# The Role of β-1,4-Galactosyltransferase-I in the Skin Wound-healing Process

*Aiguo Shen, PhD,*‡ Ji Qian, MM,† Lei Liu, MM,† Haiou Liu, MM,† Jianping Chen, MM,† Shuqiong Niu, PhM,* Meijuan Yan, MD,* Xiaodong Chen, MD,§ Congcong Shen, MM,§ Jianxin Gu, PhD,‡ and Chun Cheng, MD†*

**Abstract:** Cell-surface carbohydrate chains are known to contribute to cell migration, interaction, and proliferation. β-1,4-galactosyltransferase-I (β-1,4-GalT-I), which is one of the best-studied glycosyltransferases, plays a key role in the synthesis of type 2 chains in *N*-glycans and the core 2 branch in *O*-glycans. Recently, it has been reported that skin wound healing is significantly delayed in β-1,4-GalT-I$^{-/-}$ mice. However, the expression of β-1,4-GalT-I and its biological function in the skin wound-healing process remain to be elucidated. We used real-time polymerase chain reaction to demonstrate that the expression of β-1,4-GalT-I mRNA reached plateau values at 12 hours after skin was injured and remained elevated until 11 days after the injury. Furthermore, lectin blotting showed that β-1,4-galactosylated carbohydrate chains were also increased after skin injury. A double-staining method combining lectin-fluorescent staining with RCA-I and immunofluorescence was first used to determine the cellular localization of β-1,4-galactosylated carbohydrate chains. Morphological analysis showed that the chains were primarily expressed in neutrophils and partially expressed in macrophages, endothelial cells, and collagen. Our results suggest that β-1,4-GalT-I and β-1,4-galactosylated carbohydrate chains participate in leukocyte recruitment, angiogenesis, and collagen deposition in the skin wound-healing process.

**Key Words:** β-1,4-GalT-I, skin wound healing, β-1,4-galactosylated carbohydrate chains, RCA-I

(*Am J Dermatopathol* 2008;30:10–15)

From the *The Jiangsu Province Key Laboratory of Neural Regeneration, Nantong University, Nantong; †Department of Microbiology and Immunology, Medical School of Nantong University (Former Nantong Medical College), Nantong; ‡Gene Research Center, Medical School of Fudan University (Former Shanghai Medical University), Shanghai; and §Department of Dermatology, Affiliated Hospital of Nantong University, Nantong, People's Republic of China.

Supported by National Natural Scientific Foundation of China Grant (Number 30300099, Number 30770488), Natural Scientific Foundation of JiangSu province Grant (Number BK2003035), and College and University Natural Science Research Programme of Jiangsu province (Number 03KJB180109).

Aiguo Shen and Ji Qian have contributed equally to this work.

Reprints: Chun Cheng, MD, Department of Microbiology and Immunology, Medical School of Nantong University (Former Nantong Medical College), Nantong, 226001, People's Republic of China (e-mail: cheng_chun@yahoo.com.cn).



## INTRODUCTION

Skin wound healing starts immediately after an injury and consists of three phases: inflammation, proliferation, and maturation. These phases proceed with a complicated but well-organized interaction among various types of tissues and cells.[1,2] During the inflammatory phase, platelet aggregation at the injury site is followed by infiltration of leukocytes, including neutrophils and macrophages, into the wound site. In the proliferative phase, re-epithelialization takes place and newly formed granulation tissue begins to cover the wound area to repair tissue destruction. Thus, it is generally accepted that leukocyte infiltration is mandatory to induce wound healing. Leukocyte infiltration is known to be mediated by selectins and their oligosaccharide ligands.[3,4] Glycosyltransferases have been shown to be mainly responsible for the biosynthesis of selectin ligands.[5,6]

β-1,4-galactosyltransferase I (β-1,4-GalT-I), which is the first mammalian glycosyltransferase cDNA to be cloned, transfers galactose from uridine diphosphate-galactose donors to terminal *N*-acetylglucosamine (GlcNAc) of carbohydrate chains in β-1,4-linkage to form a Galβ1→4GlcNAc structure in the Golgi apparatus.[7] However, a portion of β-1,4-GalT-I is also found on the cell surface where it functions as a cell adhesion molecule during a variety of cellular interactions by binding GlcNAc-containing oligosaccharide substrates, or ligands, in the extracellular matrix.[8] β-1,4-GalT-I is thought to be associated with a variety of biological functions, including sperm-egg interactions, morula compaction, embryonic maturation, embryonal carcinoma cell spreading, neurite extension, and mesenchymal cell migration, in which it mediates specific cell-cell and cell-matrix adhesions.[9–14]

Previous reports have shown that acute and chronic inflammatory responses are suppressed in β-1,4-GalT-I-deficient mice, and neutrophil infiltration at the inflammatory sites is primarily reduced.[5] It has also been shown that skin wound healing is significantly delayed concomitant with reduced leukocyte infiltration at the wound site in β-1,4-GalT-I$^{-/-}$ mice.[15] However, the expression of β-1,4-GalT-I and its biological function in the skin wound-healing process in normal rats remains to be elucidated. In the present study, we created full-thickness excisional skin wounds in the normal rats and found that the expressions of β-1,4-GalT-I mRNA and β-1,4-galactosylated carbohydrate chains were increased during skin wound healing. Morphological analysis demonstrated β-1,4-galactosylated carbohydrate chains colocalized with neutrophils, macrophages, collagen, and endothelial

cells. These findings suggest that β-1,4-GalT-I and β-1,4-galactosylated carbohydrate chains participate in leukocyte recruitment, collagen accumulation, and angiogenesis in the skin wound-healing process.

## MATERIALS AND METHODS

### Animals

Sprague Dawley rats weighing from 180 to 220 g were provided by the Experimental Animal Center of Nantong University. All animals were kept under standardized laboratory conditions in an air-conditioned room with free access to food and water. All the animal tests were carried out in accordance with the US National Institute of Health Guide for the Care and Use of Laboratory Animals.

### Wound Model

Skin wounds were prepared as described previously.[16] Briefly, animals were deeply anesthetized with a cocktail of xylazine (10 mg/kg), ketamine (95 mg/kg) and acepromazine (0.7 mg/kg) administered intraperitoneally. After shaving the dorsal hair and cleaning the exposed skin with 70% ethanol, a 0.5-cm diameter circle was marked on the skin of the mid-dorsal region, and full-thickness excisional wounds, including the skin and panniculus carnosus muscle, were created using scissors. The wounds were uncovered without a dressing. Usually, six wounds were made on the same animal. In following experiments, wounds and their surrounding area, including the scab and epithelial margins, were harvested at the indicated time intervals after the rats were euthanized with an overdose of diethyl ether.

### Real-time Polymerase Chain Reaction

Total RNA was isolated using the TRIzol (Invitrogen) method according to the manufacturer's protocol. Transcript levels of β-1,4-GalT-I in skin tissues were measured in 36-well microtiter plates using Rotor-gene 3000 Sequence Detector Systems (Perkin-Elmer/Applied Biosystems, Foster City, CA). The following primers were used: β-1,4-GalT-I primers, sense 5′-TATTTGCATCCAGTCTTTCAGC-3′ and antisense 5′-CAGCTTAGCTCGATTAAACATGG-3′; and $\beta_2$-microglobulin ($\beta_2$-M) primers, sense 5′-GTCTTTCTACATCCTGGCTCACA-3′ and antisense 5′-GACGGTTTTGGGCTCCTTCA-3′. Probes were designed using Primer Express (Perkin-Elmer/Applied Biosystems, Foster City, CA). All the probes were labeled with 6-carboxytetramethyl-rhodamine at the 3′-end and FAM at the 5′-end. All runs were accompanied by the TaqMan $\beta_2$-M control reagents. The expression level of β-1,4-GalT-I was calculated by standardizing the copy number with the $\beta_2$-M copy number in a sample. Specific mRNA transcript levels were expressed as fold difference. Analysis of results was based on 6 independent experiments.

### Lectin Blotting

Wound tissues were homogenized in 10 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 1 mM ethylenediaminetetraacetic acid and centrifuged at 600*g* at 4°C for 10 minutes. The supernatant was then centrifuged at 8000*g* at 4°C for 20 minutes. The resulting pellet was defatted with acetone 3 times. The membrane proteins obtained were solubilized in 50 mM Tris-HCl buffer (pH 6.8) containing 5% β-mercaptoethanol and 2% sodium dodecyl sulfate by incubating at 100°C for 5 minutes, were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then transferred onto a polyvinylidene difluoride membrane. To determine both samples contain similar protein components, blotted membranes were stained with Coomassie Brilliant Blue (CBB). Because most Gal residues of serum glycoproteins are sialylated, the filters initially were treated with 25 mM sulfuric acid for 1 hour at 80°C before the analysis. After being blocked with 2.5% bovine serum albumin, the membrane was incubated with 1:5000 dilutions of biotinylated-RCA-I (Vector Laboratories, Burlingame, CA) for 45 minutes at room temperature. Finally, HRP-avidin (1:5000, Vector Laboratories) was added for an additional 45 minutes and the blots were developed with the ECL (Pierce Company, USA) detection system using an x-ray film.

### Double-staining Method Combining Lectin-fluorescent Staining with RCA-I and Immunofluorescence

Wound tissues were fixed overnight in 4% formaldehyde buffered with phosphate-buffered saline (pH 7.2) and embedded in paraffin. Sections (8 μm thick) were subjected to double-staining analysis. For double-staining analysis, the sections were incubated with monoclonal primary antibody for different cell markers as follows: CD31, a marker for vascular endothelial cells (1:200, Southern Biotechnology Associates, Birmingham, AL); MPO, a marker for neutrophils (1:100, NeoMarkers, CA); F4/80, a marker for macrophages (1:200, Santa Cruz, CA); precollagen I, a marker for collagen (1:200, Santa Cruz, CA) overnight at 4°C. After washing in phosphate-buffered saline for three times, the second antibodies (TRITC-Chicken anti-Rat, 1:100, Santa Cruz, CA; TRITC-Goat anti-Rabbit, 1:50, Jackson ImmunoResearch Laboratory, PA; TRITC-Donkey anti-Goat, 1:100, Jackson ImmunoResearch Laboratory, PA) and fluorescein isothiocyanate (FITC)-conjugated RCA-I (1:400, Vector Laboratories) were added in a dark room and incubated for 2 hours at 4°C. The fluorescence was detected by Leica fluorescence microscope (Germany).

### Statistical Analysis

At least three repetitive assessments were performed, and for each assessment, all groups were tested in quadruplicate. All data were given in terms of relative values and expressed as mean ± SE. One-way analysis of variance was used to compare differences between the operated groups and the control group. All statistical analyses were conducted with a STATA 7.0 software package (Stata Corp, College Station, TX), and all significance levels were set at $P < 0.05$.

## RESULTS

### β-1,4-GalT-I Expression in Skin Wound-healing Process

In our initial set of experiments, we examined β-1,4-GalT-I expression at the skin excisional wound sites. In the

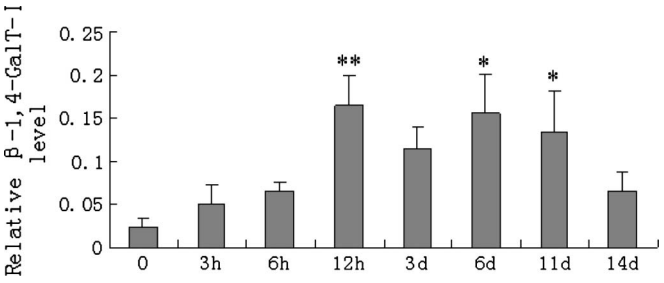


**FIGURE 1.** Expression of β-1,4-GalT-I mRNA in the skin wound-healing process by real-time polymerase chain reaction analysis. Total RNA of rat skin was isolated and subjected to real-time polymerase chain reaction analysis at each time point after the injury. For quantitative analysis, the relative β-1,4-GalT-I mRNA levels were determined by the ratio of their content relative to $\beta_2$-M in each sample. Representative results of the six animals in each group are shown. Data are expressed as the mean ± standard error of mean (n = 6). *$P < 0.01$, **$P < 0.001$.

uninjured skin of normal rats, β-1,4-GalT-I mRNA was only weakly expressed; the expression of β-1,4-GalT-I mRNA reached plateau values at 12 hours after the injury and remained elevated until 11 days after the injury (Fig. 1).

## Lectin-blotting Analysis of Glycoproteins in Rat Skin During Wound Healing

Because the gene expression levels of β-1,4-GalT-I changed in the skin wound-healing process, as revealed in Figure 1, we investigated whether the galactosylation of membrane glycoproteins in injured skin also changed in association with skin wound healing. The membrane glycoprotein samples were prepared from uninjured skin and 3 hours, 6 hours, 12 hours, 3 days, 6 days, 11 days, and 14 days after skin was injured and were subjected to lectin-blotting analysis. When blotted membranes were stained with CBB, they showed almost similar staining patterns (CBB in Fig. 2), indicating that rat injured skin contain similar protein components regardless of the stages of injury. Because some of the galactose residues were sialylated, blots were treated with acid to remove sialic acid residues and then incubated with biotinylated-conjugated RCA-I, which interacted with oligosaccharides terminating with the Galβ1→4GlcNAc group.[17] The binding patterns of protein bands to RCA-I were different among the different samples. The protein bands with molecular weights of 25 and 40 kDa (indicated with arrows at the right side of lane H of RCA-I in Fig. 2) reacted more strongly to RCA-I in rat skin from 6 hours to 6 days after injury. Because the RCA-I-binding disappeared on treatment of blots either with diplococcal β-galactosidase, which cleaves the Galβ1 → 4GlcNAc linkage but not the Galβ1 → 3GlcNAc linkage[18] or with *N*-glycanase, the lectin binding observed in Figure 2 was considered to be solely for the β-1,4-linked galactose residues attached to *N*-linked oligosaccharides. These results indicate that β-1,4-galactosylated carbohydrate chains and the galactosylation of 25- and 40-kDa glycoproteins increase after skin injured.

## Lectin-fluorescent Staining with RCA-I in Rat Injured Skin

Cell surface β-1,4-galactosylated carbohydrate chains were visualized in frozen sections of rat injured skin by use of the galactose-specific lectin, RCA-I conjugated with FITC. To further determine the kinds of cells in which β-1,4-galactosylated carbohydrate chains are expressed in the skin wound-healing phase, we used double immunofluorescent staining for more accurate localization of the molecule.

A variety of leukocytes migrate outside blood vessels and infiltrate into the wound site in the skin wound-healing process; the specific cell type(s) depends on the type of wound and the time elapsed after the injury. In the inflammatory phase, neutrophils and macrophages are the main infiltrates in the wound site. This examination revealed that in the inflammatory phase, β-1,4-galactosylated carbohydrate chains of rat injured skin colocalized with both neutrophils and macrophages (Figs. 3A, B). These results suggest that β-1,4-galactosylated carbohydrate chains are involved in leukocyte recruitment in the skin wound-healing phase.

In the proliferative phase, collagen synthesis is induced at wound sites to heal the injury, and the neovascularization is indispensable for sustaining the newly formed granulated tissues in the wound-healing process. We compared the localization of β-1,4-galactosylated carbohydrate in vascular endothelial cells and collagen. We found that 6 days after the injury, β-1,4-galactosylated carbohydrate chains of rat injured skin colocalized mostly with collagen and partially with vascular endothelial cells (Figs. 3C, D). These observations suggest that β-1,4-galactosylated carbohydrate chains participate in the collagen accumulation and angiogenesis in the proliferation phase of wound healing.

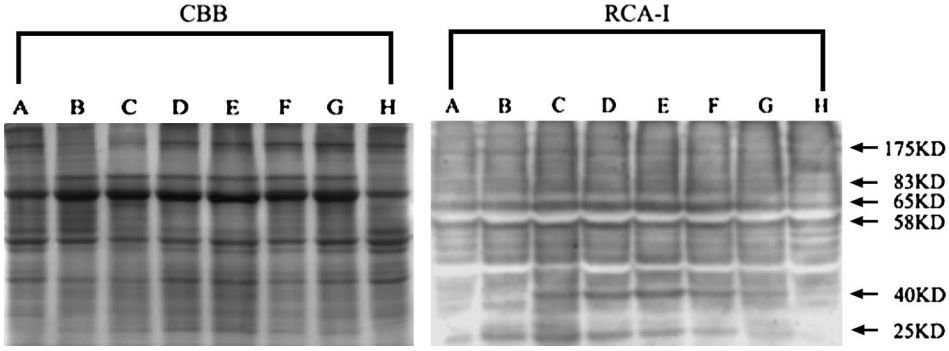


**FIGURE 2.** Lectin-blotting analysis of rat skin membrane glycoproteins in the skin wound-healing process. The blots were stained with CBB, or with RCA-I after acid treatment and then visualized as described in ''Materials and Methods.'' Samples were prepared from (A) uninjured skin, skin (B) 3 hours, (C) 6 hours, (D) 12 hours, (E) 3 days, (F) 6 days, (G) 11 days, (H) 14 days after injury.

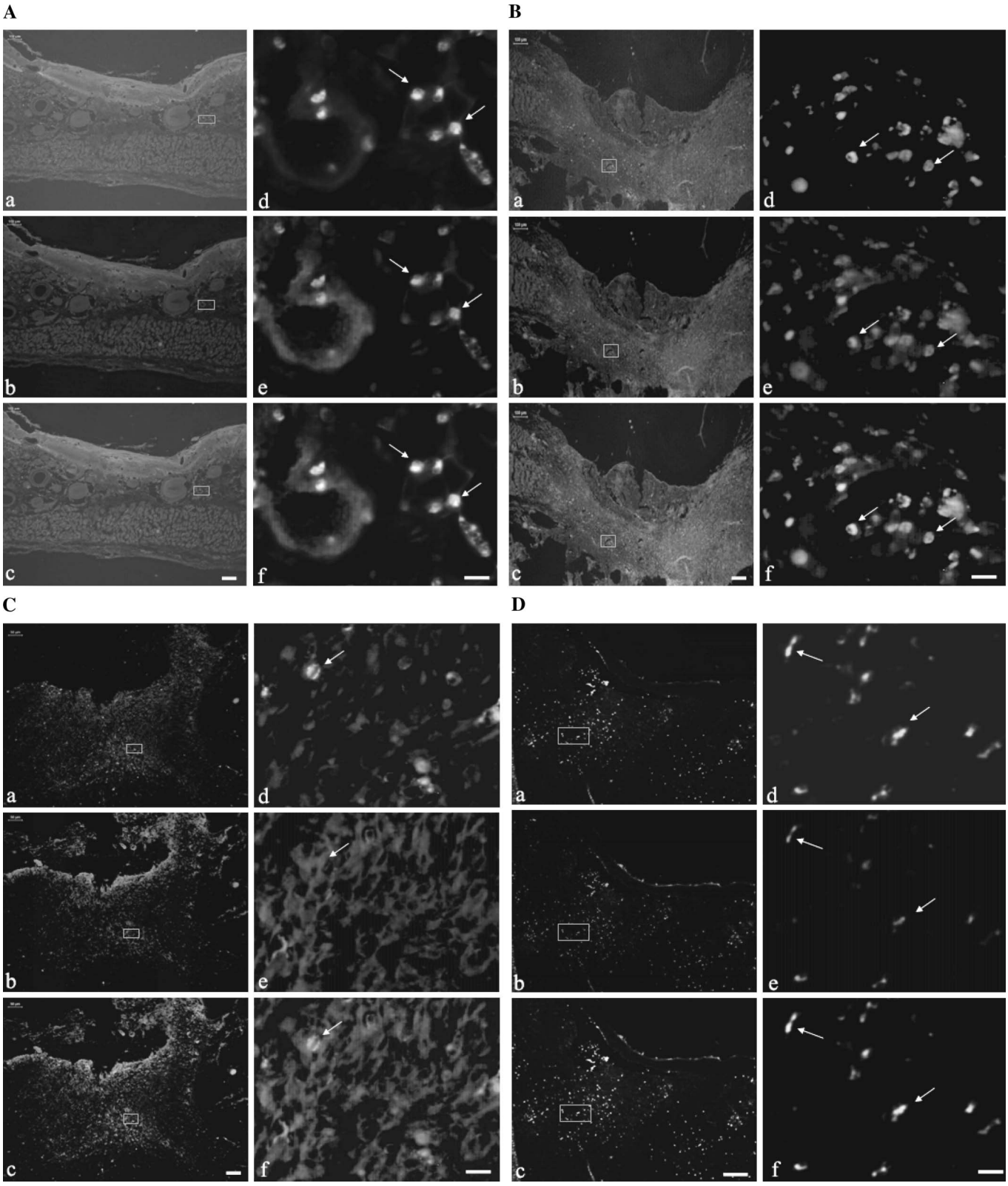


**FIGURE 3.** (Legend on next page).

## DISCUSSION

β-1,4-galactosylation of glycoproteins plays important roles in protein conformation, stability, transport, and clearance from the circulation. Recent studies have revealed that aberrant glycosylation causes various human diseases, such as metastasis of tumor cells, muscular dystrophy, human immunoglobulin A nephropathy, and dyserythropoietic anemia.[19–22] However, there are few reports of a biological role for β-1,4-GalT-I and β-1,4-galactosylation in the skin wound-healing phase. Asano et al[15] reported that acute and chronic inflammatory responses were suppressed in β-1,4-GalT-I-deficient mice. Furthermore, Mori et al[15] also found that wound closure was significantly delayed in β-1,4-GalT-I$^{-/-}$ mice. Diabetes is associated with impaired wound healing. It is established that glycation of cell membrane components and glycosylation of hemoglobin in diabetes are responsible for impaired innate host response, which leads to the development of chronic, nonhealing wounds.[23] Our findings were consistent with previous studies. We created full-thickness excisional skin wounds in the normal rats and found that the expression of β-1,4-GalT-I mRNA was increased for up to 12 hours after the injury and remained elevated until 11 days after the injury. Furthermore, β-1,4-galactosylated carbohydrate chains were also augmented, implying that β-1,4-GalT-I and β-1,4-galactosylated carbohydrate chains might play a significant role in skin wound healing, especially in the inflammation and proliferation phase.

Skin injury causes immediate clot formation and local inflammation characterized by an infiltration of neutrophils and macrophages into the wound sites.[1,2] This work demonstrated that β-1,4-galactosylated carbohydrate chains colocalized with both neutrophils and macrophages. Leukocyte emigration is known to be mediated by E- and P-selectins on endothelial cells and their oligosaccharide ligands such as sialyl Lewis$^x$ (sLe$^x$) on leukocytes.[3,4] A variety of terminal carbohydrate epitopes such as sLe$^x$ and sulfated sLe$^x$ are mainly expressed at the terminus of *N*-acetyllactosamine repeats on serine/threonine (*O*)-linked oligosaccharides (*O*-glycans).[24–26] β-1,4-GalT-I is responsible for the biosynthesis of the type 2 chains in *N*-glycans and the core 2 branch in *O*-glycans.[27] Thus, β-1,4-GalT-I plays an important role in the synthesis of selectin ligands such as sLe$^x$ and sulfated sLe$^x$. It has been reported that mice deficient in both P- and E-selectins show markedly reduced recruitment of inflammatory cells and delayed wound closure, indicating that endothelial selectins play an important role in skin wound healing.[28] Moreover, neutrophil and macrophage recruitment at the inflammatory site is reduced probably because of the reduced expression of selectin ligands in the β-1,4-GalT-I$^{-/-}$ mice.[5] Previous reports and our observations indicate that β-1,4-GalT-I and β-1,4-galactosylated carbohydrate chains were involved in neutrophils and macrophages recruiting into the wound site in the inflammatory phase of skin wound healing.

Angiogenesis and collagen deposition are indispensable for wound healing.[1,2] In this paper, it was found that β-1,4-galactosylated carbohydrate chains colocalized with vascular endothelial cells and collagen. In the β-1,4-GalT-I$^{-/-}$ mice, angiogenesis is impaired and collagen synthesis is attenuated in the proliferation phase of wound healing, accompanied by lower expression of vascular endothelial growth factor (VEGF) and transforming growth factor-β1 (TGF-β1).[15] VEGF and TGF-β1 are a potent angiogenic factor and a principal fibrogenic factor in adult skin wound healing, respectively.[29,30] Most of these factors that regulate wound healing are glycoproteins, and their glycosylation patterns affect their biological activity, stability, transport, and clearance from the circulation.[31] β-1,4-GalT-I may participate in glycosylation of these factors in the skin wound-healing process.

Endothelium is also known to be a primary site in the inflammatory phase of skin wound healing. Src-suppressed C kinase substrate is one of the major lipopolysaccharide (LPS)-responsive proteins and may participate in alteration of cytoskeletal architecture in endothelial cells during inflammation.[32] Furthermore, Src-suppressed C kinase substrate and β-1,4-GalT-I show many similarities, and they interact functionally with each other.[33] Therefore, it is reasonable to presume that β-1,4-GalT-I may participate in alteration of cytoskeletal architecture in endothelial cells in the inflammatory phase of skin wound healing.

Three phases of wound healing—inflammation, proliferation, and maturation—proceed consecutively.[1,2] β-1,4-GalT-I participated in all three phases, especially in the inflammatory phase; however, the exact mechanisms remain to be further elucidated. In the inflammatory phase, the pro-inflammatory cytokine tumor necrosis factor-α triggers endothelial cells to increase the expression of adhesion molecules, which are pivotal for the rolling, adhesion, and transmigration of leukocytes over the endothelial cell wall.[34] β-1,4-GalT-I in primary human endothelial cells is upregulated in a time- and concentration-dependent manner in response to tumor necrosis factor-α stimulation because of an increase

**FIGURE 3.** Localization of β-1,4-galactosylated carbohydrate chains in the skin wound-healing process. (A) Combined lectin-fluorescent staining and immunohistochemistry was performed on sections of injured skin using FITC-RCA- I (a, d) and antibody MPO (b, e) specific for neutrophils 12 hours after injury. (d–f) a high magnification of the inset in parts (a–c), (f) merged picture indicated that β-1,4-galactosylated carbohydrate chains were colocalized to neutrophils. (B) Combined lectin-fluorescent staining and immunohistochemistry was performed on sections of injured skin using FITC-RCA- I (a, d) and antibody F4/80 (b, e) specific for macrophages 6 days after injury. (d–f) a high magnification of the inset in parts (a–c), (f) merged picture indicated that β-1,4-galactosylated carbohydrate chains were colocalized to macrophages. (C) Combined lectin-fluorescent staining and immunohistochemistry was performed on sections of injured skin using FITC-RCA- I (a, d) and antibody CD31 (b, e) specific for vascular endothelial cells 6 days after injury. (d–f) a high magnification of the inset in parts (a–c), (f) merged picture indicated that β-1,4-galactosylated carbohydrate chains were colocalized to vascular endothelial cells. (D) Combined lectin-fluorescent staining and immunohistochemistry was performed on sections of injured skin using FITC-RCA- I (a, d) and antibody precollagen I (b, e) 6 days after injury. (d–f) a high magnification of the insets in figure parts (a–c), (f) merged picture indicated that β-1,4-galactosylated carbohydrate chains were colocalized to collagen. Scar bar: (a–c), 50 μm; (d–f), 10 μm.

in the stability of the mRNA transcript.[35] The activity and expression of β-1,4-GalT-I in HL60 cells increase after their treatment by phorbal 12-myristate 13-acetate (PMA), partly attributable to an increase of stability of the mRNA transcripts.[36] We cannot rule out the possibility that upregulation of β-1,4-GalT-I mRNA after skin injury might result from an increase of stability of the mRNA transcripts. The further dissection of the mechanisms involved in regulating the expression of β-1,4-GalT-I is important to increase our understanding of the skin wound healing and may enable the designing of more specific therapies for skin wounds.

## REFERENCES


1. Singer AJ, Clark RA. Cutaneous wound healing. *N Engl J Med*. 1999;341: 738–746.
2. Martin P. Wound healing—aiming for perfect skin regeneration. *Science*. 1997;276:75–81.
3. McEver RP, Moore KL, Cummings RD. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J Biol Chem*. 1995;270:11025–11028.
4. Lowe JB. Selectin ligands, leukocyte trafficking, and fucosyltransferase genes. *Kidney Int*. 1997;51:1418–1426.
5. Asano M, Nakae S, Kotani N, et al. Impaired selectin-ligand biosynthesis and reduced inflammatory responses in β-1,4-galactosyltransferase-I-deficient mice. *Blood*. 2003;102:1678–1685.
6. Homeister JW, Thall AD, Petryniak B, et al. The alpha (1, 3) fucosyltransferases FucT-IV and FucTVII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity*. 2001;15:115–126.
7. Furukawa K, Sato T.β-1,4-galactosylation of N-glycans is a complex process. *Biochim Biophys Acta*. 1999;1473:54–66.
8. Shur BD, Evans S, Lu Q. Cell surface galactosyltransferase: current issues. *Glycoconj J*. 1998;15:537–548.
9. Miller DJ, Macek MB, Shur BD. Complementarity between sperm surface β-l,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. *Nature*. 1992;357:589–593.
10. Hathaway HJ, Shur BD. Cell surface β-l,4-galactosyltransferase functions during neural crest cell migration and neurulation in vivo. *J Cell Biol*. 1992;117:369–382.
11. Maillet CM, Shur BD. Perturbing cell surface β-1,4-galactosyltransferase on F9 embryonal carcinoma cells arrests cell growth and induces laminin synthesis. *J Cell Sci*. 1994;107:1713–1724.
12. Huang QL, Shur BD, Begovac PC. Overexpression cell surface β-1,4-galactosyltransferase-I in PC12 cells increases neurite outgrowth on laminin. *J Cell Sci*. 1995;108:839–847.
13. Eckstein DJ, Shur BD. Cell surface β-1,4-galactosyltransferase is associated with the detergent-insoluble cytoskeleton on migrating mesenchymal cells. *Exp Cell Res*. 1992;201:83–90.
14. Evans SC, Lopez LC, Shur BD. Dominant negative mutation in cell surface β-1,4-galactosyltransferase inhibits cell-cell and cell-matrix interactions. *J Biol Chem*. 1993;120:1045–1057.
15. Mori R, Kondo T, Nishie T, et al. Impairment of skin wound healing in β-1,4-galactosyltransferase-deficient mice with reduced leukocyte recruitment. *Am J Pathol*. 2004;164:1303–1314.
16. Park SG, Shin H, Shin YK, et al. The novel cytokine p43 stimulates dermal fibroblast proliferation and wound repair. *Am J Pathol*. 2005;166:387–398.
17. Venkatesh YP, Lambert JM. Galactose-induced dimerization of blocked ricin at acidic pH: evidence for a third galactose-binding site in ricin B-chain. *Glycobiology*. 1997;7:329–335.
18. Glasgow LR, Paulson JC, Hill RL. Systematic purification of five glycosidases from Streptococcus (Diplococcus) pneumoniae. *J Biol Chem*. 1977;252:8615–8623.
19. Granovsky M, Fata J, Pawling J, et al. Suppression of tumor growth and metastasis in Mgat5-deficient mice. *Nat Med*. 2000;6:306–312.
20. Yoshida A, Kobayashi K, Manya H, et al. Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev Cell*. 2001;1:717–724.
21. Chui D, Oh-Eda M, Liao YF, et al. Alpha-mannosidase-II deficiency results in dyserythropoiesis and unveils an alternate pathway in oligosaccharide biosynthesis. *Cell*. 1997;90:157–167.
22. Nishie T, Miyaishi O, Azuma H, et al. Development of immunoglobulin A nephropathy-like disease in beta-1,4-galactosyltransferase-I-deficient mice. *Am J Pathol*. 2007;170:447–456.
23. Morain WD, Colen LB. Wound healing in diabetes mellitus. *Clin Plast Surg*. 1990;17:493–501.
24. Maemura K, Fukuda M. Poly-N-acetylactosaminyl O-glycans attached to leukosialin: the presence of sialyl Le(x) structures in O-glycans. *J Biol Chem*. 1992;267:24379–24386.
25. Wilkins PP, McEver RP, Cummings RD. Structures of the O-glycans on P-selectin glycoprotein ligand-1 from HL-60 cells. *J Biol Chem*. 1996; 271:18732–18742.
26. Hiraoka N, Petryniak B, Nakayama J, et al. High endothelial venule-specific sulfotransferase expresses 6-sulfo sialyl Lewis(x), an L-selectin ligand displayed by CD34. *Immunity*. 1999;11:79–89.
27. Kotani N, Asano M, Iwakura Y, et al. Knockout of mouse β 1,4-galactosyltransferase-1 gene results in a dramatic shift of outer chain moieties of N-glycans from type 2 to type 1 chains in hepatic membrane and plasma glycoproteins. *Biochem J*. 2001;357:827–834.
28. Subramaniam M, Saffaripour S, Van De Water L, et al. Role of endothelial selectins in wound repair. *Am J Pathol*. 1997;150:1701–1709.
29. Frank S, Madlener M, Werner S. Transforming growth factors β1, β2, and β3 and their receptors are differentially regulated during normal and impaired wound healing. *J Biol Chem*. 1996;271:10188–10193.
30. Ozawa K, Kondo T, Hori O, et al. Expression of the oxygen-regulated protein ORP150 accelerates wound healing by modulating intracellular VEGF transport. *J Clin Invest*. 2001;108:41–50.
31. Gagneux P, Varki A. Evolutionary considerations in relating oligosaccharide diversity to biological function. *Glycobiology*. 1999;9:747–755.
32. Kitamura H, Okita K, Fujikura D, et al. Induction of Src-suppressed C kinase substrate (SSeCKS) in vascular endothelial cells by bacterial lipopolysaccharide. *J Histochem Cytochem*. 2002;50:245–255.
33. Wassler MJ, Foote CI, Gelman IH, et al. Functional interaction between the SSeCKS scaffolding protein and the cytoplasmic domain of β1,4-galactosyltransferase. *J Cell Sci*. 2001;114:2291–2300.
34. Collins T, Read MA, Neish AS, et al. Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. *FASEB J*. 1995;9:899–909.
35. Garcia-Vallejo JJ, van Dijk W, van Die I, et al. Tumor necrosis factor-α up-regulates the expression of β-1,4-galactosyltransferase-I in primary human endothelial cells by mRNA stabilization. *J Biol Chem*. 2005;280: 12676–12682.
36. Pasqualetto V, Lemaire S, Neel D, et al. Phorbol ester treatment of HL 60 leukemia cells results in increase of β-(1→4)-galactosyltransferase. *Carbohydr Res*. 2000;328:301–305.


 