocytes. Keratinocytes containing Cx-43 with phosphorylated S368 also migrated faster than the control keratinocytes with Cx-43 containing unphosphorylated S386.²⁹

Contraction of the fibroblast-populated collagen lattices (PCLs) is an *in vitro* model of wound contraction and scar contracture that proceeds through the compaction of collagen fibers by fibroblasts within a connective tissue matrix. Mesenchymal cell PCL contraction is cell density dependent, where lattices with 100,000 cells contracted faster and to a greater degree than lattices with 50,000 cells,³⁰ suggesting that GJIC contributes to lattice contraction. To confirm GJIC occurring between cells incorporated within the collagen lattices, human dermal fibroblast PCLs were cast. At 24 h, an individual cell microinjected with a dye that can only pass between cells through gap junctions passed dye to the neighboring cells (Fig. 3). The microinjection of a single fibroblast, residing at the periphery of the lattice, which is at high density, and a cell residing in the center of the lattice, which is at low density, passed the dye into

neighboring cells.³¹ Fibroblasts incorporated into the collagen lattices communicate with one another through GJIC.

In another experiment, utilizing an osteoblast cell line, the importance of Cx-43 expression and GJIC was studied by measuring the differences in the contraction of transformed osteoblast PCLs.³² Transformed osteoblasts were created by pretreating them with an antisense Cx-43 cDNA sequence. These transformed osteoblasts failed to express Cx-43. Transformed osteoblast-incorporated PCLs were compared to the wild-type osteoblast cells in lattice contraction. Wild-type osteoblast PCLs were reduced to 36% of their initial area, while transformed osteoblasts that failed to express Cx-43 were reduced to only 68% of their initial size. Osteoblasts, failing to express Cx-43, were retarded in compacting collagen fibrils. Fibroblast PCLs treated with the uncouplers of gap junctions, which block GJIC, also contracted less than untreated fibroblasts PCLs.³¹

The relationship between GJIC with fibroblasts in wound healing is not as well studied as that of re-epithelialization closure of open wounds by keratinocyte migration. In human fibroblasts, cultures inhibiting GJIC between fibroblasts limit their expression of α SMA and synthesis of procollagen.³³ Cultured fibroblasts maintained in a serum-free medium, with oleamide, a natural inhibitor of GJIC, showed inhibition of Type I collagen as compared to the cells maintained in a medium without oleamide. Interfering with GJIC between fibroblasts inhibited collagen synthesis, but not

Figure 3. Fluorescent microscopy of fibroblasts in fibroblast-populated collagen lattice injected with a dye. A microneedle (on the left of the image in a V-shape) injected Lucifer Yellow dye 2 min earlier to a single fibroblast. The initial injected fibroblast located in the center of the panel shows the most-intense fluorescence. Below the brightest, injected fibroblasts are two less-intense fluorescent fibroblasts, which received dye from that first-injected fibroblast via gap-junctional intercellular communication.

fibronectin synthesis. When an oleamide-enriched medium was replaced with a oleamide-free medium, collagen synthesis was restored, showing that oleamide inhibition of collagen synthesis was reversible. These findings support the concept that collagen synthesis is optimized by GJIC between fibroblasts.³³

TGF- β and GJIC

Besides Cx-43 involvement with GJIC between fibroblasts, Cx-43 influences TGF- β intracellular signaling.³⁴ The cytokine TGF- β enhances profibrotic activities through promoting fibroblast proliferation, collagen synthesis, the transformation of fibroblasts into myofibroblasts, and fibroblast PCL contraction.^{9,35} For TGF- β to advance the transcription of genes related to these profibrotic activities, it initially binds to a specific fibroblast cell surface receptor, which combines with a second cell surface receptor. The complex initiates intracellular kinase activities that lead to the phosphorylation of members of the Smad signaling pathway family of proteins.³⁶ The activin receptor-like kinase (ALK) 5 phosphorylates Smad 2/3, a critical early step in promoting granulation tissue synthesis.³⁷ A competitive inhibitor of ALK 5 that interrupts the Smad signaling pathway blocks the phosphorylation of Smads 2/3 and TGF- β profibrotic activities.³⁸ In rat PVA sponge implants, blocking the Smad signaling pathway through inhibiting ALK 5 kinase prevents the transformation of fibroblasts into myofibroblasts and inhibits connective tissue synthesis.³⁹ TGF- β signaling through the Smad signaling pathway has a relationship with Cx-43. The transport of Cx-43 from its site of synthesis at the rough endoplasmic re-

ticulum to its insertion within a connexon on the cell plasma membrane requires direct attachment to a specific tubulin site on microtubules.⁴⁰ The amino acid sequence where Cx-43 binds to microtubules happens to be the same site, where Smad 2/3 binds. When Cx-43 binds to tubulin in microtubules, it kicks off Smad 2/3 into the cell's cytosol compartment, the cell cytoplasm's soluble compartment. If Smad 2/3 remains sequestered on microtubules, it prevents its participation in the Smad intracellular signaling pathway. Both the inhibition of Cx-43 synthesis or preventing Cx-43 attachment onto microtubules will lead to the sequestering of Smad 2/3 and its absence from the cell's cytosol, both inhibiting TGF- β intracellular signaling. The capacity of Cx-43 to positively mediate TGF- β signaling shows another possible avenue for Cx-43 influencing wound repair and scarring.

