Targeting O-glycosyltransferase (OGT) to promote healing of diabetic skin wounds

Kasper Runager§, Meryem Bektas§, Paula Berkowitz§, and David S. Rubenstein§#¶1

From the §Department of Dermatology, University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA, #Department of Pharmacology, University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA
¶Lineberger Comprehensive Cancer Center, University of North Carolina-Chapel Hill School of Medicine, Chapel Hill, NC 27599, USA

Running title: OGT in diabetic wounds

#To whom correspondence should be addressed: David S. Rubenstein, M.D., Ph.D., Department of Dermatology, The University of North Carolina School of Medicine, Suite 405 Mary Ellen Jones Bldg CB 7287, Chapel Hill, North Carolina 27599-7287, Telephone: 919-843-7092, Fax: 919-966-3898, Email: druben@med.unc.edu

Keywords: OGT, diabetes, wound healing, keratinocytes

Background: Increased intracellular protein O-GlcNAc modification may contribute to delayed wound healing in diabetes.

Results: Hyperglycemia increases intracellular protein O-GlcNAc modification and delays wound healing in keratinocytes. Targeted knockdown of OGT altered rates of wound closure.

Conclusion: OGT knockdown accelerates wound healing under both normal and hyperglycemic culture conditions.

Significance: OGT may represent a novel druggable target for promoting healing of diabetic wounds.

SUMMARY

Non-healing wounds are a significant source of morbidity. This is particularly true for diabetic patients, who tend to develop chronic skin wounds. O-GlcNAc modification of serine and threonine residues is a common regulatory post-translational modification analogous to protein phosphorylation; increased intracellular protein O-GlcNAc modification has been observed in diabetic and hyperglycemic states. Two intracellular enzymes, UDP-N-acetylglucosamine-polypeptide β-N-acetylglucosaminyl transferase (OGT) and O-GlcNAc-selective N-acetyl-β-D-glucosaminidase (OGA), mediate addition and removal, respectively, of N-acetylglucosamine (GlcNAc) from intracellular protein substrates. Alterations in O-GlcNAc modification of intracellular proteins is linked to diabetes and the increased levels of protein O-GlcNAc modification observed in diabetic tissues may in part explain some of the observed underlying pathophysiology that contributes to delayed wound healing. We have previously shown that increasing protein O-GlcNAc modification by over-expression of OGT in murine keratinocytes results in elevated protein O-GlcNAc modification and a hyper-adhesive phenotype. This study was undertaken to explore the hypothesis that increased O-GlcNAc modification of cellular proteins in diabetic skin could contribute to the delayed wound healing observed in patients with diabetic skin ulcers. In the present study, we show human keratinocytes cultured under hyperglycemic conditions display increased levels of O-GlcNAc modification as well as a delay in the rate of wound closure in vitro. We
further show that specific knock-down of OGT by RNA interference (RNAi) reverses this effect, thereby opening up the opportunity for OGT-targeted therapies to promote wound healing in diabetic patients.

INTRODUCTION

Non-healing wounds are a significant source of morbidity affecting 6.5 million patients in the United States and costing approximately $25 billion annually to treat (1). Patients with diabetes are at increased risk for developing non-healing wounds. A variety of factors likely contribute to the predisposition of diabetic patients to develop non-healing wounds including neuropathy, vasculopathy, as well as the underlying endocrine dysfunction that results in elevated glucose levels.

Like phosphorylation, intracellular protein O-GlcNAc modification is a common, dynamic post-translational modification that regulates many intracellular proteins including enzymes, transcription factors, structural and cell adhesion proteins. N-acetylglucosamine (GlcNAc) modification of serine and threonine is catalyzed by the enzyme UDP-N-acetylglucosamine-polypeptide β-N-acetylglucosaminyl transferase (O-GlcNAc transferase, OGT); whereas, GlcNAc is removed by O-GlcNAc-selective N-acetyl-β-D-glucosaminidase (GlcNAcase, OGA) (reviewed in (2)).

Hyperglycemia, excess glucose, feeds into the glucosamine pathway to provide excess UDP-GlcNAc for OGT to modify intracellular proteins (3). Excess glucose is converted to glucosamine, which is ultimately converted to UDP-N-acetylglucosamine (UDP-GlcNAc), the donor substrate for OGT modification of intracellular proteins. Consequently, hyperglycemia is associated with increased O-GlcNAc modification of a variety of proteins (3-7). The increased O-GlcNAc modification of intracellular proteins observed in hyperglycemic states including diabetes is thought to contribute to some of the pathology associated with diabetes. For example, (i) pancreatic β-cells have high levels of OGT and are sensitive to alterations in intracellular O-GlcNAc modification and (ii) over-expression of OGT in muscle and adipose tissue causes diabetes in transgenic mouse models (8). Increased O-GlcNAc modification of intracellular proteins is observed in diabetic tissue, including human diabetic tissue (9) and hyperglycemic animal models (4).

Further support for a pathologic role for intracellular O-GlcNAc modification in diabetes comes from studies demonstrating a genetic association of diabetes and mutations causing increased intracellular protein O-GlcNAc modification; a mutation that results in early termination in the gene encoding OGA has been associated with a genetic predisposition to adult onset type II diabetes in a Mexican American population (10). OGA removes O-GlcNAc from intracellular proteins and the identified OGA mutations result in increased levels of protein O-GlcNAc modification.

During wound healing, keratinocytes at the wound margin must down-regulate adhesion to adjacent cells to permit movement away from the edge and into the wound (11). Previous data from our group demonstrated that increased O-GlcNAc modification stabilizes cell-cell adhesion in part by increasing the post-translational stability of desmosome components including plakoglobin (12). By increasing cell-cell adhesion, we hypothesize that increased intracellular protein O-GlcNAc modification retards wound healing. This study was undertaken to explore the hypothesis that increased O-GlcNAc modification of cellular proteins in diabetic skin could contribute to the delayed wound healing observed in patients with chronic diabetic skin ulcers. In the present study we modeled hyperglycemia by culturing human keratinocytes in elevated glucose. Under hyperglycemic conditions, we observed (i) increased levels of O-GlcNAc modification of keratinocyte proteins and importantly (ii) delays in wound closure. Hyperglycemia induced delays in wound closure were reversed by shRNA and siRNA knockdown of OGT, the gene responsible for adding the
GlcNAc moiety to proteins. These observations suggest that targeting OGT may be beneficial for treating non-healing diabetic wounds.

**EXPERIMENTAL PROCEDURES**

**Materials.** Cell culture media were obtained from Invitrogen (Carlsbad, CA). shRNA plasmids were purchased from Open Biosystems (Thermo Fisher Scientific, Waltham, MA), and packaged into inactivated lentivirus particles at University of North Carolina at Chapel Hill Lenti-shRNA core facility. The sequences for the mature sense strands in the hairpins were: shOGT (TRCN0000035064): 5’-GCCCTAAGTTTGAGTCCAAAT-3’, and shOGA (TRCN0000134040): 5’-CCAGAAACTTTCCTTGCTAAT-3’. The TRC Lentiviral eGFP shRNA was used as a Positive Control for transduction (Open Biosystems catalog #RHS4459). Mouse monoclonal O-GlcNAc specific antibodies (clone RL2) were from Thermo Scientific (Waltham, MA). Rabbit monoclonal antibodies to GAPDH were from Cell Signaling (Danvers, MA). Mouse monoclonal antibodies to β-actin were from Sigma (St. Louis, MO). Rabbit polyclonal OGT antibodies were from Abcam (Cambridge, MA). Mouse and rabbit anti-sheep horseradish peroxidase-conjugated secondary antibodies were from GE Healthcare (Pittsburgh, PA). Control siRNA (sense strand: GCAGUUAUAAUGACUAGAU) and OGT siRNA (sense strand: GCACAAUCCUGAAAUUU) with 3’UU overhangs were purchased from Sigma-Aldrich.

**Cell culture and scratch wounding.** Untransfected and shRNA transfected HaCaT cells were cultured in normal or high glucose Dulbecco’s modified Eagle’s medium (DMEM) (5.5 mM or 25 mM glucose, respectively)(13), 1% fetal bovine serum (FBS), 1,000 units penicillin/mL, 100 µg streptomycin/mL. Media were supplemented with the amounts of glucose or inhibitor specified in the figure legends. shRNA-transfected cells were selected using 1 µg puromycin per mL medium. Puromycin-containing media were replaced six hours prior to scratching. Cells were grown for 60 hours (until confluent) before scratch assays were performed. Scratch wounds were performed by making a linear scratch across monolayers of confluent cells in 24-well culture plates followed by one wash with 1×PBS and the addition of fresh culture medium. Pictures were taken on a Nikon TE2000-U spinning disk microscope using a 10× magnification immediately after scratching, and incubated at 37°C for the amount of time stated in the figure legends before another set of pictures was taken. Wounds were subsequently analyzed using the Tscratch software package (14). Only wounds of the same initial wound-size were evaluated and compared.

**Statistical analyses.** Error bars reflect the standard error of mean (SEM). Student’s T-tests were performed as two-sided tests with unequal variance as described in the Tscratch software manual.

**Stable transduction of keratinocytes with shRNAs.** HaCaT cells were cultured in DMEM, 10% FBS to 50-60% confluency and incubated with 10 µg/mL polybrene and shRNA (shGFP, shOGT, or shOGA) using a multiplicity of infection (MOI) of two for five hours after which the medium was changed to fresh DMEM. The following day, medium containing 1 µg/mL puromycin was added to the cells to select for successfully transduced cells. Cell cultures were passaged 6-8 times under puromycin selection before they were used for experiments.

**Quantification of immunoblot signals.** Samples were equally loaded on and separated by SDS-PAGE as previously described. Immunoblotting was performed according to established protocols and developed by enhanced chemiluminescence (ECL) reaction (Amersham Biosciences). Protein bands from immunoblots were quantified using the GeneSnap software
(SynGENE, Frederick, MD). For RL2 staining, the three most prominent bands were analyzed using GeneSnap software.

**siRNA transfection of keratinocytes.** siRNA against OGT (3’-GCACAAUCCUGAUAAUUU-5’) and a scrambled control siRNA (3’-GCAGUUAUAAUGACUAGAU-5’) were synthesized with 3’-UU overhands and were diluted to 20 mM in water (working stock) and each well in a 24-well plate with 40% confluent keratinocytes was transfected using Oligofectamine (Invitrogen) according to the protocol. Briefly, 3 μL Oligofectamine (Invitrogen) was diluted in 12 μL Opti-MEM I (Invitrogen) and incubated for 8 min. In the meantime 3 μL siRNA was mixed with 50 μL Opti-MEM I and this was added to the Oligofectamine dilution and left to form complexes for 20 min. 32 μL Opti-MEM was then added to the mix and added to the cells (in 500 μL high glucose DMEM). After 48 hours the medium was changed to high glucose DMEM and at 60 hours the cells were used for scratch assay. Pictures were taken at the time points described in the figure legends.

**RESULTS**

**Hyperglycemic conditions result in elevated protein O-GlcNAc levels in human keratinocytes.** In order to investigate if increased levels of O-GlcNAc modification in diabetic skin may be linked to elevated tissue glucose levels, these conditions were mimicked in cell culture by growing human keratinocytes (HaCaT) for 48 hours with various glucose concentrations (Fig. 1). Immunoblot of cell lysates shows that increased levels of glucose indeed resulted in more O-GlcNAc modification in keratinocyte lysates, as detected by the O-GlcNAc specific antibody RL2 (Fig. 1A,B). This dose-dependent increase in O-GlcNAcylation emphasizes the link between increased glucose concentrations and intracellular protein O-GlcNAc modification in keratinocytes

**Human keratinocts exhibit delayed wound healing under hyperglycemic conditions.** We then utilized the in vitro keratinocyte scratch assay model of wound healing to test the hypothesis that hyperglycemic conditions decrease the rate of wound closure (Fig. 1C,D). The assay was performed by preincubating HaCaT cells with different amounts of glucose for 48 hours, after which a “wound” was introduced in the confluent layer of cells. Elevated levels of glucose in the culture media decreased the rate of wound closure in a dose-dependent manner (Fig. 1C,D).

**Gene knockdown of key enzymes for the O-GlcNAc pathway by RNA interference affects the rate of wound closure in human keratinocyte culture.** The apparent link between delayed wound closure and elevated levels of O-GlcNAc modification in HaCaT cells led us to further investigate the role of the enzymes responsible for the addition and removal of O-GlcNAc protein modification (OGT and OGA, respectively) in more detail. In order to do this, we stably transduced HaCaT cells with shRNAs against either enzyme and analyzed cell lysates by immunoblot analysis (Fig 2A and 2B). Immunoblot analysis of the cell lysates confirmed the impact of RNAi on O-GlcNAc levels, with shOGT displaying significantly reduced levels of O-GlcNAc modification. The shOGA transduced cells displayed levels of O-GlcNAc modification similar to both the untransduced and the shGFP controls (Fig. 2B). Scratch-wounding of shRNA-transduced cells show that knocking down OGT significantly increases the rate of wound closure (Fig. 2C and 2D). shOGA transduced cells were not significantly different from shGFP transduced controls despite evidence that targeting OGA with shRNA reduced total OGA protein levels (data not shown). Collectively, these data strongly suggest that decreasing the amount of O-GlcNAc (via shOGT) accelerates wound healing providing additional support for the relationship between O-GlcNAc protein modification and wound healing.
siRNA knock-down of OGT decreases keratinocyte O-GlcNAc modification and accelerates wound closure in hyperglycemic conditions. To investigate the therapeutic potential of siRNA knockdown to downregulated OGT expression, we tested OGT specific small interfering RNAs (siRNAs) as a means to knock down OGT and accelerate wound healing in the keratinocyte scratch model (Fig. 3). A 19mer siRNA directed against the OGT mRNA sequence was synthesized and HaCaT cells were transfected with the OGT specific silencing RNA as well as a control siRNA with a scrambled sequence. Two days post transfection, cell lysates were probed for RL2 and OGT immunoreactivity (Figs. 3A and 3B). The results show that siRNA against OGT results in a marked knock down in both OGT levels and RL2 immunoreactivity as quantified from immunoblots.

Next, siRNA transfected cells were tested in a scratch-wounding assay to examine the effect of this form of OGT RNAi on wound closure in vitro. Fig. 3C shows that wound healing at the 26-hour time point is significantly more progressed with OGT siRNA compared to both control siRNA and untreated cells (Fig 3D). These results further support the hypothesis that the level of intracellular O-GlcNAc modification in human keratinocytes is linked to wound closure rate and that this may be manipulated using OGT knockdown.

DISCUSSION

Increasing evidence from the literature suggests that alterations in the hexosamine pathway play a key role in the pathophysiology of diabetes. For example, over-expression of OGT in mice results in a diabetic phenotype (8) and increased levels of O-GlcNAc modification have been observed in cells and tissue from type 2 diabetes patients relative to healthy controls (9,15). Previously, we had reported that overexpression of OGT in keratinocytes (i) increases GlcNAc modification of cellular proteins and (ii) markedly enhances cell-cell adhesion (12). Consistent with these observations, we observed a dose dependent increase in protein O-GlcNAc modification in human keratinocyte cultures grown in increasing concentrations of glucose. Furthermore, increasing concentrations of glucose and O-GlcNAc protein modification was associated with delayed wound closure in a dose dependent fashion. Significantly, silencing OGT activity with either OGT specific shRNA or siRNA decreases GlcNAc modification of keratinocyte proteins and promotes wound healing in a scratch model assay, even in the presence of elevated glucose concentrations. Collectively, these observations suggest that increased intracellular O-GlcNAc modification, mediated by the enzyme OGT, likely contributes to delayed wound healing in non-healing diabetic skin wounds.

The effects of increased OGT activity on promoting cell adhesion and delaying wound healing may in part be due to regulation of keratinocyte cell adhesion components, including desmosomes, adherens junctions, and cytoskeletal elements as we have previously reported (12). In this context, we previously showed that plakoglobin, a component of both adherens junction and desmosome cell-cell adhesion complexes, is post-translationally stabilized by increased O-glycosylation in OGT overexpressing keratinocytes. This increased plakoglobin protein level drove formation of desmosomes and plakoglobin based adherens junctions and markedly enhanced cell-cell adhesion (12). These observations indicate that in keratinocytes, O-GlcNAc modification functions in part to regulate plakoglobin’s post-translational stability and significantly, to regulate keratinocyte cell-cell adhesion. During wound healing, keratinocytes migrate into the wound to promote re-epithelialization. Keratinocytes at the wound margin must down-regulate adhesion to adjacent cells at the trailing margin to permit movement away from the edge and into the wound. By increasing cell-cell adhesion, we suggest that increased intracellular protein O-GlcNAc modification retards wound
healing; whereas, down-regulation of intracellular protein O-GlcNAc modification promotes wound healing.

It is worth noting that O-GlcNAc is a ubiquitous intracellular modification. In addition to modifying cell adhesion and structural proteins, transcription factors and regulatory enzymes are also modified by OGT catalyzed addition of GlcNAc to serine and threonine residues. Thus, the effects of OGT activity are likely to be pleiotropic. In addition to its effects on adhesion, altering levels of intracellular protein O-GlcNAc modification may also impact cell proliferation and chemotaxis and it may be the combination of these effects that contribute to the observed delayed wound healing.

