O-GlcNAc, proteinski vezan višefunkcijski mehanizam u staničnoj signalizaciji te njegova uloga u patogenezi šećerne bolesti, stresa i zloćudnih bolesti

Protein-associated O-GlcNAc, a multifunctional mechanism in cell signaling and its role in the pathogenesis of diabetes, stress and malignant diseases

Tamas Nagy[1], Attila Miseta[1,2], Gabor L. Kovacs[1,2]

[1]Institut za laboratorijsku medicinu, Medicinski fakultet, Pečuh, Mađarska
[1] Institute of Laboratory Medicine, Faculty of Medicine, Pecs, Hungary
[2]Znanstveni centar MEDIPOLIS, Sveučilište u Pečuhu, Pečuh, Mađarska
[2] MEDIPOLIS Knowledge Centre, University of Pecs, Pecs, Hungary

Sažetak

Sve veći broj dokaza ukazuje na to da put biosinteze heksozamina (HPB, engl. *hexosamine biosynthesis pathway)* ima značajnu ulogu u modulaciji unutarstaničnih putova preoblikovanja signala. Njegov krajnji produkt, tj. UDP-GlcNAc, je supstrat kod spajanja O-vezanog β-N-acetilglukozamina (O-GlcNAc) s ostatcima Ser/Thr. To spajanje regulira široki raspon proteina preko interferencije s fosforilacijom. O-GlcNAc je dinamična posttranslacijska modifikacija koja je bitna za funkciju normalne stanice u sisavaca; njeno je, međutim, najveće značenje utvrđeno u patološkim procesima. Kako HBP iziskuje glukozu, veliki unos glukoze znatno povećava protok kroz HBP te također povećava omjer proteina povezanih s O-GlcNac. To, pak, utječe na različite funkcije stanice koje uključuju tradicijski prihvaćene štetne učinke u šećernoj bolesti i njenim komplikacijama ili, kao što je nedavno nađeno, O-GlcNAc bi mogao biti koristan kod ishemijskih/reperfuzijskih ozljeda. U ovom pregledu sažeto prikazujemo trenutne spoznaje u istraživanju O-GlcNAc vezane za njegovo sudjelovanje u signalnim putovima i staničnim procesima. Također se usredotočujemo na utjecaj O-GlcNAc u bolestima kao što su šećerna bolest, upala, razvoj zloćudnih bolesti ili ozljede uzrokovane hipoksijom.

Ključne riječi: O-GlcNAc, Ca^{2+}, šećerna bolest, odgovor na stres, zloćudna bolest

Abstract

Growing evidence suggests that hexosamine biosynthesis pathway (HBP) plays a significant role in the modulation of intracellular signaling transduction pathways. Its end product, UDP-GlcNAc is a substrate for the addition of O-linked β-N-acetylglucosamine (O-GlcNAc) to Ser/Thr residues. This process regulates a wide range of proteins usually by interfering with phosphorylation. O-GlcNAc is a dynamic posttranslational modification, which is essential in normal mammalian cellular function; however, its main significance has been revealed in pathological processes. Since HBP requires glucose, high glucose intake considerably increases the flux through HBP and also increases the ratio of O-GlcNAc-associated proteins. This has an impact on various cellular functions, involving either the traditionally recognized detrimental effects in diabetes and diabetic complications or, as found recently, O-GlcNAc might be beneficial in ischemia/reperfusion injuries. In this review we summarize the current findings in O-GlcNAc research concerning its participation in signaling pathways and cellular processes. We also focus on the impact of O-GlcNAc in diseases such as diabetes, inflammation, development of malignancies or hypoxia-induced injuries.

Keywords: O-GlcNAc, Ca^{2+}, diabetes, stress response, malignancy

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Uvod

Osim što je izvor energije koja se dobiva glikolizom i citratnim ciklusom, glukoza sudjeluje u mnogim drugim unutarstaničnim metaboličkim putovima kao što je pentoza-fosfatni put, glikogeneza, sinteza nukleotidnih šećera itd. Jedan od tih putova je i put biosinteze heksozamina (HBP) te njegov krajnji produkt: proteinski vezana O-gliko-

Introduction

Glucose, apart from being energy source via glycolysis and citrate cycle, also participates in many other intracellular metabolic pathways such as pentose-phosphate shunt, glycogenesis, nucleotide sugar synthesis, etc. One of them is the hexosamine biosynthesis pathway (HBP) and its end-product: protein-associated O-glycosylation

zilacija. Tijekom posljednjih su godina heksozaminski put i O-GlcNAc postali predmetom intenzivnog istraživanja, posebice stoga što bi mogli interferirati s kinazama i fosforilacijom u nekoliko signalnih putova (1). O-glikozilacija se odigrava unutar citoplazme i jezgre i uključuje osjetljive ciljne proteine zbog specifičnog i reverzibilnog enzimskog prijenosa metabolita heksozaminskog puta: UDP-N-acetilglukozamina na OH-skupinu aminokiselina proteina serina ili treonina.

O-GlcNAc je prvi put opisan 1984. godine (2) i otada je njegova važnost spoznata u nekoliko staničnih procesa kao što su prepoznavanje hranjivih tvari (3), regulacija staničnog ciklusa (4) ili modifikacija nekoliko čimbenika prijepisa (5-9). S obzirom na tako široku funkcionalnost O-GlcNAc ne iznenađuje da je povezan s razvojem brojnih patofizioloških procesa. Najočitije i dobro poznato značenje O-GlcNAc ustanovljeno je u kroničnim komplikacijama šećerne bolesti i inzulinske rezistencije (pregled pod 10); međutim, prilagodba na stres, osobito s obzirom na srce, odnedavno je također u središtu pozornosti (11,12). Donekle je paradoksno da dok je s jedne strane učinak povećanog protoka kroz heksozaminski put štetan kod šećerne bolesti, s druge se strane čini da je povećani O-GlcNAc blagotvoran kod bolesti zglobova kao što je osteoartritis, ili u situacijama akutnog stresa kao što je ishemijski srčani napadaj (13,14). Naš je cilj dati prošireni pregled mehanizama i teškoća kod signalnih putova, osvrnuti se na trenutno razumijevanje uloge GlcNAc u određenim bolestima, te postići što dublji uvid u dvostrana svojstva O-GlcNAc.

Put HBP

Približno 2-4% glukoze koja pristigne u stanicu metabolizira se putem biosinteze heksozamina (HBP). Ključni enzim je glutamin-fruktoza-6P-amidotransferaza (GFAT) koja katalizira reakciju: L-glutamin + D-fruktoza-6P = L-glutamat + D-glukozamin-6P. Tu je reakciju moguće zaobići tako što se glukozamin neposredno dodaje stanicama i time se povećava protok HBP. Nakon dva kasnija metabolita u protoku (N-acetilglukozamin-6P, N-acetilglukozamin-1P), krajnji produkt HBP je UDP-N-acetilglukozamin (UDP-GlcNAc).

UDP-GlcNAc se koristi u sintezi peptidoglikana, u sintezi lipo- i mukopolisaharida, biosintezi proteoglikana N-tipa (vezanog na amid „N" na pokrajnjem lancu Asn) ili O-tipa (najčešće se prvi N-acetilgalaktozamin (galNAc) veže α-glikozidnom vezom na OH-skupinu Ser/Thr, a zatim se spajaju dodatne ugljikohidratne molekule kao npr. GlcNAc), te biosintezi povezanoj glikozil-fosfatidilinozitolom (kojom se osigurava učvršćenje proteina na membrane). Proteinski povezan O-GlcNAc (Slika 1.) pripada O-tipu glikozilacije, no on dodaje samo jednu O-vezanu molekulu b-N-acetilglukozamina serinskim i treoninskim ostatcima ciljnih proteina (15). O-GlcNAc je također jedinstven

(O-GlcNAc). In the last few years, hexosamine pathway and O-GlcNAc became an intensively investigated topic, in particular because they might interfere with kinases and phosphorylation in a number of signaling pathways (1). O-glycosylation takes place and remains inside the cytoplasm and the nucleus on susceptible target proteins due to a specific and reversible enzymatic transfer of the hexosamine pathway metabolite: UDP-N-acetylglucosamine to the OH group of serine or threonine amino acids of these proteins.

O-GlcNAc was first described in 1984 (2) and since then its importance in several cellular processes has been recognized, such as nutrient sensing (3), cell-cycle regulation (4), or modification of several transcriptional factors (5-9). Given that O-GlcNAc has such widespread functionality it is not surprising that it has been associated with the development of numerous patho-physiological processes. The most obvious and well known significance of O-GlcNAc has been established in chronic complications of diabetes mellitus and insulin resistance (reviewed in 10); however, stress adaptation, especially regarding the heart, has lately also come in the spotlight (11,12). It is somewhat paradoxical that while, on the one hand, the effect of increased flux through the hexosamine pathway in diabetes is deleterious, on the other hand increased O-GlcNAc seems to be beneficial in joint diseases such as osteoarthritis, or in acute stress-situations such as an ischemic heart attack (13,14). Our goal was to give an extensive summary on the mechanisms and involvements in signaling pathways, to review the current understanding of the role of GlcNAc in certain diseases and to get a deeper insight of the Janus faced properties of O-GlcNAc.

HBP pathway

Approximately 2-4% of the glucose entering the cell is metabolized through the hexosamine biosynthetic pathway (HBP). The key enzyme is glutamine-fructose-6P amidotransferase (GFAT) which catalyzes the L-glutamine + D-fructose-6P = L-glutamate + D-glucosamine-6P reaction. This reaction can be bypassed by directly adding glucosamine to cells and thus increasing the HBP flux. Following the two subsequent downstream metabolites (N-acetylglucosamine-6P, N-acetylglucosamine-1P), the end-product of HBP is UDP-N-acetylglucosamine (UDP-GlcNAc).

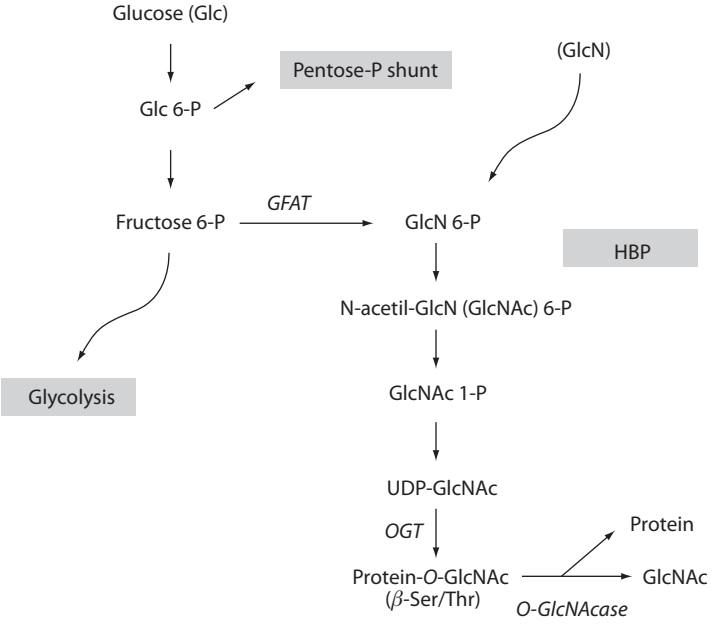
UDP-GlcNAc is utilized in peptidoglycan synthesis, in lipo- and mucopolysaccharide synthesis, N-type (linked to the amide "N" on the sidechain of Asn) or O-type proteoglycan biosynthesis (most commonly, first N-acetylgalactosamine (galNAc) is linked by an α-glycosidic linkage to the OH group of Ser/Thr, then additional carbohydrate molecules are attached such as GlcNAc), glycosyl-phosphatidylinositol (GPI)-linked biosynthesis (provides the

po tome što se pojavljuje poglavito u citoplazmi i jezgri. O-vezani GlcNAc predstavlja reverzibilnu reakciju dok se preostale poslijetranslacijske glikozilacije, koje su trajne, zbivaju u endoplazmatskom retikulu (ER) ili Golgijevom aparatu. O-GlcNAc je brz, dinamičan proces; kratkotrajna primjena glukozamina ili izlaganje raznolikim uvjetima stresa mogu dovesti do povišenih razina proteinski vezanog O-GlcNAc (11,16). S druge strane, razine O-GlcNAc nakon oporavka od podražaja poprimaju prijašnje vrijednosti u relativno kratkom vremenskom razmaku (11). S obzirom da treba OH-ostatke Ser/Thr, O-GlcNAc može konkurirati fosforilaciji i time ukazuje na mogućnost preinačivanja signalnih slijedova (kaskada) ovisnih o fosforilaciji (1,17-19). Postoje, međutim, također dokazi da mjesta za fosforilaciju kao i za O-GlcNAc mogu istodobno biti prisutni na istom proteinu i u tom slučaju utjecati na funkcije proteina u suradnji, a ne na konkurentski način (20,21).

Zanimljivo je da pored mnoštva kinaza samo jedan gen kodira O-GlcNAc-transferazu (OGT) koja je smještena na kromosomu X (22). Regulacija OGT zasad nije shvaćena u potpunosti, no postoje dokazi da je sama OGT meta kako fosforilacije, tako i O-GlcNAc (23). Uklanjanje N-acetilglukozaminske skupine iz proteina katalizira i specifičan enzim nazvan O-GlcNAkaza, a njegov je gen smješten na 10. kromosomu (24). Dokazivanje da je protein *in vivo* zaista O-glikoziliran iziskuje sveobuhvatne, višestruke pristupe kao što su imunoprecipitacija, dvodimenzionalna elektroforeza i spektrometrija masa tako da se istraživači u većini studija odlučuju za istodobno mjerenje ukupnih razina O-GlcNAc i aktivnosti i razina izražaja proteina od interesa.

anchoring of proteins to membranes). Protein-associated O-GlcNAc (Figure 1.) is an O-type glycosylation, but it only adds a *single* O-linked β-N-acetylglucosamine molecule to the serine and threonine residues of target proteins (15). O-GlcNAc is also unique in terms that O-GlcNAc occurs mainly in the cytoplasm and nucleus. O-linked glcNAc is a reversible reaction while the remaining posttranslational glycosylations take place in the ER or Golgi and are permanent. O-GlcNAc is a fast, dynamic process; short treatment of glucosamine or exposition to a wide variety of stress conditions can lead to increased levels of protein-associated O-GlcNAc (11,16). On the other hand, after recovery from the stimuli, O-GlcNAc levels return to previous values over a relatively short period of time (11). Since O-GlcNAc needs Ser/Thr OH-residues, it can compete with phosphorylation indicating a possibility to modulate the phosphorylation-dependent signaling cascades (1,17-19). However there is also evidence that both phosphorylation sites and O-GlcNAc sites can co-exist on the same protein, influencing the protein's function in this case in a co-operative rather than a competitive way (20, 21).