Mast cells communicate with fibroblasts by gap junctions

The coculture of mast cells with fibroblasts in collagen lattices enhances lattice contraction.⁴¹ GJIC between fibroblasts and a human mast cell line, HMC-1,⁴² was shown as the possible mechanism for mast cell enhancement of cocultured PCL contraction.⁴³ Loading a fluorescent dye, Calcein AM green, which can only escape from loaded cells through gap junctions into a coupled cell, demonstrates GJIC between mast cells and fibroblasts. The plasma membranes of mast cells collected from a rat's peritoneal cavity fluid were initially, permanently tagged fluorescently red. The cytoplasm of the red-fluorescent-tagged mast cells was then loaded with Calcein AM green dye, which can only

Figure 4. Rat mast cell-1 (RMC-1) in culture viewed by phase-contrast microscopy, immunostained for Chymase, and dye-loaded RMC-1 passing dye to fibroblasts in monolayer culture. **(A)** A confluent monolayer of RMC-1 having a cobblestone appearance, which is typical of cultured mast cells. **(B)** RMC-1 that were immune-stained for the proteinase marker of mast cells, Chymase. **(C)** Two round intense brightly appearing RMC-1 that were placed in cultured fibroblasts 3 h before taking the photograph. The two cultured fibroblasts are fluorescent as a result of the dye passing from the RMC-1 to the fibroblasts via gap-junction channels.

escape from mast cells via GJIC. The fluorescent red- and green-tagged rat peritoneum-derived mast cells were injected into 7-day-old rat PVA sponge implants. The green dye can pass between coupled cells through GJIC channels, but red fluorescence cannot escape from the plasma membrane of tagged mast cells. One day later, the implants were harvested, frozen sections cut, nuclei stained with fluorescent blue, and sections viewed with a fluorescent microscope. All cells populating the granulation tissue within sponge implants had fluorescent blue nuclei. The generation of GJIC between mast cells and fibroblasts was documented by the presence of cells with fluorescent blue nuclei and a green fluorescence cytoplasm, but no red fluorescence plasma membrane. The double-fluorescent-tagged mast cells, displaying both red and green fluorescence, were distinguished from the green fluorescence fibroblasts.¹⁴ The study that implicates GJIC between fibroblasts and mast cells is responsible for the transfer of green dye from mast cells into fibroblasts.

Our laboratory established a mast cell line derived from mast cells collected from the rat peritoneal cavity washes.²¹

The freshly harvested rat peritoneal cells were placed in culture dishes with a medium supplemented with a spent medium from cultured human HMC-1 mast cells and bovine serum to maintain their viability. No cytokine supplements were required to promote the growth of these rat mast cells. After 4 weeks, rat mast cells (named RMC-1) were the exclusive cell type growing in the culture dishes. Phase-contrast microscopy revealed a confluent cobblestone layer of cells displaying prominent cytoplasmic granules (Fig. 4A). Characterization of cells as mast cells was the presence of c-kit on the cell's plasma membranes as well as immune histological staining of the mast cell proteinase Chymase (Fig. 4B). Identifying these cells as MC-TC mast cells is documented by the presence of the Chymase within cell granules as well as by western blot analysis. The RMC-1 was maintained in a basic culture medium with supplements limited to antibiotics and bovine serum. No cytokine supplements were required to maintain the viability of RMC-1. The coculture of RMC-1 with fibroblasts in monolayer culture showed the development of GJIC between these different cell types (Fig. 4C). Fibroblasts in coculture with RMC-1

TAKE-HOME MESSAGES

- In addition to the influence of cytokines binding to fibroblasts and keratinocytes at specific receptor sites, which affects the repair process, keratinocytes and fibroblasts are influenced by GJIC. In regard to keratinocytes, the absence of gap junctions promotes their migration and the closure of open wounds by re-epithelialization. In regard to fibroblasts within granulation tissue, GJIC between wound fibroblasts promotes the generation of granulation tissue with the synthesis and deposition of connective tissue.
- A major cytokine in fibrosis is TGF- β . TGF- β promotes profibrotic activities by utilizing the intracellular Smad signaling pathway. A critical pair of Smad proteins is sequestered on microtubules, where they are prevented from participating in TGF- β intracellular signaling. Their release from microtubules into the cell cytosol compartment is required for their participation in TGF- β intracellular signaling. The transport of Cx, the protein critical for creating connexons or hemichannels, which form gap-junction channels, utilizes the same binding site on microtubules as Smad 2/3. The binding of Cx to tubulin within microtubules releases bound Smad 2/3 into the cell's cytosol compartment. TGF- β intracellular signaling requires Smad 2/3 localized within the fibroblast's cytosol.
- Hypertrophic scar is an example of excessive fibrosis. Hypertrophic scar is a common problem in patients who have recovered from severe burns. The success of controlling this problem may involve controlling GJIC forming between wound fibroblasts and mast cells, which are the major inflammatory cells identified in developing hypertrophic scars. Either eliminating mast cell populations in developing hypertrophic scars or preventing their association with fibroblasts through GJIC are two possible approaches for the control of excessive scarring.

showed enhanced profibrotic activities; instigated through GJIC, as demonstrated by increases in collagen synthesis, fibroblast proliferation, and collagen lattice contraction, as well as promoting the transformation of fibroblasts into myofibroblasts. To eliminate the role of cytoplasmic granules in promoting profibrotic activities, granule-free RMC-1 were generated by pretreating RMC-1 with the Secretagogue Compound 48/80, which purges mast cells cytoplasmic granules, creating degranulated mast cells.⁴⁴ Intact RMC-1 or degranulated RMC-1 were introduced to the human dermal fibroblasts in monolayer culture. Fibroblasts cocultured with RMC-1 devoid of cytoplasmic granules promoted increases in collagen synthesis, fibroblast proliferation, collagen lattice contraction, and the transformation of fibroblasts into myofibroblasts, which were the identical profibrotic activities generated by human fibroblasts cocultured with intact RMC-1. This finding does not support a role for mast cell cytoplasmic granules in promoting profibrotic activities, when cocultured with fibroblasts. To implicate the involvement of GJIC between fibroblasts with RMC-1 in promoting profibrotic activities, RMC-1 were pretreated with

a long-acting GJIC inhibitor that generated the intracellular accumulation of oleamide, a GJIC uncoupler. RMC-1 with high levels of oleamide that were unable to form GJIC with fibroblasts failed to enhance the profibrotic activities in RMC-1–fibroblast cocultures. The failure to form GJIC between RMC-1 and fibroblasts substantiates a possible role for GJIC between mast cells and wound fibroblasts in the development of profibrotic activities, required for the development of hypertrophic scars.^{21,45}

The knockdown of Cx-43 expression in RMC-1 by Cx-43 small interfering RNA (siRNA) completely blocked GJIC between RMC-1. siRNA knockdown of Cx-43 in human dermal fibroblasts blocked the expression of Cx-43, but only dampened GJIC between fibroblasts.⁴⁶ In fibroblasts treated with Cx-43 siRNA, the expression of other Cxs has the capacity to substitute for Cx-43 in GJIC.⁴⁷ These other Cxs were able to retain a low level of GJIC between fibroblasts; on the other hand, RMC-1 only express Cx-43 and cannot engage in GJIC. siRNA-treated RMC-1 in coculture with fibroblasts were unable promote profibrotic activities, such as the expression of α SMA within fibroblasts. GJIC between mast cells and fibroblasts promotes profibrotic activities in cocultured fibroblasts.⁴⁶ Cx-43 is critical for GJIC between mast cells and fibroblasts. Since Cx-43 expression in RMC-1 is responsible for GJIC, controlling Cx-43 expression in mast cells is a possible approach for preventing the stimulation of profibrotic activities by mast cells in the development of hypertrophic scar. Early in wound healing, the re-epithelialization of an open wound by keratinocytes benefits from the absence of Cx-43 expression. During the remodeling phase of repair, inhibiting Cx-43 expression in wound

mast cells should prevent mast cell promotion of profibrotic activities in resident fibroblasts.

SUMMARY

In summary, experimental evidence derived from both cell culture and animal studies demonstrate that GJIC participates in wound repair. Gap junctions have been established between keratinocytes, fibroblasts, as well as between fibroblasts and mast cells. In re-epithelialization wound closure, optimal keratinocyte migration proceeds in the absence of Cx-43 expression and the loss of GJIC between keratinocytes. In granulation tissue, GJIC, between wound fibroblasts, has been visually demonstrated by the passager of dye between coupled cells and by the transmission electron microscope. GJIC optimizes the deposition of granulation tissue. Recently, the identification of GJIC between fibroblasts and mast cells has produced evidence, suggesting mast cells promoting excessive fibrosis associated with hypertrophic scarring via GJIC. There is experimental evidence suggesting a role for the formation of connexons, hemichannels, on the fibroblast's plasma membrane and the Smad signaling pathway.

AUTHOR DISCLOSURE AND GHOSTWRITING

The author has no commercial associations that might create conflict of interest with the article. No ghostwriters were used in preparation of the article.

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