Diabetic wounds represent a significant health care burden. The incidence and social and financial cost of treating these wounds is likely to increase as the incidence of diabetes rises due to the obesity epidemic and aging populations. We have demonstrated that decreasing the global level of O-GlcNAc modification through knockdown of OGT using RNAi accelerates wound healing in a hyperglycemic keratinocyte culture model. Collectively, these data show that locally targeting OGT may prove an effective approach to promote healing in diabetic ulcers. As it has previously been demonstrated that the impaired barrier function in wounds allows for transfection with oligonucleotides (16), we suggest that topical administration of siRNAs or antisense oligodeoxynucleotides against OGT may be an effective treatment to promote healing of diabetic skin wounds.
REFERENCES

1. Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., Hunt, T. K., Gottrup, F., Gurtner, G. C., and Longaker, M. T. (2009) Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen* 17, 763-771
2. Hart, G. W., Housley, M. P., and Slawson, C. (2007) Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature* 446, 1017-1022
3. Konrad, R. J., Janowski, K. M., and Kudlow, J. E. (2000) Glucose and streptozotocin stimulate p135 O-glycosylation in pancreatic islets. *Biochem Biophys Res Commun* 267, 26-32
4. Liu, K., Paterson, A. J., Chin, E., and Kudlow, J. E. (2000) Glucose stimulates protein modification by O-linked GlcNAc in pancreatic beta cells: linkage of O-linked GlcNAc to beta cell death. *Proc Natl Acad Sci U S A* 97, 2820-2825
5. Dentin, R., Hedrick, S., Xie, J., Yates, J., 3rd, and Montminy, M. (2008) Hepatic glucose sensing via the CREB coactivator CRTC2. *Science* 319, 1402-1405
6. Konrad, R. J., Tolar, J. F., Hale, J. E., Knierman, M. D., Becker, G. W., and Kudlow, J. E. (2001) Purification of the O-glycosylated protein p135 and identification as O-GlcNAc transferase. *Biochem Biophys Res Commun* 288, 1136-1140
7. Konrad, R. J., Mikolaenko, I., Tolar, J. F., Liu, K., and Kudlow, J. E. (2001) The potential mechanism of the diabetogenic action of streptozotocin: inhibition of pancreatic beta-cell O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase. *Biochem J* 356, 31-41
8. McClain, D. A., Lubas, W. A., Cooksey, R. C., Hazel, M., Parker, G. J., Love, D. C., and Hanover, J. A. (2002) Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. *Proc Natl Acad Sci U S A* 99, 10695-10699
9. Park, K., Saudek, C. D., and Hart, G. W. (2010) Increased expression of beta-N-acetylglucosaminidase in erythrocytes from individuals with pre-diabetes and diabetes. *Diabetes* 59, 1845-1850
10. Lehman, D. M., Fu, D. J., Freeman, A. B., Hunt, K. J., Leach, R. J., Johnson-Pais, T., Hamlington, J., Dyer, T. D., Arya, R., Abboud, H., Goring, H. H., Duggirala, R., Blangero, J., Konrad, R. J., and Stern, M. P. (2005) A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase is associated with type 2 diabetes in Mexican Americans. *Diabetes* 54, 1214-1221
11. Gurtner, G. C., Werner, S., Barrandon, Y., and Longaker, M. T. (2008) Wound repair and regeneration. *Nature* 453, 314-321
12. Hu, P., Berkowitz, P., Madden, V. J., and Rubenstein, D. S. (2006) Stabilization of plakoglobin and enhanced keratinocyte cell-cell adhesion by intracellular O-glycosylation. *J Biol Chem* 281, 12786-12791
13. Clark, R. J., McDonough, P. M., Swanson, E., Trost, S. U., Suzuki, M., Fukuda, M., and Dillmann, W. H. (2003) Diabetes and the accompanying hyperglycemia impairs cardiomyocyte calcium cycling through increased nuclear O-GlcNAcylation. *J Biol Chem* 278, 23199-23206
14. Geback, T., Schulz, M. M., Koumoutsakos, P., and Detmar, M. (2009) TScratch: a novel and simple software tool for automated analysis of monolayer wound healing assays. *Biotechniques* 46, 265-274
15. Jensen, R. V., Zachara, N. E., Nielsen, P. H., Kimose, H. H., Kristiansen, S. B., and Botker, H. E. (2013) Impact of O-GlcNAc on cardioprotection by remote ischaemic preconditioning in non-diabetic and diabetic patients. *Cardiovasc Res* 97, 369-378
16. Wang, C. M., Lincoln, J., Cook, J. E., and Becker, D. L. (2007) Abnormal connexin expression underlies delayed wound healing in diabetic skin. *Diabetes* 56, 2809-2817
CONFLICT OF INTEREST
David S. Rubenstein is an inventor on a patent on compositions and methods for targeting OGT and promoting wound healing. Kasper Runager has contributed to the above referenced invention and has a percentage share. Neither licensing agreements nor financial benefit have accrued to either individual.

ACKNOWLEDGEMENTS
This work was supported by USA National Institutes of Health Grant RO1 AI49427 (to D.S.R.).
FIGURE LEGENDS

Figure 1. Hyperglycemia increases O-GlcNAc modification and retards wound healing in human keratinocytes. HaCaT cells were cultured in media supplemented with glucose to the final concentrations indicated. A. Representative immunoblot of cell lysates separated by SDS-PAGE and probed with antibodies to (i) RL2, which recognizes the O-GlcNAc modification, and (ii) GAPDH as a loading control. B. The RL2 signal was quantified relative to the GAPDH loading control (n=3). C, D. Scratch assays; human keratinocyte monolayers were incubated in DMEM with the indicated glucose concentrations. Cells were scratched with a pipet tip and micrographs were made at 0 h and 16 h. Wound sizes were then measured using image analysis software. Representative micrographs shown in C and quantified in D. N=11 for all conditions. Error bars reflect the standard error of mean (SEM). * indicates P-value <0.07 and ** indicates P-value <0.0005 compared to normal glucose levels (5.5 mM). The P-value between 25 and 100 mM is <0.01.

Figure 2. OGT knockdown using shRNA accelerates wound healing. HaCaT cells were stably transduced with shRNA targeting OGT, OGA, and GFP (control) and grown to confluency. A. OGT knockdown decreases protein O-GlcNAcylation. Cell lysates were analyzed by immunoblot with antibodies to (i) O-GlcNAc (RL2), (ii) OGT, and (iii) actin (loading control). B. RL2 reactivity was quantified compared to actin. C, D. OGT knockdown promotes wound healing in hyperglycemic cultures. Transduced cells were grown to confluency in growth medium with 25 mM glucose and scratched to introduce wounds. Representative micrographs obtained at 0 h and 12 h are shown in panel C, while the quantification of open wound areas is shown in panel D. In the scratch wounding assay N=18 for untreated cells, N=10 for shGFP, N=8 for shOGT, and N=14 for shOGA. Error bars reflect the standard error of mean (SEM). Asterisks (*) denote results with p-values < 0.05.

Figure 3. OGT knockdown using siRNA accelerates wound healing. HaCaT cells were transfected with 100 nM siRNA against OGT or a scrambled control siRNA. A, B. Cell lysates of confluent cultures were subjected to immunoblot with antibodies to (i) O-GlcNac (RL2), (ii) OGT, and (iii) GAPDH (control) (A) and quantified (B). C, D. 60 hrs after transfection with siRNAs, the confluent cultures were scratched and micrographs obtained at 0 h, 16 h, and 26 h (C). The open wound area was quantified using image analysis software (D). N=7 for the untransfected cells, N=7 for control siRNA, and N=6 for OGT siRNA. Error bars reflect the standard error of mean (SEM). Asterisks (*) denote p < 0.05 compared to controls.
FIGURE 1

A

IB: RL2

GAPDH

Glc (mM) 5.5 25 50 100

kDa

140

100

B

Relative RL2 signal

5.5 25 50 100

[Glc] (mM)

C

Glc (mM) 0 hr 16 hrs

5.5

25

100

D

% open wound area

5.5 25 100

[Glc] (mM)

* **
FIGURE 3

A

IB:

RL2

OGT

GAPDH

B

RL2 reactivity

Untr

Control siRNA

OGT siRNA

kDa

1250

130

100

35

C

0 h

16 h

26 h

Untr

Control siRNA

OGT siRNA

D

% open wound area

Untreated

Control siRNA

OGT siRNA

16 h

26 h
Targeting O-glycosyltransferase (OGT) to promote healing of diabetic skin wounds
Kasper Runager, Meryem Bektas, Paula Berkowitz and David S. Rubenstein

J. Biol. Chem. published online January 7, 2014

Access the most updated version of this article at doi: 10.1074/jbc.M113.513952

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts