O-GlcNAcylation regulates integrin-mediated cell adhesion and migration via formation of focal adhesion complexes

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O-GlcNAcylation is a post-translational modification of a protein serine or threonine residue catalyzed by O-GlcNAc transferase (OGT) in the nucleus and cytoplasm. O-GlcNAcylation plays important roles in the cellular signaling that affect the different biological functions of cells, depending upon cell type. However, whether or not O-GlcNAcylation regulates cell adhesion and migration remains unclear. Here, we used the doxycycline-inducible short hairpin RNA (shRNA) system to establish an OGT knockdown (KD) HeLa cell line and found that O-GlcNAcylation is a key regulator for cell adhesion, migration, and focal adhesion (FA) complex formation. The expression levels of OGT and O-GlcNAcylated proteins were remarkably suppressed 24 h after induction of doxycycline. Knockdown of OGT significantly promoted cell adhesion, but it suppressed the cell migration on fibronectin. The immunostaining with paxillin, a marker for FA plaque, clearly showed that the number of FAs was increased in the KD cells compared with that in the control cells. The O-GlcNAcylated levels of paxillin, talin, and focal adhesion kinase were down-regulated in KD cells. Interestingly, the complex formation between integrin β1, focal adhesion kinase, paxillin, and talin was greatly increased in KD cells. Consistently, levels of active integrin β1 were significantly enhanced in KD cells, whereas they were decreased in cells overexpressing OGT. The data suggest a novel regulatory mechanism for O-GlcNAcylation during FA complex formation, which thereby affects integrin activation and integrin-mediated functions such as cell adhesion and migration.

O-GlcNAcylation is controlled by OGT3 and is a specific type of post-translational modification that consists of the covalent attachment of single GlcNAc to the nucleus and cytoplasm of the serine or threonine residue of an extremely large family of target proteins (1, 2). This post-translational modification is essential for cell survival and division (3), and aberrant O-GlcNAcylation provokes tumorigenesis, diabetes, and Alzheimer’s disease by regulating cell signaling, transcription, metabolism, and cytoskeletal formation (4–7). The increased O-GlcNAcylation seems to be a general characteristic of cancer cells. For example, higher levels of O-GlcNAcylation expression have been observed in cancers of the liver (8), lung, colon (9), and breast (10). Furthermore, numerous breast cancer cell lines have shown higher levels of O-GlcNAcylation, and the levels of OGT expression in aggressive breast cancer cell lines are much higher than those seen in less aggressive breast cancer cell lines (11). O-GlcNAc modifications have also been observed in important target proteins, such as p53 (12), HIF-1α (13), β-catenin (10), and G6PD (14), which are involved in the regulation of malignant cancer characteristics by controlling cellular metabolism and proliferation. On the other hand, the suppression of OGT expression in breast or liver cancer cell lines decreases cell motility, which suggests that O-GlcNAcylation could be involved in cell migration (10, 15).

Cell migration is a highly integrated multistep process that includes the development of cytoplasmic protrusions, attachment, and spreading (16). The migratory capacity of cancer cells is initially mediated by alterations in the expression of cell surface molecules known as integrins (17). It is becoming increasingly clear that integrins are crucial for cell migration in the tumor microenvironment (18). Following ligand binding, integrins cluster into focal contacts that contain different focal adhesion (FA)-associated proteins, such as α-actinin, vinculin, talin, FAK, and paxillin, which link the integrins to the actin cytoskeleton (19). The processes of adhesion formation and disassembly drive the migration cycle through ligand binding, which in turn regulates integrin activity and cytoskeletal complex formation as well as adhesion dynamics (20). O-GlcNAcylation appears to occur in actin cytoskeletal regulatory proteins, such as paxillin (21) and talin (22), as well as in microtubule assembly proteins, such as tubulin (23), and in microtubule-associated proteins (24). However, whether and how O-GlcNAcylation impacts cell migration remains unclear.

In the present study, we used the doxycycline shRNA-inducible system to knock down the OGT gene to identify the biological functions of O-GlcNAcylation and its regulatory mechanisms in cell adhesion and migration. We found that the knockdown of OGT aberrantly increased cell adhesion, FA formation, and integrin β1 activation, which in turn decreased cell migration. Thus, our findings may provide
FAK was modified by O-GlcNAc

Figure 1. Knockdown of OGT suppressed O-GlcNAc modification and enhanced cell spreading in HeLa cells. A and B, the expression levels of OGT and O-GlcNAc from cell lysates of DOX-controlled OGT KD HeLa cells were verified with concentrations of DOX at 0, 0.1, 0.5, 1.0, and 5.0 μg/ml for 72 h (A) or at the indicated time with 0.1 μg/ml DOX (B). The control (Ctrl) indicates the cells treated without DOX. Cell lysates from the indicated cells were subjected to Western blotting with the O-GlcNAc (CTD110.6), OGT, and α-tubulin antibodies. C, representative images of cell spreading are shown after incubation for 48 h. Cells were incubated with (KD) or without (Ctrl) 0.1 μg/ml DOX for 24 or 48 h on a normal culture dish, after which the cell areas were measured. Values represent the mean ± S.E. (error bars) (n = 50). **, p < 0.01 (Welch’s correction t test). Scale bars, 15 μm. Experiments were independently repeated at least two times.

new insight into integrin-mediated cell migration and explain why O-GlcNAc modification is usually highly expressed in some malignant cancers.

Results

Established OGT knockdown (KD) cells

A growing number of studies have shown that O-GlcNAc modification plays a critical role in the regulation of tumor cell growth (11) and cancer metastasis (25, 26). To investigate the effects of O-GlcNAc expression on cell adhesion and migration, we used the DOX-dependent inducible shRNA KD system to establish OGT KD HeLa cells. In this cellular system, OGT and O-GlcNAc were expressed at normal levels in the absence of DOX, whereas both expressions were drastically suppressed in the presence of DOX in the culture medium at the indicated concentrations, as shown in Fig. 1A. Furthermore, similar suppression levels were observed even following incubation at the lowest concentration of 0.1 μg/ml after 24 h (Fig. 1B), suggesting an effective KD of OGT and a rapid turnover of O-GlcNAc levels in HeLa cells. After culture for 48 h, elongated cell shapes were converted to a more-rounded morphology, and the KD cells showed significantly increased cell spreading areas compared with those in the control cells (Fig. 1C). These observations suggest the impact that O-GlcNAc modification exerts on cell morphology.

Knockdown of O-GlcNAc enhanced cell adhesion and FA formation and suppressed cell motility

Next, we used a fibronectin (FN)-coated dish to investigate the effects of OGT KD on cell adhesion, FA formation, and cell motility. To verify the initial stage of cell adhesion, we performed a 20-min cell adhesion assay on FN. Interestingly, the number of adhered cells was drastically increased in the KD cells compared with that in the control cells (Fig. 2A). During cell adhesion, integrins and cytoplasmic proteins such as paxillin, talin, and FAK become clustered in the plane of the cell membrane and in well-developed aggregates, the so-called FA plaque, which can be detected by immunofluorescence microscopy (27). Consistent with their enhancement of cell adhesion, in the present study, OGT KD cells also promoted an increase in FA formation, by comparison with the activity in control cells (Fig. 2B). By contrast, the KD cells showed a significant reduction in cell motility, as observed by video microscopy (Fig. 2C). These data indicate that a loss of O-GlcNAc modification promotes cell adhesion and focal contact formation while suppressing cell migration.

Talin, FAK, and paxillin were O-GlcNAc–modified proteins

Previous studies have revealed that some forms of protein FA plaque, such as paxillin and talin, are modified by O-GlcNAc (21, 22). In the present study, we investigated whether O-GlcNAc modification of those target proteins also occurred in HeLa cells in this system. Consistent with previous studies,
O-GlcNAc modifications were detected on both paxillin and talin, whereas O-GlcNAcylation levels for both proteins were significantly decreased in KD cells, compared with that seen in control cells (Fig. 3, A and B). Importantly, we also found that FAK, a key molecule for integrin-mediated signaling, was also a target protein for O-GlcNAcylation, which was decreased in the KD cells (Fig. 3C). The suppression of O-GlcNAcylation on FAK and talin was also confirmed in DOX-induced OGT KD 293T cells (data not shown). To further establish the occurrence of O-GlcNAcylation in these proteins, we conducted a chemoenzymatic labeling assay using an azido-GalNAc sugar, as described under “Experimental procedures.” Clearly, talin, FAK, and paxillin were labeled, which proved that they are O-GlcNAcylated proteins (Fig. 4, A–C). These results suggest that O-GlcNAcylation may affect both integrin β1–mediated complex formation and FA formation, which confirms this process as a regulator of cell adhesion and migration.

Reduction of O-GlcNAcylation promoted complex formation

FAK is a key component of the signal transduction pathways triggered by integrins. When cells bind to the extracellular matrix (ECM), FAK is usually recruited to integrin-mediated nascent FA, because it interacts directly through the cytoskeletal proteins talin and paxillin, with the cytoplasmic tail of integrin β1 (28). Therefore, we compared the ability of control and KD cells to form FA complexes. As shown in Fig. 5A, the complexes immunoprecipitated with anti-FAK antibody showed higher levels of paxillin in KD cells than in control cells. Consistently, KD cells demonstrated a greater number of complex formations composed of both β1 integrin and talin (Fig. 5B) and talin and FAK (Fig. 5C). A similar phenomenon was also confirmed in OGT-KD 293T cells (data not shown).

Knockdown of O-GlcNAcylation activated integrin β1

Given the increase in FA complex formation in KD cells, it is reasonable to speculate that OGT-KD may affect integrin activation. Integrin-mediated adhesion can recruit FA proteins to form FA plaque and then trigger conformational activation, so-called inside-out signaling, of integrin β1 in the ectodomain, which then can be recognized by a specific antibody (29, 30) that we used to examine the expression levels of active integrin β1 in both control and KD cells. The expression levels of active β1 in immunostaining (Fig. 6A) or cell lysates (Fig. 6B) were clearly up-regulated in the KD cells compared with control cells. In contrast to KD cells, the expression levels of active β1 were suppressed in the OGT-overexpressing HeLa cells, which further suggested that O-GlcNAcylation negatively regulates integrin-mediated inside-out signaling. Thus, we were convinced that OGT could be a novel regulator for FA complex formation and integrin activation by dynamically regulating cell adhesion and migration.

Discussion

In the present study, we clearly showed that O-GlcNAcylation negatively regulates integrin-mediated cell adhesion and FA complex formation as well as integrin activation, which results in the control of cell migration on the ECM (Fig. 7). Our findings are the first to demonstrate that OGT may function as a key regulator of FA complex formation during cell–ECM adhesion. These results provide clues to understanding the roles of O-GlcNAcylation in cell migration. Cell migration is a central process in the development and maintenance of multicellular organisms (16). Although the detailed mechanisms underlying cell migration remain unclear, it is reasonable to postulate that integrin-mediated cell adhesion could regulate migration, which would allow communication between cell–ECM contact and the actin cytoskeleton through focal adhesions (31). The dynamic balance between adhesion receptors and the binding of ECM ligands provides FA turnover that regulates adhesion formation and disassembly (32). In the framework of this model, an imbalance in the processes of attachment and detachment leads to conformational changes that mediate abnormal adhesion (19). In the present study, we clearly demonstrated that the suppression of O-GlcNAcylation inhibited HeLa cell migration, whereas it enhanced cell–ECM adhesion (Fig. 2), which indicated that O-GlcNAcylation is involved in the regulation of integrin-mediated cell adhesion. Consistently, FAK serves as a key regulator of FA assembly and disassembly processes that are fundamental for efficient cell migration (33). Indeed, there were more stress fibers and focal adhesions in FAK-deficient cells, whereas cell motility was inhibited (34). Aberrant cell–ECM adhesiveness is likely to suppress cell migration, and proper cell adhesion is an important determinant for cell migration (35). Thus, our data are reasonable in that the knockdown of O-GlcNAcylation aberrantly increased cell adhesion, as well as spreading and FA complex formation, which in turn decreased cell migration. Consistently, a loss of paxillin phosphorylation at Ser-250 markedly inhibits focal adhesion turnover and cell migration (36).
We were intrigued as to why a knockdown of OGT would enhance integrin activation. Integrins are the major cell surface receptors used to assemble and recognize a functional ECM and to facilitate cell signaling and migration (37). The organization of cell adhesions is complex and includes a number of cytoplasmic proteins, such as paxillin, talin, FAK, vinculin, and \( \alpha \)-actinin (29). Integrin activation is associated with an array of biological and pathological conditions involving both outside-in and inside-out signaling (38). Accumulating data have indicated that the cytoplasmic domain of the integrin \( \beta 1 \) subunit cooperatively promotes integrin activation through the binding of talin (39). Consequently, our results clearly showed the interaction of integrin \( \beta 1 \) with talin, and the association of FAK, paxillin, and/or talin both were greatly increased in the KD cells, which suggests that the KD of OGT promotes inside-out signaling (Fig. 5). A reciprocal relationship between O-GlcNAcylation and O-phosphorylation has been observed in the specific serine or threonine residue of particular proteins (40,41), and therefore, how O-GlcNAcylation affects the O-phosphorylation of FA complex proteins is worthy of clarification.

The O-GlcNAcylation of FAK is noteworthy. Integrins do not possess enzymatic activity; rather, they associate with a number of cytoplasmic protein kinases, such as FAK and Src. Tyrosine-phosphorylated FAK is well-known to be a promoter of interactions with various Src homology 2– and 3–containing proteins and to initiate enzymatic cascades via these associated kinases that ultimately lead to changes in cell behavior (42). By contrast, serine or threonine phosphorylation on FAK is not well-understood. FAK phosphorylation at either Ser-732 or Ser-722 has recently been recognized as important for microtubule organization, nuclear movement, and neuronal migration during cell adhesion (43, 44). Interestingly, phosphorylation of both Ser-843 and Ser-910 on FAK exhibited synchronized phosphorylation during cell mitosis (45), which may be related to O-GlcNAcylation because expression levels of OGT change during mitosis (46). Furthermore, a cluster of ser-

![Figure 4. Confirmation of O-GlcNAcylation on talin, FAK, and paxillin.](image)

![Figure 5. Increased focal adhesion complex formation in OGT KD cells.](image)
ine phosphorylation sites was recently identified at the initiation of the FA-targeting domain in FAK (47), which may suggest that some of those sites could be modified by O-GlcNAcylation. Thus, to elucidate the roles of OGT in cell biology, it is necessary to identify the specific sites and functions of O-GlcNAcylation in FAK.

Our results indicate that O-GlcNAcylation plays important roles in regulating cell adhesion, FA complex formation, and cell migration. Emerging data have already established that O-GlcNAc modification has a critical role in the progress of human diseases, and particularly diseases such as cancer, diabetes, and Alzheimer’s (7). Intriguingly, FAK has been associated with insulin resistance in adipocytes in the early stages of type II diabetes (48, 49) and has also been implicated in the deposition of β-amyloid plaque (50, 51). It would be reasonable to assume that dynamic regulation of FAK O-GlcNAcylation with phosphorylation may partially serve as a possible explanation for a number of diseases.

Experimental procedures

Antibodies and reagents

Experiments were performed using the following antibodies: mAb against O-GlcNAc (CTD110.6, 9875S) and peroxidase-conjugated secondary antibody against rabbit (7074S) from Cell Signaling Technology; the rabbit polyclonal antibody against OGT (O0164) and mAb against α-tubulin (T6199) and VSV (V5507) from Sigma; mAb against integrin β1 (610468) and paxillin (610052) from BD Biosciences; mAb against active integrin β1 (HUTS-4; 2079Z) and peroxidase-conjugated secondary antibodies against mouse (AP124P) and goat (AB324P) from Millipore; Alexa Fluor 488–conjugated anti-mouse (A11029) from Invitrogen; TO-PRO-3 (T3605) from Molecular Probes, Inc.; and GFP-agarose (MBL, D153-8) and goat anti-body against GFP (Rockland, 600-101-215). The mAb against human β1 (P5D2) was obtained from the Developmental Studies Hybridoma Bank, University of Iowa. Human FN and doxycycline hyclate (D9891) were from Sigma-Aldrich. An ABC kit was acquired from Vector Laboratories, and Ab-Capcher Mag was from ProteNova (Takamatsu, Japan).
FAK was modified by O-GlcNAc

Cell culture and expression plasmids

HeLa and 293T cell lines (RIKEN, Japan) were maintained at 37 °C in DMEM high-glucose (Invitrogen) supplemented with 10% fetal bovine serum under a humidified atmosphere that contained 5% CO₂. To express GFP-tagged talin (52) and 2× VSV-tagged FAK, expression vector pEGFP-N1-talin-GFP (Addgene 26724) and pRKVSV-FAK were kindly provided by Dr. Anna Hüttenlocher (52) and Dr. Kenneth Yamada (53), respectively. The pcDNA3.1/myc-his expression vector containing human OGT was kindly provided by Dr. Yuanyuan Ruan (School of Basic Medical Sciences, Fudan University, Shanghai, China). Transfection was performed using PEI MAX (molecular mass, 40 kDa; Polysciences Inc.) and following the dictates of the United States patent application (number US20110020927A1) with minor modifications. Briefly, 24 h prior to transfections, cells were seeded on a 10-cm dish, and expression vectors with PEI MAX (1 mg/ml in 0.2 M hydrochloric acid) were preincubated for 15 min at a 1:3 ratio in 2,000 μl of a solution that contained 20 mM CH₃COONa buffer, pH 4.0, and 150 mM NaCl. Cells and DNA complexes were further incubated for 24 h with 10 ml of normal culture medium to promote expression.

Establishment of doxycycline-inducible OGT knockdown cells

We used CS-RfA-ETBsd DOX-dependent inducible RNAi mediated by a single lentivirus vector (RIKEN) for the knockdown experiment (54). The following oligonucleotides were inserted into pENTR/H1/TO (sense, CACCGCTGAGCAGTCGTTATCCTCGAGGAAACTCGAGTTTCTCGGAATCTGCTCAGC; antisense, AAAAGGCTGACGATATTCCCGAGAAAATCCTCGAGGAAACTCGAGTTTCTCGGAATCTGCTCAGC) with minor modification from a procedure established in a previous report (13). Using LR clonase, inserted oligonucleotide was then transferred to CS-RfA-ETBsd, which encodes tetracycline-dependent transactivators for shRNA expression. To prepare the viruses, PEI MAX was used to transfect the resultant vector into 293T cells with packaging plasmids. HeLa and 293T cells were then infected by the obtained viruses and selected for stable integration with 10 μg/ml blasticidin. The shRNA-mediated silencing of OGT was induced by the addition of DOX in the established cell line, and the cells cultured by DOX-free medium were used as the control in the present study.

Immunoprecipitation and Western blotting

The cells were washed with PBS, and lysed in lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Triton X-100) with protease and phosphatase inhibitors (Nacalai Tesque, Kyoto, Japan). The supernatants were collected, and the protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce). Equal amounts of proteins were subjected to SDS-PAGE and then transferred to polyvinylidene difluoride membranes. To detect active integrin β1, we prepared samples under nonreducing conditions. The membranes were blocked either with 5% nonfat milk in TBST or with 3% BSA for 2 h at room temperature, and then the probes were probed with antibodies against O-GlcNAc, OGT, α-tubulin, active integrin β1 (HUTS-4) (55, 56), integrin β1 (Millipore), paxillin, VSV, and GFP. After being washed, the membranes were incubated with horseradish peroxidase–conjugated secondary antibodies. Detection was accomplished using a horseradish peroxidase substrate (Millipore) according to the manufacturer’s instructions. For immunoprecipitation, the supernatant (500 μg of protein) was incubated with an anti-VSV or an anti-paxillin with an Ab-Capcher Mag. GFP-talin was immunoprecipitated with GFP-conjugated beads. The immunoprecipitates were washed with lysis buffer and subjected to SDS-PAGE. The immunocomplexes then were detected using the indicated antibodies. An mAb against α-tubulin was used as the loading control.

Cell adhesion assay

Cell adhesion assays were performed in a 96-well CellCarrier (PerkinElmer Life Sciences) coated with FN (5 μg/ml) overnight. HeLa cells were pretreated with or without DOX (0.1 μg/ml) for 24 h. Cells were replated at a density of 10⁴ cells/well in plates using serum-free DMEM with 0.1% BSA, followed by incubation at 37 °C for 20 min. Nonadherent cells were removed by washing three times with PBS. Cells were fixed with 4% formaldehyde and stained 4′,6-diamidino-2-phenylindole (Invitrogen) and were then imaged by fluorescent microscopy using an Operetta CLS (PerkinElmer Life Sciences). To count the number of nuclei in the each well, images were analyzed using Harmony software (PerkinElmer Life Sciences).

Immunofluorescence

Cells were plated onto FN-coated glass coverslips (MatTek Corp., Ashland, MA) for 1 h, washed with PBS, and fixed with 4% paraformaldehyde. For permeabilization, the cells were treated with 0.1% Triton X-100 in PBS. The cells were blocked with 0.1% Tween 20 and 3% BSA in PBS and then stained with paxillin, active β1 (HUTS-4), total β1 (P5D2), and OGT antibodies overnight at 4 °C. The samples were followed by incubation with anti-mouse Alexa Fluor 488–conjugated secondary antibody and were then incubated with TO-PRO-3. Images were acquired by sequential excitation using an Olympus FV1000 laser-scanning confocal microscope with an UPlanSApo ×60/1.35 oil objective and high-sensitivity gallium arsenide photodiode detector units operated by F10-ASW version 4.02 software. To count the number of FAs, we followed a protocol previously described using ImageJ (57), excluding focal adhesions that were less than 0.2 μm², because these disappeared quickly (58). OGT-overexpressing cells were identified via co-immunostaining with OGT. The relative fluorescence intensities of active integrin β1 and total integrin β1 were quantified using ImageJ software.

Video microscopy

Glass-bottom dishes (Asahi Glass, Shizuoka, Japan) were precoated with FN (10 μg/ml) in PBS, let stand at 4 °C overnight, and were then blocked with 1% BSA. Ten thousand cells were suspended in 2 ml of DMEM containing 3% fetal bovine serum medium, which was then added to each FN-coated glass-bottom dish and monitored for 12 h using AxioVision equipment (Carl Zeiss, Oberkochen, Germany). Images were acquired using an inverted microscope (Axio Observer.D1, Carl Zeiss) every 10 min with 5% CO₂ at 37 °C in a heated chamber equipped with temperature and CO₂ controllers.
FAK was modified by O-GlcNAc

(Onpu-4 and CO2; AR BROWN, Tokyo, Japan) during time-lapse imaging. Cell motility was evaluated using an AxioVision Tracking module (Carl Zeiss).

**Chemoenzymatic labeling assay**

Chemoenzymatic labeling and biotinylation of proteins in cell lysates was carried out using the Click-iT O-GlcNAc enzymatic labeling system (Invitrogen). Briefly, the whole-cell lysate of 293T cells transfected with an expression plasmid for VSV-FAK or GFP-talin (500 μg of 293T cells transfected with an expression plasmid for VSV-FAK) and HeLa cells were immunoprecipitated and then labeled with labeling enzyme GaIT and UDP-GalNAz according to the Click-iT O-GlcNAc enzymatic labeling system protocol (Invitrogen). Labeled proteins were conjugated with an alkyne-biotin compound following the Click-iT protein analysis detection kit protocol (Invitrogen). Control experiments were performed in the absence of GaIT and UDP-GalNAz. Biotinylated and control samples were then subjected to SDS-PAGE and transferred to a polyvinylidene difluoride membrane for further detection using an ABC kit (Vector Laboratories).

**Statistics**

All results shown are the results of at least two independent experiments and are shown as representative data. The values represent the mean ± S.E. p values were calculated using Welch’s correction t test using GraphPad Prism version 5 (*, p < 0.05; **, p < 0.01).

**Author contributions**—T. I. and J. G. designed the research; Z. X. and T. I. performed all experiments; T. F. and Y. W. assisted with experiments; T. I., T. F., Y. W., and J. G. analyzed and interpreted the data; Z. X., T. I., Y. W., and J. G. wrote and revised the manuscript; and all authors approved the final version of the manuscript.

**References**

1. Zachara, N. E., and Hart, G. W. (2006) Cell signaling, the essential role of O-GlcNAc! Biochim. Biophys. Acta 1761, 599–617 CrossRef Medline
2. Vosseller, K., Sakabe, K., Wells, L., and Hart, G. W. (2002) Diverse regulation of protein function by O-GlcNAc: a nuclear and cytoplasmic carbohydrate post-translational modification. Curr. Opin. Chem. Biol. 6, 851–857 CrossRef Medline
3. Tan, E. P., Duncan, F. E., and Slawson, C. (2017) The sweet side of the cell cycle. Biochem. Soc. Trans. 45, 313–322 CrossRef Medline
4. Singh, J. P., Zhang, K., Wu, J., and Yang, X. (2015) O-GlcNAc signaling in cancer metabolism and epigenetics. Cancer Lett. 356, 244–250 CrossRef Medline
5. Itkonen, H. M., Minner, S., Guldvik, I. J., Sandmann, M. J., Tsourlakis, M. C., Berge, V., Svinland, A., Schloem, T., and Mills, I. G. (2013) O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer cells. Cancer Res. 73, 5277–5287 CrossRef Medline
6. Slawson, C., and Hart, G. W. (2011) O-GlcNAc signaling: implications for cancer cell biology. Nat. Rev. Cancer 11, 678–684 CrossRef Medline
7. Copeland, R. J., Han, G., and Hart, G. W. (2013) O-GlcNAcomics: revealing roles of O-GlcNAcylation in disease mechanisms and development of potential diagnostics. Proteomics Clin. Appl. 7, 597–606 CrossRef Medline
8. Zhang, X., Qiao, Y., Wu, Q., Chen, Y., Zou, S., Liu, X., Zhu, G., Zhao, Y., Chen, Y., Yu, Y., Pan, Q., Wang, J., and Sun, F. (2017) The essential role of YAP O-GlcNAcylation in high-glucose-stimulated liver tumorigenesis. Nat. Commun. 8, 15280 CrossRef Medline
9. Mi, W., Gu, Y., Han, C., Liu, H., Fan, Q., Zhang, X., Cong, Q., and Yu, W. (2011) O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy. Biochim. Biophys. Acta 1812, 514–519 CrossRef Medline
10. Gu, Y., Mi, W., Ge, Y., Liu, H., Fan, Q., Han, C., Yang, J., Han, F., Lu, X., and Yu, W. (2010) GlcNAcylation plays an essential role in breast cancer metastasis. Cancer Res. 70, 6344–6351 CrossRef Medline
11. Caldwell, S. A., Jackson, S. R., Shahriari, K. S., Lynch, T. P., Sethi, G., Walker, S., Vosseller, K., and Regnato, M. J. (2010) Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. Oncogene 29, 2831–2842 CrossRef Medline
12. Yang, W. H., Kim, J. E., Nam, H. W., Ju, J. W., Kim, H. S., Kim, Y. S., and Cho, J. W. (2006) Modification of p53 with O-linked-N-acetylgalcosamine regulates p53 activity and stability. Nat. Cell Biol. 8, 1074–1083 CrossRef Medline
13. Ferrer, C. M., Lynch, T. P., Sodi, V. L., Falcone, J. N., Schwab, L. P., Peacock, D. L., Vocadlo, D. J., Seagroves, T. N., and Regnato, M. J. (2014) O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. Mol. Cell 54, 820–831 CrossRef Medline
14. Rao, X., Duan, X., Mao, W., Li, X., Li, Z., Li, Q., Zheng, Z., Xu, H., Chen, M., Wang, P. G., Wang, Y., Shen, B., and Yi, W. (2015) O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth. Nat. Commun. 6, 8468 CrossRef Medline
15. Xu, W., Zhang, X., Wu, J. L., Fu, L., Liu, K., Liu, D., Chen, G. G., Lai, P. B., Wong, N., and Yu, J. (2017) O-GlcNAc transferase promotes fatty liver-associated liver cancer through inducing palmitic acid and activating endoplasmic reticulum stress. J. Hepatol. 67, 310–320 CrossRef Medline
16. Franz, C. M., Jones, G. E., and Ridley, A. J. (2002) Cell migration in development and disease. Dev. Cell 2, 153–158 CrossRef Medline
17. Hood, J. D., and Cheres, D. A. (2002) Role of integrins in cell invasion and migration. Nat. Rev. Cancer 2, 91–100 CrossRef Medline
18. Guo, W., and Giancotti, F. G. (2004) Integrin signaling during tumour progression. Nat. Rev. Mol. Cell Biol. 5, 816–826 CrossRef Medline
19. Parsons, J. T., Horwitz, A. R., and Schwartz, M. A. (2010) Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat. Rev. Mol. Cell Biol. 11, 633–643 CrossRef Medline
20. Webb, D. J., Parsons, J. T., and Horwitz, A. F. (2002) Adhesion assembly, disassembly and turnover in migrating cells: over and over and over again. Nat. Cell Biol. 4, E97–E100 CrossRef Medline
21. Kwak, T. K., Kim, H., Jung, O., Lee, S. A., Kang, M., Kim, H. J., Park, J. M., Kim, S. H., and Lee, J. W. (2010) Glucoseamine treatment-mediated O-GlcNAc modification of paxillin depends on adhesion state of rat insulinoma INS-1 cells. J. Biol. Chem. 285, 36021–36031 CrossRef Medline
22. Hagmann, J., Grob, M., and Burger, M. M. (1992) The cytoskeletal protein talin is O-glycosylated. J. Biol. Chem. 267, 14424–14428 Medline
23. Ji, S., Kang, I. G., Park, S. Y., Lee, J., Oh, Y. J., and Cho, J. W. (2011) O-GlcNAcylation of tubulin inhibits its polymerization. Amino Acids 40, 809–818 CrossRef Medline
24. Ding, M., and Vandré, D. D. (1996) High molecular weight microtubule-associated proteins contain O-linked-N-acetylgalcosamine. J. Biol. Chem. 271, 12555–12561 CrossRef Medline
25. Ferrer, C. M., Lu, T. Y., Bicagulapa, Z. A., Katsetos, C. D., Sinclair, D. A., and Regnato, M. J. (2017) O-GlcNAcylation regulates breast cancer metastasis via SIRT1 modulation of FOXM1 pathway. Oncogene 36, 559–569 CrossRef Medline
26. Jiang, M., Xu, B., Li, X., Shang, Y., Chu, Y., Wang, W., Chen, D., Hu, S., Zhang, S., Li, M., Wu, K., Yang, X., Liang, J., Nie, Y., and Fan, D. (2018) O-GlcNAcylation promotes colorectal cancer metastasis via the miR-101-O-GlcNAc/EZH2 regulatory feedback circuitry. Oncogene, in press CrossRef Medline
27. Giancotti, F. G., and Ruoslahti, E. (1999) Integrin signaling. Science 285, 1028–1032 CrossRef Medline
28. Chen, H. C., Appeddu, P. A., Parsons, J. T., Hildebrand, J. D., Schaller, M. D., and Guan, J. L. (1995) Interaction of focal adhesion kinase with cytoskeletal protein talin. J. Biol. Chem. 270, 16995–16999 CrossRef Medline
FAK was modified by O-GlcNAc

29. Valdembri, D., and Serini, G. (2012) Regulation of adhesion site dynamics by integrin traffic. Curr. Opin. Cell Biol. 24, 582–591 CrossRef Medline

30. Askari, J. A., Tynan, C. J., Webb, S. E., Martin-Fernandez, M. L., Ballestrem, C., and Humphries, M. J. (2010) Focal adhesions are sites of integrin extension. J. Cell Biol. 188, 891–903 CrossRef Medline

31. Collins, C., and Nelson, W. J. (2015) Running with neighbors: coordinating cell migration and cell-cell adhesion. Curr. Opin. Cell Biol. 36, 62–70 CrossRef Medline

32. Wolfram, T., Spatz, J. P., and Burgess, R. W. (2008) Cell adhesion to agar presented as a nanopatterned substrate is consistent with an interaction with the extracellular matrix and not transmembrane adhesion molecules. BMC Cell Biol. 9, 64 CrossRef Medline

33. Parsons, J. T., Martin, K. H., Slack, J. K., Taylor, J. M., and Weed, S. A. (2000) Focal adhesion kinase: a regulator of focal adhesion dynamics and cell movement. Oncogene 19, 5606–5613 CrossRef Medline

34. Ilic´, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nomura, S., Fujimoto, J., Okada, M., and Yamamoto, T. (1995) Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 377, 539–544 CrossRef Medline

35. Hang, Q., Isaji, T., Hou, S., Wang, Y., Fukuda, T., and Gu, J. (2017) A key regulator of cell adhesion: identification and characterization of important N-glycosylation sites on integrin α5β1 for cell migration. Mol. Cell. Biol. 37, e00558-16 CrossRef Medline

36. Quizi, J. L., Baron, K., Al-Zahrani, K. N., O’Reilly, P., Sriram, R. K., Conway, J., Laurin, A. A., and Sabourin, L. A. (2013) SLK-mediated phosphorylation of paxillin is required for focal adhesion turnover and cell migration. Oncogene 32, 4656–4663 CrossRef Medline

37. Anthis, N. J., and Campbell, I. D. (2011) The tail of integrin activation. Trends Biochem. Sci. 36, 191–198 CrossRef Medline

38. Wolfenson, H., Lavelin, I., and Geiger, B. (2013) Dynamic regulation of the structure and functions of integrin adhesions. Dev. Cell 24, 447–458 CrossRef Medline

39. Shattil, S. J., Kim, C., and Ginsberg, M. H. (2010) The final steps of integrin activation: the end game. Nat. Rev. Mol. Cell Biol. 11, 288–300 CrossRef Medline

40. Comer, F. I., and Hart, G. W. (2000) O-Glycosylation of nuclear and cytosolic proteins: dynamic interplay between O-GlcNAc and O-phosphate. J. Biol. Chem. 275, 29179–29182 CrossRef Medline

41. Yang, X., and Qian, K. (2017) Protein O-GlcNAcylation: emerging mechanisms and functions. Nat. Rev. Mol. Cell Biol. 18, 452–465 CrossRef Medline

42. Mitra, S. K., Hanson, D. A., and Schlaepfer, D. D. (2005) Focal adhesion kinase: in command and control of cell motility. Nat. Rev. Mol. Cell Biol. 6, 56–68 CrossRef Medline

43. Xie, Z., Sanada, K., Samuels, B. A., Shih, H., and Tsai, L. H. (2003) Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration. Cell 114, 469–482 CrossRef Medline

44. Bianchi, M., De Lucchini, S., Marin, O., Turner, D. L., Hanks, S. K., and Villa-Moruzzi, E. (2005) Regulation of FAK Ser-722 phosphorylation and kinase activity by GSK3 and PK1 during cell spreading and migration. Biochem. J. 391, 359–370 CrossRef Medline

45. Ma, A., Richardson, A., Schaefer, E. M., and Parsons, J. T. (2001) Serine phosphorylation of focal adhesion kinase in interphase and mitosis: a possible role in modulating binding to p130Cas. Mol. Biol. Cell 12, 1–12 CrossRef Medline

46. Sakabe, K., and Hart, G. W. (2010) O-GlcNAc transferase regulates mitotic chromatin dynamics. J. Biol. Chem. 285, 34460–34468 CrossRef Medline

47. Grigera, P. R., Jeffery, E. D., Martin, K. H., Shabanowitz, J., Hunt, D. F., and Parsons, J. T. (2005) FAK phosphorylation sites mapped by mass spectrometry. J. Cell Sci. 118, 4931–4935 CrossRef Medline

48. Lachén-Montes, M., González-Morales, A., de Morentin, X. M., Pérez-Valderrama, E., Ausín, K., Zelaya, M. V., Serna, A., Aso, E., Ferrer, L., Fernández-Irigoyen, J., and Santamaría, E. (2016) An early dysregulation of FAK and MEK/ERK signaling pathways precedes the β-amyloid deposition in the olfactory bulb of APP/PS1 mouse model of Alzheimer’s disease. J. Proteomics 148, 149–158 CrossRef Medline

49. Grace, E. A., and Busciglio, J. (2003) Aberrant activation of focal adhesion proteins mediates fibrillar amyloid β-induced neuronal dystrophy. J. Neurosci. 23, 493–502 CrossRef Medline

50. Franco, S. J., Rodgers, M. A., Perrin, B. J., Han, J., Bennin, D. A., Critchley, D. R., and Huttonlocher, A. (2004) Calpain-mediated proteolysis of talin regulates adhesion dynamics. Nat. Cell Biol. 6, 977–983 CrossRef Medline

51. Segarra, M., Vilardell, C., Matsumoto, K., Espana, J., Lozano, E., Serrapipes, C., Munoz-Urmenegil, G., Yamada, K. M., and Cid, M. C. (2005) Dual function of focal adhesion kinase in regulating integrin-integrated MMP-2 and MMP-9 release by human T lymphoid cells. FASEB J. 19, 1875–1877 CrossRef Medline

52. Du, J., Chen, X., Liang, X., Zhang, G., Xu, J., He, L., Zhan, Q., Feng, X. Q., Chien, S., and Yang, C. (2011) Integrin activation and internalization on soft ECM as a mechanism of induction of stem cell differentiation by ECM elasticity. Proc. Natl. Acad. Sci. U.S.A. 108, 9466–9471 CrossRef Medline

53. McFarlane, S., McFarlane, C., Montgomery, N., Hill, A., and Waugh, D. J. (2015) CD44-mediated activation of α5β1-integrin, cortactin and paxillin signaling underpins adhesion of basa-like breast cancer cells to endothelium and fibronectin-enriched matrices. Oncotarget 6, 36762–36773 CrossRef Medline

54. Horzman, U., Ozdil, B., and Pesen-Ozkur, D. (2014) Step-by-step quantitative analysis of focal adhesions. MethodsX 1, 56–59 CrossRef Medline

55. Berginski, M. E., Vitriol, E. A., Hahn, K. M., and Gomez, S. M. (2011) O-GlcNAc mediated signaling is crucial for glucose-stimulated insulin secretion and involves activation of focal adhesion kinase and paxillin. Diabetes 60, 1146–1157 CrossRef Medline

56. Rivera, A., Torres, C., and Humphries, M. J. (2010) Focal adhesion remodeling is crucial for glucose-stimulated insulin secretion and involvement of focal adhesion kinase and paxillin. Oncogene 29, 1885–1892 CrossRef Medline

57. Horzum, U., Ozdil, B., and Pesen-Ozkur, D. (2014) Step-by-step quantitative analysis of focal adhesions. MethodsX 1, 56–59 CrossRef Medline

58. Berginski, M. E., Vitriol, E. A., Hahn, K. M., and Gomez, S. M. (2011) High-resolution quantification of focal adhesion spatiotemporal dynamics in living cells. PLoS One 6, e22025 CrossRef Medline