Interestingly, while there is an abundance of kinases, only one gene encodes O-GlcNAc transferase (OGT) which is located on the X chromosome (22). The regulation of OGT has not been well understood yet, but there is evidence that OGT itself is a target for both phosphorylation and O-GlcNAc (23). The removal of the N-acetylglucosamine group from proteins is also catalyzed by a specific enzyme called O-GlcNAcase and its gene is localized on the 10th



Slika 1. Odvijanje biosinteze heksozamina

Figure 1. The hexosamine biosynthesis pathway

Najčešće korištena specifična antitijela protiv O-GlcNAc-proteina nazivaju se CTD110.6 i RL-2 (25,26). Postoji više načina da se na razine O-GlcNAc utječe eksperimentalno; npr. pretjeranim izražajem ili brisanjem/prigušivanjem gena ključnih enzima kao što su GFAT, OGT i O-GlcNAkaza (11,27-29). Treba istaknuti da unatoč tome što je moguće proizvesti stanične linije s pogreškom u HBP, prisutnost O-GlcNAc je od vitalnog značenja tako da genetički manipulirane životinje s OGT umiru u embrionalnom stadiju (22). Radi oponašanja dijabetičkih uvjeta protok kroz HBP može se povećati dodavanjem glukozamina izvana ili visokih koncentracija (25-30 mM) šećera (29). Kod korištenja vrsti stanica koje su ovisne o inzulinu te bez drugih dostupnih izvora energije (npr. laktata) posebna je pozornost potrebna kad su stanice izložene glukozaminu tijekom duljeg razdoblja jer glukozamin može iscrpsti razine ATP-a (30).

U studijama je također opisano da primjena glutamina ima sličan učinak (povećanjem aktivnosti GFAT) (31). Azaserin i 6-diazo-5-oksonorleukin (DON) inhibiraju GFAT te time smanjuju protok kroz HBP, dok aloksan inhibira OGT smanjujući time samo razine O-GlcNAc, uz relativno nepromijenjene ostale metabolite HBP (32). O-(2-acetamido-2-deoksi-D-glukopirnosiliden)amino-N-fenil-karbamat (PUGNAc) i streptozotocin (STZ) blokiraju O-GlcNAkazu, a time i uklanjanje O-GlcNAc iz proteina (33,34). Treba napomenuti da su aloksan i STZ nespecifični inhibitori; iako posebice STZ ima prijeporan učinak, on je unatoč tome lijek u širokoj uporabi u studijama na životinjama za izazivanje šećerne bolesti tipa 1 preko razaranja β-stanica gušterače.

Broj identificiranih proteina koji omogućuju poslijetranslacijsku O-glikozilaciju ubrzano raste te danas obuhvaća više od 400 staničnih proteina kao što su NF-κB, aneksin, endotelna dušična oksid-sintaza, αB-kristalin, OGT, α-tubulin, c-myc, protein 70 toplinskog šoka itd. Kako bi potpomogao istraživanje O-GlcNAc, Centar za analizu bioloških sekvenci ima internetske stranice dostupne na: http://www.cbs.dtu.dk/services/YinOYang/ gdje iznosi predviđanja vezana za živčanu mrežu i mjesta spajanja O-β-GlcNAc u eukariotskim proteinskim sekvencama.

Utjecaj O-GlcNAc na funkciju proteina

Modifikacija proteina preko O-GlcNAc definitivno izaziva promjene u njihovoj funkcionalnosti. Prva i temeljito istražena funkcija O-GlcNAc je odnos prema fosforilaciji (35,36). Wells i sur. su pokazali da OGT i proteinska fosfataza 1 koegzistiraju u zajedničkom kompleksu (37). U nekim su proteinima snižene razine O-GlcNAc povezane s povišenim razinama fosforilacije (Tau iz moždanog tkiva bolesnika s Alzheimerovom bolešću, 38). Nedavna je studija također dokazala da je fosforilacija p38 podložna modifikaciji pomoću GlcNAc (39). Dokazano je da inhibicija kinaza

chromosome (24). To prove that a protein in vivo is indeed O-glycosylated requires extensive, multiple approaches such as immunoprecipitation, 2D electrophoresis and mass spectrometry, so that in most studies researchers decide for the measurement of the overall O-GlcNAc levels simultaneously with the activity and expressional levels of the proteins of interest. The most commonly used specific antibodies against O-GlcNAc proteins are called CTD110.6 and RL-2 (25,26). There are multiple ways to influence O-GlcNAc levels experimentally: e.g. by overexpression or by gene deletion/gene silencing of key enzymes such as GFAT, OGT and O-GlcNAcase (11,27-29). It has to be noted that even though HBP-defected cells lines can be created, the presence of O-GlcNAc is vital and OGT-knocked-out animals die at embryonic stage (22). To simulate diabetic conditions, the flux through HBP can be increased by adding external glucosamine or high levels (25-30 mM) of sugar (29). When working with insulin-dependent cell types and with no alternate energy source available (e.g. lactate), care should be taken when cells are exposed to glucosamine for a prolonged period of time since glucosamine may deplete ATP levels (30).

It has also been reported that glutamine treatment has similar effect (through enhancing GFAT activity) (31). *Azaserine* and 6-*diazo-5-oxonorleucine* (DON) inhibit GFAT, thus decreasing the flux through HBP, while *alloxan* inhibits OGT, decreasing only the levels of O-GlcNAc and leaving the other metabolites of HBP relatively unchanged (32). *O-(2-Acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenyl-carbamate* (PUGNAc) and *streptozotocin* (STZ) block O-GlcNAcase and thus inhibit the removal of O-GlcNAc from proteins (33,34). It should be noted that alloxan and STZ are non-specific inhibitors; although STZ especially has a debatable effect, it is a widely used drug in animal models to induce type 1 diabetes by destroying pancreatic β-cells.

The number of identified proteins capable of posttranslational O-glycosylation is quickly growing, to date they include more than 400 cellular proteins, such as NF-κB, annexin, endothelial nitric oxide synthase, αB-crystallin, OGT, α-tubulin, c-myc, heat shock protein 70 etc. To aid O-GlcNAc research, Center for Biological Sequence Analysis web site, available at: http://www.cbs.dtu.dk/services/YinOYang/ produces neural network predictions for O-ß-GlcNAc attachment sites in eukaryotic protein sequences.

Influence of O-GlcNAc on protein function

O-GlcNAc modification of proteins definitely induces changes in the functionality of these proteins; the first and most thoroughly investigated function of O-GlcNAc is the relation to phosphorylation (35,36). Wells and co-workers have shown that OGT and protein phosphatase1 co-exist

kao što su PKC i PKA može povećati razine O-GlcNAc (35). Ako se ti podatci razmotre zajedno, čini se da je najuobičajenija interakcija između fosforilacije i O-GlcNAc recipročna.

O-GlcNAc može također priječiti razgradnju proteina, bilo blokiranjem fosforilacijskih mjesta nužnih da bi pospješio razgradnju (npr. estrogenski receptor - ER - β koji ima sekvencu PEST = kratak život proteina signalizira područje bogato aminokiselinama prolinom - P, glutamičnom kiselinom - E, serinom - S, i treoninom - T), ili O-GlcNAc izravno blokira ciljna mjesta razgradnje proteina (npr. Sp1) (40,41). Postoje također dokazi koji ukazuju da je proteazom O-glikoziliran te da razina O-GlcNAc na proteazomima ovisi o prehrambenom stanju stanica (42). Prema toj hipotezi koncentracije glukoze te stoga i protok u HBP i O-GlcNAc smanjuju se tijekom izgladnjelosti stanice, pa se proteazom time rješava inhibicije i omogućuje da razgradnja proteina bude izvor energije.

Osim gore navedenih mehanizama, O-GlcNAc može također regulirati interakcije protein-protein i lokalizacije proteina (pregled u 1, 43). S obzirom da je ugljikohidrat, dodatavanje O-GlcNAc može promijeniti hidrofobnost proteina. Danas se malo zna o učinku O-GlcNAc na hidrofobnost proteina, premda postoje određeni dokazi da O-GlcNAc može promijeniti hidrofobne reakcije između proteina (42). Također, naši su nedavni, no još neobjavljeni rezultati, pokazali izmijenjenu osmotsku rezistenciju i difuziju unutarstanične vode nakon primjene glukozamina.

Uloga O-GlcNAc u unutarstaničnim procesima

Prepoznavanje hranjivih tvari

Uvjerljivi dokazi ukazuju da HBP i O-GlcNAc značajno sudjeluju u prepoznavanju hranjivih tvari (3). Visoke koncentracije glukoze uzrokuju povećani protok kroz HBP i kasnije povećavaju O-GlcNAc koji smanjuje iskorištenje glukoze negativnim mehanizmom povratne sprege. Protok kroz HBP, međutim, ne povećava samo glukoza već i slobodne masne kiseline, glutamin i glukozamin (3,44-46). Povišeni O-GlcNAc najprije inhibira ulazak glukoze kroz staničnu membranu povišenjem inzulinske rezistencije. Točan mehanizam koji je u osnovi inzulinske rezistencije nije još uvijek potpuno jasan, no postoji nekoliko studija koje otkrivaju oštećenje translokacije prijenosnika glukoze GLUT4 na staničnoj membrani kod primjene visokih koncentracija glukoze ili glukozamina (47,48). Ta se pojava može, primjerice, dovesti u vezu s poremećenom aktivacijom AKT za koju se pretpostavlja da je nužna za translokaciju GLUT4 ovisnu o inzulinu (vidjeti u nastavku).

O-GlcNAc također modulira sintezu glikogena (49). Glikogen-sintazu (GS) deaktivira glikogen-sintaza-kinaza 3 (GSK-3). Inzulin preko PI3-kinaze, AKT i PKC inhibira GSK-3

in a common complex (37). In some proteins, reduced O-GlcNAc levels correlate with increased phosphorylation levels (Tau from the brain tissue of Alzheimer's disease patients (38)). Also, a recent report has shown p38 phosphorylation to be a subject to O-GlcNAc modification (39). It has been shown that inhibition of kinases such as PKC and PKA may increase O-GlcNAc levels (35). If these data are taken together, the most common interaction of phosphorylation and O-GlcNAc seems to be reciprocal.

O-GlcNAc may also block protein degradation, either by blocking phosphorylation sites which are required to promote the degradation (e.g. estrogen receptor (ER)-β which has a PEST sequence = The short life-time of a protein is signaled by a region rich in the amino acids proline (P); glutamic acid (E); serine (S); and threonine (T)) or O-GlcNAc directly blocks the degradation target sites of the proteins (e.g. Sp1) (40,41). There is also evidence suggesting that the proteasome is O-glycosylated and the level of O-GlcNAc on proteasomes depends on the nutritional state of cells (42). According to this hypothesis, glucose levels and consequently HBP flux and O-GlcNAc decrease during cell-starvation and thus the proteasome would be liberated from the inhibition allowing protein degradation to provide energy.

Apart from the above mentioned mechanisms, O-GlcNAc may also regulate protein-protein interactions and protein localizations (reviewed in 1, 43). As a carbohydrate, the addition of O-GlcNAc may change the hydrophobicity of a protein. To date, little is known about the effect of O-GlcNAc on protein hydrophobicity, although there is some evidence that O-GlcNAc may change the hydrophobic interactions between proteins (42). Also, our recent, yet unpublished results showed altered osmotic resistance and intracellular water diffusion following glucosamine treatment.

The role of O-GlcNAc in intracellular processes

Nutrient sensing

Strong evidence suggests that HBP and O-GlcNAc take a significant part in nutrient sensing (3). High glucose levels generate increased flux through the HBP and subsequently elevate O-GlcNAc, which downregulates glucose uptake by a negative feedback mechanism. However, not only glucose but free fatty acids, glutamine and glucosamine also increase HBP flux (3,44-46). Elevated O-GlcNAc first inhibits glucose entrance through the cell membrane by increasing the insulin resistance. The exact mechanism underlying insulin resistance is not completely understood, yet there are several studies showing that the translocation of glucose transporter GLUT4 to the cell membrane is damaged when high levels of glucose or glucosamine

i time uzrokuje pojačanu aktivnost GS (50). Jedan način reguliranja O-GlcNAc jest blokiranje signalnog puta potaknutog inzulinom. Kao što je već navedeno, studije su već opisale da je nakon porasta O-GlcNAc aktivnost AKT bila smanjena bilo izravnom inhibicijom fosforilacije ili prethodnom inhibicijom kinaze (36). S druge je pak strane prikazano da sama GS može biti O-glikozilirana te da ta modifikacija inhibira njenu funkciju baš kao i fosforilacija pomoću GSK3 (21). Mehanizam koji je vjerojatan u tom slučaju predstavlja dobar primjer koji ukazuje da fosforilacija i O-GlcNAc zajednički djeluju u istom proteinu kako bi blokirali njegovu aktivnost.

Stanični ciklus

Postoji mnogo proteina koji su podložni O-GlcNAc, a koji su uključeni u stanični ciklus, kao što je to protoonkogen c-myc (51) ili citoskeletni proteini (regulacija diobenog vretena tijekom citokineze) poput α-tubulina i keratina 8,13,18 (52-54). O-GlcNAc također modificira YY1 (55), protein koji sudjeluje u preslikavanju DNA, staničnom rastu i diferencijaciji. Manjkava regulacija staničnog ciklusa predstavlja najznačajniji čimbenik u razvoju karcinoma te je stoga razumijevanje uloge O-GlcNAc iznimno bitno. Prekid HBP (npr. delecijom gena glukozamin-6P-acetiltransferaze) rezultira značajno smanjenim ukupnim koncentracijama O-GlcNAc. Zatajenje HBP je smrtonosno za eksperimentalne životinje u embrionalnom stadiju (22), no održanje staničnih linija s manjkavim HBP je moguće. Ipak, takve su stanične linije obilježene sporijim brzinama rasta i izmijenjenim staničnim ciklusima (56). S obzirom da povišeni O-GlcNAc također remeti stanični rast, čini se da se regulacija O-GlcNAc razlikuje u različitim stadijima staničnog ciklusa. Slawson i sur. su nedavno pokazali da je ispravno tretiranje O-GlcNAc nužno za normalan stanični ciklus te da je O-GlcNAc potreban za usklađivanje napredovanja M-faze, citokinezu i fosforilaciju proteina u mitozi (4).

Čimbenici prijepisa

Općenito, glavnina proteina koje modificira O-GlcNAc smještena je u jezgri i vezana za kromatin (57). U O-GlcNAc je također obilno prisutan kompleks jezgrenih pora (58), što ukazuje da O-GlcNAc modulira promet kroz jezgru. Ipak, nedavno je opisano da prisutnost O-GlcNAc nije nužna za prijenos kroz pore (59). Najvažnija uloga O-glikozilacije u jezgri je regulacija prijepisa (transkripcije). Popis poznatih čimbenika prijepisa koje O-GlcNAc povisuje ili smanjuje svakim je danom sve veća; Whelan i sur. su nedavno objavili obnovljeni popis (60). Neki od primjera tih čimbenika su Sp1 (41), p53 (8), CREB (5) i NF-κB (7). O-GlcNAc služi kao signal za lokalizaciju proteina u jezgri (17), a također i kao modifikacija u odnosu na transkripcijsku aktivnost. Modifikacija O-GlcNAc može povećati (p53 (8)) ili smanjiti (CREB (5)) transkripcijsku aktivnost, ili obo-

are administered (47,48). This phenomenon can be linked to, e.g., impaired AKT activation which is presumably required for insulin-dependent GLUT4 translocation (see below).

Glycogen synthesis is also modulated by O-GlcNAc (49). Glycogen Synthase (GS) is deactivated by Glycogen synthase kinase 3 (GSK-3). Insulin, through PI3kinase, AKT and PKC inhibits GSK-3 thus causing increased GS activity (50). One way of O-GlcNAc regulation is to block the signaling pathway induced by insulin. As mentioned, it has been reported that upon increased O-GlcNAc, AKT activity was reduced either by direct inhibition of phosphorylation or by upstream kinase inhibition (36). On the other hand, it has been shown that GS itself can be O-glycosylated, and this modification inhibits its function just like phosphorylation by GSK3 (21). The putative mechanism here is a good example to show that phosphorylation and O-GlcNAc co-operate in the same protein to block its activity.

Cell cycle

There are many O-GlcNAc susceptible proteins that are involved in cell cycle, such as the proto-oncogen c-myc (51), or cytoskeletal proteins (regulation of the mitotic spindle during cytokinesis) as α-tubulin and keratin 8, 13, 18 (52-54). YY1 (55), a protein involved in DNA replication, cell growth and differentiation is also O-GlcNAc modified. Inadequate regulation of cell cycle is a major factor in the development of cancer, so that the understanding of the role of O-GlcNAc is essential. Disruption of the HBP (e.g. by gene-deletion of glucosamine-6P-acetyltransferase) results in significantly lowered overall O-GlcNAc levels. Failing HBP is lethal for experimental animals in embryonic phase (22), yet HBP-defective cell lines can be maintained. Still, these cell lines have slower growth rates and altered cell cycles (56). Since elevated O-GlcNAc also disturbs cell growth, O-GlcNAc regulation seems to be different during the various phases of the cell cycle. Slawson et al. have recently shown that proper O-GlcNAc processing is required for normal cell cycle and that O-GlcNAc is necessary for the fine-tuning of the M-phase progression, cytokinesis, and mitotic protein phosphorylation (4).

Transcriptional factors

In general, the bulk of O-GlcNAc-modified proteins are localized in the nucleus, associated to chromatin (57). The nuclear pore complex is also abundant in O-GlcNAc (58), suggesting that O-GlcNAc modulates nuclear trafficking. However, it has been shown recently that the presence of O-GlcNAc is not necessary for pore transport (59). The most important role of O-glycosylation in the nucleus is the regulation of transcription. The list of known transcriptional factors that can be up- or downregulated by O-GlcNAc is growing every day; Whelan et al. have recen-

je (Sp1) (41,61,62). Moguće objašnjenje za takvu raznoliku funkcionalnost jest da bi na tim proteinima mogla biti prisutna višestruka mjesta za O-GlcNAc koja su odgovorna bilo za odgođenu razgradnju proteina (posljedica pojačane razgradnje je smanjena aktivnost) ili regulaciju (pozitivno ili negativno) aktivnosti prijepisa. NF-κB (vidjeti u nastavku) je prisutan u svim stanicama i aktivira ga širok raspon podražaja: stres, citokini, slobodni radikali ili antigeni. NF-κB ima važnu ulogu u imunom odgovoru, upali, autoimunim bolestima, šećernoj bolesti, karcinomu, te odgovoru na srčani stres tako da je modifikacija O-GlcNAc svakako od osobitog značenja (7,63,64).

Tretiranje Ca^{2+}

Uzevši u obzir njegovu široku staničnu primjenjivost, vjerojatno je da bi O-GlcNAc mogao ometati regulaciju unutarstaničnog Ca^{2+}, osobito kod stresa i epizoda ishemije/reperfuzije u kojima je $[Ca^{2+}]_i$ ključni element preoblikovanja signala. Međudjelovanje između O-GlcNAc i fosforilacije je nepobitno dokazano; do sada, međutim, samo neizravni dokazi naznačuju takvo međudjelovanje s homeostazom $[Ca^{2+}]_i$ (65).

Već je odavno poznato da je tzv. glukoza-inzulin-kalij (engl. *glucose-insulin-potassium*, GIK) blagotvoran za bolesnike zahvaćene ishemijom/reperfuzijom (66). Štoviše, u ispitivanjima na životinjama kratkotrajna hiperglikemija ili primjena glukozamina štiti od opterećenja s Ca^{2+} potaknutog ishemijom/reperfuzijom (67). Čini se da glukozamin također priječi ulaz kapacitacijskog kalcija (engl. capacitative calcium entry, CCE) koji predstavlja povećanje Ca^{2+} koje potiče IP_3 (16,68). Kao što smo nedavno i dokazali, ta se inhibicija u kardiomiocitima zbiva preko O-GlcNAc (16) iako specifični ciljni proteini nisu poznati. Regulacija $[Ca^{2+}]_i$ tijekom ishemije/reperfuzije u srcu je po sebi složen mehanizam koji nije poznat u svim detaljima; međutim, gore navedeni neizravni dokazi ukazuju da HBP i/ili O-GlcNAc moduliraju homeostazu $[Ca^{2+}]_i$.

Tijekom epizode ishemije ili nakon podražaja agonista kao što je angiotenzin II (AngII) aktivira se fosfolipaza C (PLC) koja stvara dva sekundarna glasnika, tj. inozitol-trifosfat (IP_3) i diacil-glicerol (DAG). Porast Ca^{2+} može nastati i pomoću puta IP_3 i puta DAG/PKC. IP_3 otpušta Ca^{2+} iz ER (što je popraćeno ulaskom drugog Ca^{2+} iz izvanstaničnog prostora (nazvanog CCE)), dok PKC/DAG aktivira Ca^{2+}-kanale u staničnoj membrani (L-tipa i vjerojatno druge Ca^{2+}-kanale također). Nekoliko je članaka pokazalo da skupina TRPC (engl. *transient receptor protein channel*, kanal kratkotrajnog receptorskog proteina) proteina membrane ima važnu ulogu u regulaciji $[Ca^{2+}]_i$ u srcu bilo preko aktivacije IP_3 ili PKC-a (69).

O-GlcNAc može na nekoliko razina interferirati s tom regulacijom $[Ca^{2+}]_i$, npr. u PLC ili nakon receptora PLC: IP_3 i/ili PKC i drugih kinaza. Uklanjanje i ponovni unos Ca^{2+} (izmjenjivači Na^+/Ca^{2+}, SERCA (sarko/endoplasmatska Ca^{2+} -ATPa-

tly published an updated list (60). Some examples are Sp1 (41), p53 (8), CREB (5) and NF-κB (7). O-GlcNAc serves as a signal to localize a protein to the nucleus (17), and also as a modification in relation to transcriptional activity. O-GlcNAc modification can increase (p53 (8)), or decrease (CREB (5)) transcriptional activity, or both (Sp1) (41,61,62). A possible explanation for the diverse functionality is that on these proteins multiple O-GlcNAc sites could be present that are responsible for either delayed protein degradation (increased degradation results in lower activity) or the regulation (positively or negatively) of transcriptional activity. NF-κB (see below) is present in all cells and is activated upon a wide range of stimuli; stress, cytokines, free radicals, or antigens. NF-κB plays a significant role in immune response, inflammation, auto-immune diseases, diabetes, cancer, and cardiac stress response so that its O-GlcNAc modification has a special importance (7, 63,64).

Ca^{2+} handling

Considering the wide cellular applicability of O-GlcNAc, it is plausible that it might also interfere with intracellular Ca^{2+} regulation, especially in stress and ischemia/reperfusion episodes where $[Ca^{2+}]_i$ is a crucial signal transduction element. The interaction of O-GlcNAc with phosphorylation has been well established; however, so far only indirect evidence suggests that it is also involved in $[Ca^{2+}]_i$ homeostasis (65).

It has long been known that the so-called glucose-insulin-potassium (GIK) is beneficial for patients affected by ischemia/reperfusion (66). Moreover, in animal models, short term hyperglycemia or glucosamine treatment protects from ischemia-reperfusion induced Ca^{2+} -overload (67). Glucosamine also seems to inhibit Capacitative Calcium Entry (CCE) – which is an IP_3 induced Ca^{2+}-increase (16, 68). As we have shown recently, this inhibition in cardiomyocytes occurs via O-GlcNAc (16), although specific target proteins are unknown. The regulation of $[Ca^{2+}]_i$ during ischemia/reperfusion in the heart is in itself a complex mechanism not known in every detail; however, the above mentioned indirect evidence suggests that HBP and/or O-GlcNAc modulate the $[Ca^{2+}]_i$ homeostasis.

During an ischemic episode or upon stimuli by an an agonist such as Angiotensin II (AngII), phospholipase C (PLC) is activated and generates two secondary messengers, inositol triphosphate (IP_3) and diacyl-glycerol (DAG). Ca^{2+} increase can be mediated by both the IP_3 and the DAG/PKC route. IP_3 releases Ca^{2+} from the ER (which is followed by a second Ca^{2+} influx from the extracellular space (called CCE)) while PKC/DAG activates Ca^{2+} channels in the cell membrane (L-type and probably other Ca^{2+} channels as well). A number of papers has shown that the TRPC (transient receptor protein channel) membrane protein family plays an important role in the regulation of $[Ca^{2+}]_i$ in the heart either through IP_3 or PKC activation (69).

se), mitohondriji) mogu također biti zahvaćeni. Zapravo, nedavno je opisano da je O-GlcNAc smanjio aktivnost PLC te se time ukazalo na PLC kao moguću metu O-GlcNAc (65). Proteini TRPC su također vjerojatni kandidati za O-GlcNAc, npr. analiza proteinskog slijeda za TRPC1 ukazuje na visokoafinitetno mjesto za O-GlcNAc blizu završnog područja NH_2.

Proteini O-GlcNAc povezani s $[Ca^{2+}]_i$ koje smo do sada razmatrali utječu uglavnom na kratkotrajne poslijetranslacijske modifikacije. Alternativno, dugotrajna izloženost visokim koncentracijama glukoze također omogućuje promjene u izražaju jer modifikacija čimbenika prijepisa potaknuta s O-GlcNAc može utjecati na razine izražaja proteina uključenih u postupke s $[Ca^{2+}]_i$. Zapravo, za SERCA2a je izviješteno da ima smanjen izražaj nakon dulje inkubacije visoke glukoze i ta se promjena pripisala razini prijepisa (Sp1), a ne poslijetranslacijskoj modifikaciji (29). Zbog toga, čak i kad visoke koncentracije glukoze mogu u određenim okolnostima biti korisne za preživljenje stanica, prethodna povijest dugotrajne izloženosti visokim koncentracijama glukoze vjerojatno zasjenjuje blagotvorne učinke u ovom slučaju.

Uloga O-GlcNAc u patogenezi

Šećerna bolest

Šećernu bolest tipa 2 obilježavaju povišene koncentracije glukoze u krvi zbog inzulinske rezistencije perifernih stanica, kao i komplikacije šećerne bolesti prouzročene duljom izloženosti visokoj glukozi. Premda je još potrebno čekati na objašnjenje točnih mehanizama, glavnina studija slaže se u tome da O-GlcNAc doprinosi kako inzulinskoj rezistenciji, tako i razvoju komplikacija šećerne bolesti.

Inzulinska rezistencija

Smanjeni prijenos glukoze kroz membranu stanica rezultira inzulinskom rezistencijom. Uzrok tome je poremećena translokacija prijenosnika glukoze GLUT4 (koji je vjerojatno protein O-GlcNAc (70)). Tu translokaciju (kao i modeliranje i fuziju s membranom) reguliraju višestruki mehanizmi, uz inzulin i aktivaciju inzulinskog receptora kao najvažnije čimbenike. Sljedeći korak uključuje IRS-1 i IRS-2 (supstrati inzulinskog receptora) čiji je kapacitet vezanja O-GlcNAc nepobitno potvrđen (19,71,72). Nakon IRS;PI3kinaze, Akt, (smanjene aktivnosti kod inzulinske rezistencije) PKC, p38 i NF-κB (povećane aktivnosti kod inzulinske rezistencije) uključeni su u slijed (kaskadu) inzulinskog signalnog puta. Od navedenih su glasnika IRS (72) i PI3kinaza (19) O-GlcNAc-proteini, dok su vjerojatno Akt (72), p38 (39) i NF-κB (7) također kandidati za O-glikozilaciju. Iako je O-glikozilacija NF-κB još uvijek pretpostavka, aktivacija NF-κB u šećernoj bolesti je dobro opisana (7). Osim pomoću O-GlcNAc, NF-κB se može aktivirati pomoću AngII ili

O-GlcNAc could interfere at several levels of this $[Ca^{2+}]_i$ regulation, for example in PLC, or downstream of PLC: IP_3 receptor and/or PKC and other kinases. The elimination and re-uptake of Ca^{2+} (Na^+/Ca^{2+} exchangers, SERCA (sarco/endoplasmic Ca^{2+} -ATPase), mitochondria) might also be affected. In fact, it has recently beeen shown that PLC activity was downregulated by O-GlcNAc, suggesting that PLC is a possible O-GlcNAc target (65). TRPC proteins are also likely candidates for O-GlcNAc, e.g. the analysis of the protein sequence for TRPC1 suggests a high-affinity site for O-GlcNAc, close to the NH_2-terminal region.

The O-GlcNAc-proteins concerning $[Ca^{2+}]_i$ discussed so far affect mainly short-term posttranslational modifications. Alternatively, long term exposure to high levels of glucose also enables expressional changes since the O-GlcNAc modification of transcriptional factors can influence the expressional levels of proteins involved in $[Ca^{2+}]_i$ handling. Indeed, SERCA2a was reported to have a decreased expression after prolonged high glucose incubation and this change was attributed to the transcriptional level (Sp1) rather than to posttranslational modification (29). Thus, even if high glucose levels can be useful for cell survival under certain circumstances, previous history of long-term high glucose exposure probably overshadows the beneficial effects in this case.

The role of O-GlcNAc in pathogenesis

Diabetes

Type 2 diabetes is characterized by increased levels of blood glucose via insulin resistance of peripheral cells, and by diabetic complications caused by prolonged exposure to high glucose. Although the exact mechanisms await further clarification, the majority of reports concur that O-GlcNAc contributes to both insulin resistance and to the development of diabetic complications.

Insulin resistance

Reduced glucose transport through cell membrane results in insulin resistance. This is caused by impaired translocation of GLUT4 glucose transporter (which is probably an O-GlcNAc protein (70)). This translocation (and also the docking and the fusion with the membrane) is regulated by multiple mechanisms, with the most important factor being insulin and insulin receptor activation. The next step involves IRS-1 and -2 (insulin receptor substrates) whose O-GlcNAc binding capacity has been well established (19,71,72). Downstream of IRS; PI3kinase, Akt, (decreased activity in insulin resistance) PKC, p38 and NF-κB (increased activity in insulin resistance) have been implicated in the insulin signaling pathway cascade. From these messengers, IRS (72), PI3kinase (19) are O-GlcNAc proteins, and putatively Akt (72), p38 (39), and NF-κB (7)

slobodnih radikala, što također doprinosi inzulinskoj rezistenciji.
Funkcionalnost Munc18c, regulatora modeliranja/fuzije vezikula (koji npr. sadrže GLUT4) na plazmatsku membranu, poremećena je kod primjene glukozamina ili visokih koncentracija glukoze (73). Munc18c je također podložan O-glikozilaciji. Iako navedeni dokazi ukazuju da O-GlcNAc ima ključnu ulogu u inzulinskoj rezistenciji, O-GlcNAc nije ni u kom slučaju nužan za razvoj tog poremećaja (74).

Komplikacije šećerne bolesti

Brownlee (75) je objavio najsveobuhvatniju pretpostavku kao objašnjenje temeljnoga molekularnog patomehanizma svih komplikacija šećerne bolesti kao što su ubrzana ateroskleroza, zatajenje perifernih živaca, te komplikacije vezane za bubrege i mrežnicu uzrokovane mikrovaskularnim oštećenjem. Prema tom autoru pretjerano stvaranje mitohondrijskog superoksida potaknuto hiperglikemijom blokira gliceraldehid-3P-dehidrogenazu, ključni enzim u glikolizi. Zbog toga su kasniji metaboliti usmjereni na druge putove; poliolni put, krajnje produkte glikacije (engl. *advanced end-glycation products*, AGE), aktivaciju PKC (zbog povećanog stvaranja DAG), te povećani protok kroz HBP. Kod komplikacija šećerne bolesti, HBP je povezan s povećanim izražajem TGFα (engl. *transforming growth factor*, transformirajući čimbenik rasta), TGF-β1 i PAI-1 (engl. *plasminogen activator inhibitor*, inhibitor aktivatora plazminogena) (62,76,77). Točna veza između TGF i HBP nije još otkrivena (pretpostavlja se da je to PKC (78)); međutim, čimbenik prijepisa Sp1, koji je O-GlcNAc-protein, povećava izražaj PAI-1.
Endotelna dušično-oksidna sintaza (eNOS) također je podložna O-glikozilaciji; činjenica da O-GlcNAc prikriva fosforilacijska mjesta Akt na eNOS-u sprječava aktivaciju Akt i time smanjuje koncentracije NO koji je snažan vazodilatator (19,79). HBP neizravno aktivira i put PKC, premda ne samom neposrednom O-glikozilacijom PKC već vjerojatno uključivanjem drugih kasnijih kinaza (80). Ako se razmotre zajedno, ti dokazi uvjerljivo ukazuju da O-glikozilacija proteina regulira mnogo staničnih procesa povezanih s šećernom bolesti, te da dugotrajni poremećaj HBP i/ili postupaka vezanih za O-GlcNAc dovodi do teških komplikacija šećerne bolesti.

Odgovor na stres

Kao što je već prethodno navedeno, glukozamin i visoka glukoza sprječavaju ishemijsku/reperfuzijsku ozljedu i paradoks vezan za Ca^{2+}. Nedavno je pokazano da selektivno povećanje razina O-GlcNAc ima sličan učinak (14,16). Bilo u prokrvljenom srcu ili u štakora s traumatskim krvarenjem, glukozamin uzrokuje i povećane koncentracije O-GlcNAc kao i istodobno smanjenje ishemijskog oštećenja (14,81). PUGNAc, specifičan inhibitor O-GlcNAkaze, također su kandidati za O-glikozilaciju. Iako je O-glikozilacija NF-κB još uvijek nedokazana, aktivacija NF-κB u dijabetesu je dobro opisana (7). Osim O-GlcNAc, NF-κB može biti aktiviran i AngII ili slobodnim radikalima, također doprinoseći inzulinskoj rezistenciji.

are also candidates for O-glycosylation. Although the O-glycosylation of NF-κB is putative yet, the activation of NF-κB in diabetes has been well described (7). Apart from O-GlcNAc, NF-κB can be also activated by AngII or by free radicals, also contributing to insulin resistance.
The functionality of Munc18c, a regulator of docking/fusion of vesicles (containing e.g. GLUT4) to plasma membrane is disturbed when treated with glucosamine or high levels of glucose (73). Munc18c is also subject to O-glycosylation. Although all this evidence suggests that O-GlcNAc plays a major part in insulin resistance, O-GlcNAc is not by all means necessary for its development (74).

Diabetic complications

The most comprehensive hypothesis to explain the underlying molecular pathomechanism of all diabetic complications, such as accelerated atherosclerosis, peripheral nerve failure, renal and retinal complication caused by microvascular damage, was published by Brownlee (75). According to this author, hyperglycemia-induced mitochondrial superoxide overproduction blocks glyceraldehyde-3P-dehydrogenase, a key enzyme in glycolysis. Therefore the upstream metabolites are diverted to other pathways: the polyol way, the advanced end-glycation products (AGE), the activation of PKC (by increased production of DAG), and increased flux through the HBP. The HBP is associated in diabetic complications with increased expression of TGFα (transforming growth factor), TGF-β1 and PAI-1 (plasminogen activator inhibitor) (62,76,77). The exact link between TGF and HBP has not been revealed yet (PKC is assumed (78)); however, PAI-1 expression is increased by Sp1 transcriptional factor which is an O-GlcNAc protein.
Endothelial nitric oxide synthase (eNOS) is also O-glycosylated, the O-GlcNAc masking of the Akt phosphorylation sites on eNOS prevents its activation, thus decreasing the levels of the NO which is a strong vasodilatator (19,79). HBP indirectly activates also the PKC pathway, although apparently not by direct O-glycosylation of PKC itself, but probably by the involvement of other upstream kinases (80). Taken together, these pieces of evidence strongly suggest that protein O-glycosylation regulates a great number of cellular processes related to diabetes, and that the long term disturbance in HBP and/or O-GlcNAc-handling leads to severe diabetic complications.

Stress response

As mentioned above, glucosamine and high glucose prevent ischemia/reperfusion injury and Ca^{2+} paradox. Recently, it has been shown that selective increase in the levels of O-GlcNAc has a similar effect (14,16). Either in perfused heart or in trauma-hemorrhage rat models, glucosamine caused both increased levels of O-GlcNAc and simultaneously reduced ischemic damage (14,81). In

đer štiti od hipoksijskog oštećenja u izoliranim kardiomiocitima (82).

S druge strane, stanice su bez ikakvog vanjskog zahvata sklone povećati svoje koncentracije O-GlcNAc kod stresa. Kao što su pokazali Hart i sur., odgovor na nekoliko različitih stresova (vrućina, hipoksija, osmotski stres) uključivao je povišeni O-GlcNAc (11). Slabljenje OGT ne samo da je poništilo O-GlcNAc povišen zbog stresa već je smanjilo i podnošenje stresa te preživljenje stanice. Čini se da takav rezultat podupire pretpostavku da je O-GlcNAc nužan element normalnog odgovora na stres.

Koji su specifični ciljni proteini povezani s O-glikozilacijom aktiviranom stresom? Čini se da su proteini toplinskog šoka (Hsp, engl. *heat shock proteins*) prvi razumni odgovor, jer zaista nekoliko takvih proteina predstavlja kandidate za O-GlcNAc (51,83). Također je ukazano da je izražaj Hsp70 pojačan nakon modifikacije O-GlcNAc (11). Glede O-GlcNAc, homeostaza $[Ca^{2+}]_i$ bi mogla biti još jedan predmet regulacije. Hipoksija ili stres povećavaju $[Ca^{2+}]_i$, a Ca^{2+} je posrednikom u nekoliko štetnih učinaka ukoliko se početni stres ubrzano ne ukloni. Povećani $[Ca^{2+}]_i$ aktivira unutarstanične glasnike kao što su kalcineurin, kalmodulin, NF-AT, PKC i kaspaze. Posljedica toga je aktivacija nekoliko čimbenika prijepisa tako da stanice umiru uslijed hipertrofije ili apoptoze. Zanimljivo je da hipoksija izaziva prijenos glukoze od strane Ca^{2+} (84), što je neizravan dokaz veze između $[Ca^{2+}]_i$ i regulacije O-GlcNAc. Kao što je ranije spomenuto, pokazali smo da je manipuliranje O-GlcNAc utjecalo na regulaciju $[Ca^{2+}]_i$ u kardiomiocitima (16). Povećane koncentracije O-GlcNAc u kardiocitima, nastale bilo povećanjem protoka kroz HBP zbog primjene glukozamina ili inhibiranja O-GlcNAkaze pomoću PUGNAc, sprječavaju porast bazalnog $[Ca^{2+}]_i$ koji izaziva AngII (Slika 2). Moguće je da se taj inhibicijski učinak O-GlcNAc na razinu $[Ca^{2+}]_i$ razvija preko višestrukih meta, npr. PLC ili kanala TRPC.

Uloga O-GlcNAc u odgovoru na stres može se opisati na sljedeći način: stres aktivira nekoliko signalnih putova, od kojih je najvažniji Ca^{2+} koji zaista na početku služi kao prirodan i nužan mehanizam prilagodbe. $[Ca^{2+}]_i$ zatim olakšava ulazak glukoze u stanice, čime osigurava dodatni izvor energije. Međutim, mali udio glukoze prolazi kroz HBP i izaziva modulaciju i smanjenje $[Ca^{2+}]_i$ te odgovora na stres od strane O-GlcNAc. Ako je stresni podražaj kratkotrajan ili ograničen, HBP može spriječiti pretjeranu reakciju stanice, no ako je podražaj dulji (no još uvijek nije smrtonosan) fiziološki porast O-GlcNAc često nije dovoljan da bi poništio štetne učinke preopterećenja s Ca^{2+}-.

O-GlcNAc se također dobro uklapa u teoriju preduvjetovanja: kratka, blaga ishemija ili stres smanjuju rizik i ozbiljnost kasnijeg ishemijskog napadaja (85). Preduvjetovanost se pripisuje i određenom broju signalnih putova kao što su Akt/PI3kinaza ili PKC (86). S obzirom da mu stres povećava koncentraciju, O-GlcNAc raste tijekom prvog podražaja

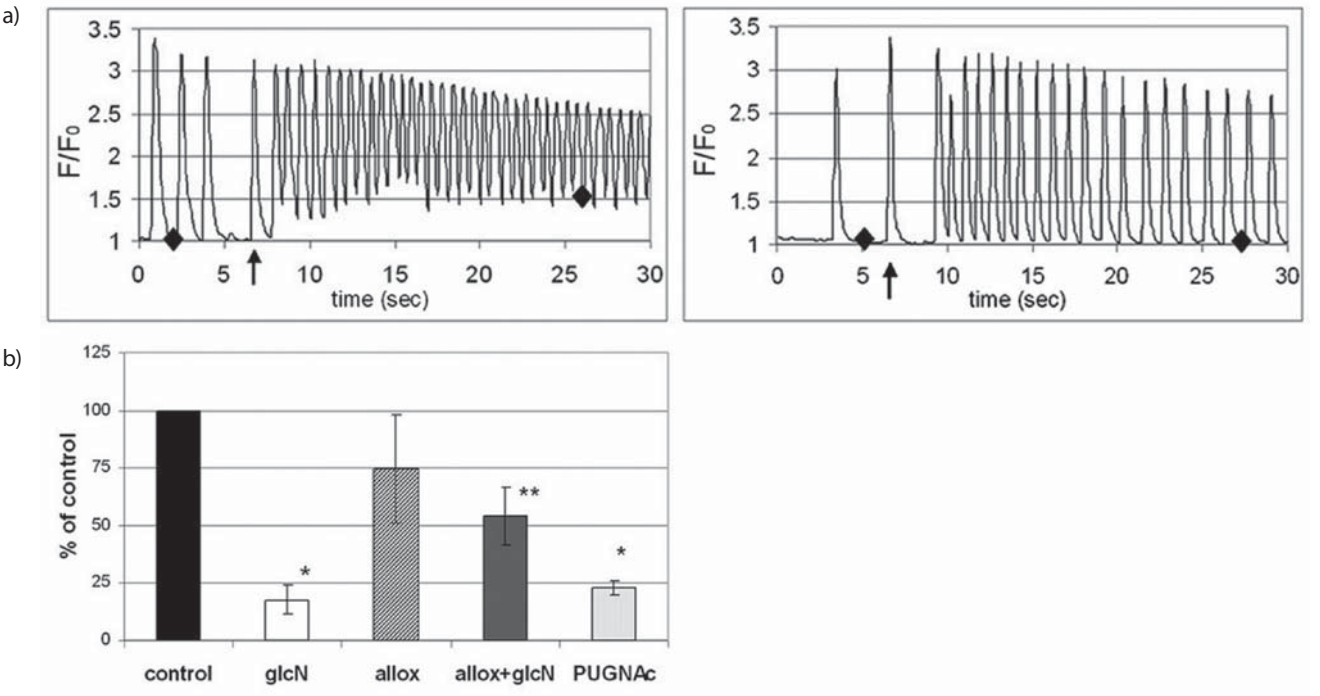
isolated cardiomyocytes, PUGNAc, a specific inhibitor of O-GlcNAcase, also protected from hypoxic damage (82).

On the other hand, without any external intervention cells tend to increase their O-GlcNAc levels upon stress. As it was shown by Hart and coworkers, the stress response to a number of different stress (heat, hypoxia, osmotic stress) included elevated O-GlcNAc (11). Blunting OGT not only abolished the stress-elevated O-GlcNAc but reduced the stress tolerance and cell survival. This result seems to support the hypothesis that O-GlcNAc is a necessary element of normal stress response.

Which are the specific target proteins related to stress activated O-glycosylation? Heat shock proteins seem to be the first reasonable answer, indeed a number of Hsp-s are candidates for O-GlcNAc (51,83). It was also shown that the expression of Hsp70 is increased after O-GlcNAc modification (11). For O-GlcNAc, another subject of regulation could be the $[Ca^{2+}]_i$ homeostasis. Hypoxia or stress elevates $[Ca^{2+}]_i$, and Ca^{2+} mediates a number of deleterious effects unless the initial stress is removed quickly. Elevated $[Ca^{2+}]_i$ activates intracellular messengers such as calcineurin, calmodulin, NF-AT, PKC and caspases. This results in the activation of several transcriptional factors and the cells end up either in hypertrophy or apoptosis. Interestingly, hypoxia induces glucose transport by Ca^{2+} (84) which is an indirect evidence for the link between $[Ca^{2+}]_i$ and O-GlcNAc regulation. As mentioned above, we have shown that manipulations with O-GlcNAc influenced $[Ca^{2+}]_i$ regulation in cardiomyocytes (16). In cardiomyocytes, elevated levels of O-GlcNAc, achieved either by increasing the flux through the HBP by glucosamine treatment or inhibiting O-GlcNAcase with PUGNAc, prevent the increase of basal $[Ca^{2+}]_i$ induced by AngII (Figure 2). This inhibitory effect of O-GlcNAc on $[Ca^{2+}]_i$ level develops possibly via multiple targets, e.g. PLC or TRPC channels.

The role of O-GlcNAc in stress response could be described as follows: stress activates several signaling pathways, most importantly Ca^{2+} which indeed at first serves as a natural and necessary adaptation mechanism. $[Ca^{2+}]_i$ then facilitates the entering of glucose into cells, which provides additional energy source. However, a small portion of the glucose will flux through the HBP, inducing the modulation and downregulation of $[Ca^{2+}]_i$ and stress response by O-GlcNAc. If the stress stimulus is short-term or limited, HBP can prevent the cells to over-react, however if the stimulus is prolonged (but still sub-lethal), the physiological O-GlcNAc elevation is often not sufficient to counter the deleterious effects of Ca^{2+}-overload.

O-GlcNAc also fits well into the preconditioning theory: a short, mild ischemia or stress reduce the risk and seriousness of a subsequent ischemic attack [85]. Preconditioning was attributed to a number of signaling pathways, such as Akt/PI3kinase or PKC (86). Since stress increases O-GlcNAc levels, O-GlcNAc elevates during the first stimuli and by the second exposure it could help to reduce



Slika 2. Povećane koncentracije O-GlcNAc inhibiraju porast $[Ca^{2+}]_i$ izazvan od AngII. A) Lijevo: Primjena AngII (naznačena strelicom) uzrokuje ubrzani porast dijastoličkog $[Ca^{2+}]_i$ u neonatalnim kardiomiocitima štakora. