Role of galectins in re-epithelialization of wounds

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Abstract: Re-epithelialization is a critical contributing process in wound healing in the human body. When this process is compromised, impaired or delayed, serious disorders of wound healing may result that are painful, difficult to treat, and affect a variety of human tissues. Recent studies have demonstrated that members of the galectin class of β-galactoside-binding proteins modulate re-epithelialization of wounds by novel carbohydrate-based recognition systems. Galectins constitute a family of widely distributed carbohydrate-binding proteins with the affinity for the β-galactoside-containing glycans found on many cell surface and extracellular matrix (ECM) glycoproteins. There are 15 members of the mammalian galectin family that so far have been identified. Studies of the role of galectins in wound healing have revealed that galectin-3 promotes re-epithelialization of corneal, intestinal and skin wounds; galectin-7 promotes re-epithelialization of corneal, skin, kidney and uterine wounds; and galectins-2 and -4 promote re-epithelialization of intestinal wounds. Promising prospects for developing novel therapeutic strategies for the treatment of problematic, slow- or non-healing wounds are implicit in the findings that galectins stimulate the re-epithelialization of wounds of the cornea, skin, intestinal tract and kidney. Molecular mechanisms by which galectins modulate the process of wound healing are beginning to emerge and are described in this review.

Keywords: Wound healing; non-healing wounds; chronic wounds; re-epithelialization; galectins; carbohydrate-based recognition

Re-epithelialization of wounds

Relevant in a variety of clinical scenarios, impaired wound healing remains among medicine’s most frustrating therapeutic challenges. Healing defects may occur in organ systems as different as cornea, skin and gastrointestinal (GI) tract (1-6). These are but a representative three of the number of human organ systems which may be threatened by impaired or delayed re-epithelialization which results in persistent epithelial defects. This defines a condition with serious medical implications. However, scientific effort has yet to comprehensively explain the failure of some, and not other, wounds to heal within a reasonable course of time. Meanwhile, patients with debilitations caused by a range of wounds, from relatively obscure to commonplace causes, disease-associated, accidental, surgical or inflicted (for example of combat), rely on what we know to support their treatment. Resolution of chronic wounds of various etiologies can be frustrating and may not always be successful. Around the world, millions of individuals are affected and in the United States alone, combat-related and other traumatic wounds cause over 300,000 hospitalizations each year (7,8).

Persistent corneal epithelial defects may undermine the integrity of the anterior stroma, produce ulceration and in the direst cases cause perforation of the stromal tissue with significant visual loss. The insidious damage of delayed re-epithelialization and resultant persistent epithelial defects are also evident in the chronic wounds of the elderly, decubitus ulcer, and venous stasis ulcer of the skin. Impairment of the intestinal surface barrier and related damage are frequently observed in a number of GI ailments including inflammatory bowel diseases (IBDs). In these conditions, the treatment goal is prompt re-epithelialization of the wound, essential
for rapid resealing of the epithelial surface barrier to control inflammation and to restore intestinal homeostasis. Delayed re-epithelialization of intestinal wounds in IBDs gives rise to uncontrolled intestinal inflammation and general immune responses (9,10).

In general, failure to re-epithelialize is caused more by a reduced potential of the epithelium to migrate across the wound bed than inadequate cell proliferation (11-13). Cell migration requires sequential adhesion to and release from the substrate, representing a complex process of cell-matrix interactions (14-17). Recent studies indicate that members of the galectin class of β-galactoside-binding proteins play a critical role in modulating cell-matrix interactions and re-epithelialization of wounds by novel carbohydrate-based recognition systems (18-26).

**Galectins**

Galectins are a family of widely distributed carbohydrate-binding proteins defined by their affinity for the β-galactoside-containing glycans which are present on various cell surface and extracellular matrix (ECM) glycoproteins (27,28). There are 15 presently identified members of the galectin family in mammals, ranging in subunit size from 14 to 39 kDa. Each galectin contains a canonical carbohydrate recognition domain (CRD) of ~130 amino acids. Galectins can be expressed both intracellularly and extracellularly. Galectins do not contain a classical signal sequence or a transmembrane domain and are secreted from the cell via nonclassical pathways. Some galectins such as galectins-1, -3, -8 and -9 have wide tissue distribution, whereas others, such as galectins-4, -5 and -6, exhibit tissue specificity. The current interest in delineating the function of galectins is explained by studies demonstrating that many critical cellular response including cell adhesion (29-31), migration (18,32), immune response (33,34) and angiogenesis (35-41) are modulated by this class of lectins.

**Carbohydrate-binding specificity of galectins**

All galectins specifically recognize galactose-containing glycans, yet each galectin has unique, fine specificity for more complex galactose-containing oligosaccharides, a consequence of variability in the CRD sequence. Each galectin associates with certain types of glycans for signaling based on differences in the carbohydrate-binding specificities (42,43). The sugar-binding specificity of different members of the galectin family can differ greatly, e.g., galectin-1 (Gal1) recognizes α2,3 sialylated, but not α2-6 sialylated, glycans; Gal2 does not bind glycans that are sialylated with either linkage; Gal3 binds internal N-acetyllactosamine (LacNAc) within polyLacNAc (42); and depending on cellular microenvironment, sialylation may also affect Gal3 binding and signaling (44). Thus, on the basis of fine distinctions in carbohydrate-binding specificities, each galectin may interact with a discrete spectrum of glycoprotein receptors, with resulting specific downstream effects. For example, the affinity of Gal1 for the blood group A tetrasaccharide is approximately 100-fold lower than that for Gal3 (45), and only Gal8, but not Gal1, Gal2, Gal3, or Gal7, interact with the glycans of podoplanin, a lymphatic vessel glycoprotein (46).

**Galectin-glycan lattices**

All lectins are either dimers or oligomers, and this multivalency enables formation of lectin-carbohydrate lattices to cross-link and clusterize cell surface receptors including growth factor receptors and integrins. The diverse functions of galectins are thought to result from the formation of galectin-glycan lattice (47-49), by which the glycoprotein receptors are trapped, and as a result, prevented from undergoing endocytosis (50). By this mechanism, the interactions between galectins and N-glycans of the cell surface receptors regulate the density and distribution of cell surface receptors as well as cell responsiveness to the receptor ligand (47-50). Thus, Gal3 interacts, in a carbohydrate-dependent manner with the N-glycans of the epidermal growth factor (EGF) receptor which defers its constitutive endocytic removal and promotes EGF signaling (50). Likewise, studies in our laboratory have shown that Gal3 stimulates epithelial cell migration and formation of lamellipodia by activating α3β1-integrin-Rac1 signaling, and carbohydrate-mediated interaction between Gal3 and complex N-glycans on the α3β1 integrin is inherent in Gal3-induced lamellipodia formation and cell migration (18). It should be noted that cytoplasmic Gal3 also promotes re-epithelialization of wounds, however, by mechanisms that are independent of galectin-glycan lattices (51).

**Role of galectins in wound healing**

**Galectin-3 (Gal3)**

Gal3 expression occurs in inflammatory cells, epithelia,
Role of Gal3 in corneal wound healing

Gal3 is present in high density at sites of corneal epithelial cell-matrix adhesion (25), an ideal placement for influence on cell-matrix interactions and cell migration. To examine whether Gal3 plays a role in re-epithelialization of corneal wounds and to determine whether the rate of wound closure is impaired in Gal3-deficient mice, we utilized two different models of corneal wound healing. In this study, corneas with either excimer laser ablations or alkali-burns were allowed to partially heal in vivo or in vitro for up to 22 h, at which time remaining wound areas were quantitated and compared among the study groups. Whether the corneas were injured by excimer laser or by alkali treatment and whether the corneas healed in vivo or in vitro, epithelial wound closure rate (mm²/h) was significantly slower in Gal3−/− mice compared with Gal3+/+ mice (Figure 1A-E) (25). However, no differences were found in the wound closure rates between Gal1+/+ and Gal1−/− groups (Figure 1F). Whether delayed re-epithelialization of corneal wounds in Gal3−/− mice is due to a deficiency in the rate of corneal epithelial cell proliferation is the question addressed by the next experiment. In order to identify cells undergoing DNA synthesis, normal and healing Gal3+/+ and Gal3−/− corneas were labeled with BrdUrd. This study found no significant difference in the number BrdUrd-labeled cells between Gal3+/+ and Gal3−/− corneas (25). Thus the rate of corneal epithelial cell proliferation seemed not to be perturbed in Gal3−/− mice. It follows that delayed re-epithelialization of corneal wounds found in Gal3−/− mice is more likely caused by impairment in the cell migration process. The next experiments set out to learn whether exogenous Gal3 would stimulate re-epithelialization of corneal wounds. In this study, Gal3+/+ mouse corneas with alkali-burn wounds were incubated in serum-free media with varying amounts of recombinant Gal3. The remaining wound areas were quantified following the healing period of 22-24 h. The rate of wound closure was stimulated by exogenous Gal3 in a concentration-dependent manner.
in Gal3−/− mice (Figure 2). In the presence of 10 and 20 μg/mL Gal3, the acceleration rate of re-epithelialization of wounds was 43% and 71%, respectively over control corneas incubated in media alone without Gal3. It was further shown that a competing disaccharide, β-lactose, but not an irrelevant disaccharide, sucrose, can nearly completely undermine the stimulatory effect of Gal3 on the rate of corneal epithelial wound closure, indicating that the lectin CRD is directly involved in the positive effect of the exogenous lectin on the wound closure. Parallel experiments demonstrated that recombinant Gal1 did not increase the healing rate of corneal epithelial wound. Other studies have subsequently revealed that exogenous Gal3 advances re-epithelialization of wounds in rat corneas (52), monkey corneas (53) as well as in a rat dry eye model (54).

**Role of Gal3 in intestinal wound healing**

Gal3 is expressed to a high degree in enterocytes and subepithelial macrophages of the GI tract (55,56), and is thought to have a wound healing function. Scratch wound-healing assays in which colonic epithelial cells (T84 cells) were treated with Gal3 for 24 hours demonstrated improved healing with a 60.4%±4.4% reduction in wound width (20). The Gal3-induced reduction in wound width was inhibited by a pan-inhibitor of galectins, β-lactose, and an anti-Gal3 neutralizing antibody (−9.8%±24.8%).

It is of interest to note that epithelia derived from IBD tissues (57-59) have reduced levels of Gal-3, but it is yet to be shown whether this is a causative factor in the wound healing related complications of patients with IBD. However, from in vitro experiments, it was learned that matrix metalloproteinase-7 (MMP7), highly expressed in IBD tissues (57,58), cleaves Gal3, and the addition of MMP7 to Gal3 abrogates the wound healing and cell migration induced by Gal3 (20). Based on these findings, Puthenedam and colleagues (20) proposed that cleavage of Gal3 may be one mechanism by which MMP7 inhibits wound healing. This study is important to our understanding of delayed wound healing in chronic intestinal diseases such as intestinal ulcers and IBD, in which MMP7 protein expression is elevated, with an accompanying decrease in Gal3 protein expression.

**Role of Gal3 in skin wound healing**

In a recent study, using Gal3−/− mice and cells isolated from these mice, Liu et al. (51) demonstrated that the absence of Gal3 impairs keratinocyte migration and skin wound re-epithelialization. Interestingly, in this study,
the promigratory function of the lectin was attributed to cytosolic GaL3, and, therefore, is likely to be carbohydrate independent.

**Galectin-7 (Gal7)**

As a prototype galectin that forms homodimers (60), Gal7 can cross-link cell surface receptors. Gal7 is expressed preferentially in stratified epithelia including epidermis, oral cavity, cornea, esophagus and ano-rectal epithelium (61). During re-epithelialization of corneal wounds, and in some cancers such as skin tumors (62), significant changes in the levels of Gal7 expression have been detected. Gal7 is considered a marker for stratified epithelia (61). Nevertheless, this lectin has been found to be present in cilia isolated from cultured human airways, and in most of the cilia of multiciliated cells in human airway epithelia primary cultures (61,63,64). Gal7 expression is evident as well in the primary cilia of Madin-Darby canine kidney (MDCK) cells (65), LLC-PK1 porcine kidney, and mpkCCD14 mouse kidney cells and on cilia in the rat renal proximal tubule (19). Gal7 plays a role in wound healing of not only stratified epithelium which lack cilia such as that of cornea and skin, but also of simple epithelia such as that of kidney epithelium (19).

**Role in corneal wound healing**

Gal7 expression is upregulated substantially in mouse corneas upon injury and exogenous Gal7 was shown to stimulate corneal wound re-epithelialization in organ culture specimens (24). The stimulation of wound closure by Gal7 is partly undermined by β-lactose, a competing disaccharide, but not by sucrose, an irrelevant disaccharide, again suggesting that the Gal7 CRD is directly involved in stimulatory effect of the exogenous lectin in promoting wound closure.

**Role in skin wound healing**

Gendronneau et al. (23) have used Gal7 knockout mice to assess the role of this lectin in skin wound healing. Superficial scratches were made along the sagittal axis of the tail of Gal7−/− and Gal7+/− adult mice. Tissue sections of healing tails at 24 and 48 h after experimental injury were stained with hematoxylin and eosin and distance between the two wound margins was measured. The process of wound closure was judged to be less efficient in the Gal7−/− mice compared to the Gal7+/− mice. Additionally, according to an ex vivo wound healing assay, outgrowth of keratinocyte from Gal7−/− skin explants was also reduced in comparison with the Gal7+/+ controls. It was further shown that Gal7 accumulates in podosomes, which are specialized cell-matrix adhesion complexes connecting the ECM to the microfilament network, and that distribution of cortactin, an actin-binding protein implicated in membrane ruffle formation, is severely affected in migrating keratinocytes lacking Gal7, suggesting that that the formation and/or stabilization of actin-based lamellipodia is abnormal in Gal7 null keratinocytes. In the in vivo model, even when proliferation was blocked by mitomycin-C, the rate of wound closure rate was slower in Gal7 null mice, supporting a conclusion that as shown with Gal3, Gal7 also promotes re-epithelialization of skin wounds by influencing cell migration and not cell proliferation.

**Role of Gal7 in wound repair of polarized kidney epithelial cells**

Rondanino et al. (19) compared the length of cilia and wound closure rate between the Gal7 shRNA knockdown and control kidney epithelial cells. In this study, the control cells exhibited significantly longer cilia than Gal7 knockdown cells. A 33% reduction in wound healing was observed in scratch wound assays for Gal7 knockdown cells compared to control cells (19).

**Role of Gal7 in uterine repair**

Gal7 is also thought to be important for normal uterine repair following menstruation (66). Gal7 immunoreactivity is detected in the endometrial luminal and glandular epithelium during the late secretory and menstrual phases, and exogenous Gal7 enhances endometrial epithelial wound repair in vitro. Also, Gal7 immunoreactivity is significantly reduced in the endometrium of women with amenorrhoea compared with normally cycling women, suggesting the putative role of Gal7 in uterine repair.

**Galectins-2 and -4**

Gal2 and Gal4 are of particular interest relative to the GI tract. Both are expressed specifically in GI tissues, but not in various other tissues including brain, kidney, skeletal muscle, liver, or lung tissues (67).

**Role of galectins-2 and -4 in intestinal epithelial wound healing**

In several models of intestinal inflammation, exogenous Gal2 was demonstrated to ameliorate colitis (68). In an effort to elucidate the function of Gal2 in wound healing,
Paclik et al. (21) employed scratch wound assays to assess the influence of exogenous Gal2 on cell migration. Confluent monolayers of Caco-2 cells were injured with a surgical blade and incubated for 24 hours either with or without 50 μg/mL Gal2, after which wound closure rate was quantified. Gal-2 significantly enhanced epithelial cell migration over the wound edge (21). In the study by Paclik et al. (21), exogenous Gal4 also promoted wound closure, whereas Gal1 did not. Both Gal2 and -4 promoted cell migration as well as proliferation of Caco-2 cells suggesting that both processes may be involved in resealing the disrupted epithelial barrier in GI disorders (21). In contrast, as described above, Gal3 and Gal7 promote cell migration, but not cell proliferation of corneal and skin epithelial cells.

**Molecular mechanism by which galectins modulate wound healing**

*Gal3 promotes wound healing by activating α3β1-integrin-Rac1 signaling*

Cell migration is complex and requires first, the extension of protrusions, e.g., lamellipodia or filopodia, from the cell; secondly, the interaction of the surface molecules of these protrusions with the permissive ligands in the underlying matrix to create transient cell-matrix adhesions; and thirdly, actomyosin-mediated cell contraction and forward movement with a concurrent detachment of adhesions at the rear end (69). Contributing to regulating the cell migration process are transmembrane integrin receptors that mediate cell-matrix adhesions and intracellular signaling pathways, leading to cytoskeletal reorganization and cell motility (69). Nearly all integrins are glycosylated proteins, and various recent studies have demonstrated modulation of transmembrane signaling by integrin glycans (70,71). That interactions between integrin glycans and carbohydrate-binding proteins, galectins, have an essential function in integrin-dependent cell adhesion and migration has specifically been demonstrated (44,72–78). Lagana et al. (74) demonstrated that Gal3 interactions with N-acetylglucosaminyltransferase V (GnT-V)-modified N-glycans on mammary carcinoma cell surface support α3β1 integrin activation and cell motility. Studies in our laboratory aimed at characterizing the molecular mechanism by which Gal3 promotes epithelial cell migration during corneal wound closure, have demonstrated that Gal3, by interacting with GnT-V-modified complex N-glycans, activates α3β1-integrin-Rac1 signaling to induce formation of lamellipodia in epithelial cells, and, this in turn, promotes cell migration and re-epithelialization of wounds (18).

**Galectin-3 promotes wound healing by interacting with N-glycans of laminin-332**

Laminin-332 (Lm332; also known as laminin-5), a component of basement membranes in the cornea, skin and other stratified squamous epithelial tissues (79–81), is overexpressed at the leading edge of wounds during healing and promotes cell migration (82–84). It is believed to have a critical role in wound re-epithelialization. A null mutation of Lm332 causes a lethal blistering disease of the skin. Laminins are heavily glycosylated; nevertheless, the role of Lm332 has not been widely studied relative to its glycosylation pattern. The glycosyltransferase GnT-V catalyzes addition of the β1,6-linked GlcNAc branch which serves as a substrate for polylactosamine, the high affinity ligands for Gal3. By contrast, GnT-III adds GlcNAc to the inner β-linked mannose to form bisecting GlcNAc, which suppresses both further processing by branching enzymes, such as GnT-V, and elongation of N-glycans (85–87), resulting in downregulation of interaction with Gal3 with a concomitant reduction in cell migration and cancer metastasis (88). Therefore, it may be inferred that the sugar chains are an on/off switch for galectin binding during wound healing. This is particularly relevant since changes in glycosylation are observed during re-epithelialization of wounds. Kariya et al.’s elegant study (84) has shown that Gal3 binds to Lm332 coated wells, which greatly enhances Lm332-dependent keratinocyte motility. On the other hand, exogenous Gal3 did not induce an increase in cell migration on GnT-III-Lm332 substratum because modifying Lm332 by GnT-III diminishes its ability to bind to Gal3. These results led authors to propose that Gal3 may be a cofactor for Lm32-induced cell motility during wound healing and squamous cell carcinoma tumor progression, conditions that are associated with GnT-V overexpression (84).

**Galectin-3 promotes wound healing by interacting with N-glycans of CD147**

Subsequent to the injury, epithelial cells are required to change their shape and rearrange their position to assume a migratory phenotype. It is well established that induction of matrix metalloproteinase activity contributes to the
disassembly of intercellular junctions and the degradation of the ECM to mitigate the physical constraint to cell movement. CD147 (EMMPRIN) is a widely distributed cell surface glycoprotein highly enriched on the surface of keratinocytes during wound healing. A major function of CD147 is stimulation of MMP synthesis through homophilic interactions involving both heterotypic and homotypic cell-cell interactions. In a recent study, Mauris and colleagues (26) have demonstrated that Gal3 plays a key in destabilizing cell-cell interactions by interacting with and clustering CD147 on the epithelial cell surface. In this study, the authors identified CD147 as a membrane receptor for galectin-3 in human keratinocytes and demonstrated that Gal3 initiates keratinocyte cell-cell disassembly by inducing MMP expression in a CD147-dependent manner. Thus, one of the mechanisms by which Gal3 promotes cell migration and re-epithelialization of wound is by destabilizing cell-cell contacts to promote the epithelial rearrangement and cellular plasticity that are associated with cell motility.

**Galectin-3 promotes cell migration by interaction with Alix**

Alix, is a protein component of the endosomal sorting complex required for transport (ESCRT) machinery (89) and has been reported to attenuate EGFR endocytosis (90). Galectin-3 is an intracellular partner of Alix (91) and Liu and colleagues (51) have demonstrated that cytoplasmic Gal3 promotes keratinocyte migration and skin wound re-epithelialization by modulating intracellular trafficking of EGFR by interacting with Alix. Unlike Gal3 interactions with the glycans of cell surface receptors, interactions between intracellular Gal3 and Alix are likely to be carbohydrate-independent. Thus, both extracellular and intracellular galectins play a role in wound healing by distinct mechanisms.

**Therapeutic implications**

As described in the introduction, there is an ongoing and expanding need for effective treatment of chronic wounds in the elderly, decubitus ulcers, and venous stasis ulcers of the skin. Paralleling this need, wound healing related complications in various GI diseases including IBDs remain a major clinical challenge, as does the treatment of persistent epithelial defects of the cornea. In ophthalmology we find an example of a contemporary development that expands the scope of the challenge to find the key to wound healing. It has been estimated that in the United States alone in a given year nearly half a million excimer laser keratectomy procedures are performed to obviate the need for eyeglasses and contact lenses to correct myopia (92). Considering that over 25-30% of the adult population worldwide is myopic, the potential number of myopia surgeries is enormous. In some cases following excimer laser surgery, there is a delay in epithelial healing, which puts the pre-surgically healthy cornea at risk of developing postoperative haze, infectious keratitis, and ulceration.

The quest has led to investigations of EGF, transforming growth factor-α, fibroblast growth factor, keratinocyte growth factor, and hepatocyte growth factor, all of which are known to stimulate cell proliferation, as possible drug targets to promote wound healing. Generally, the results have been disappointing (1,5,93-96). The extent of acceleration of re-epithelialization of wounds was far less in most of these studies using growth factors (92,94) than that observed with galectins in some of the studies discussed above. Additionally, it was found that treating corneas with growth factors such as EGF resulted in hyperplastic epithelium, a clearly undesirable condition (93,97,98). In this respect, the lectins Gal3 and Gal7 do not induce cell mitosis in healing corneas and skin, implying that galectin-based drugs may be more attractive as they do not have the disadvantage of causing epithelial hyperplasticity. In summary, findings that galectins stimulate the re-epithelialization of corneal, dermal, intestinal and kidney wounds provide the basis for developing novel therapeutic strategies for the treatment of nonhealing wounds.

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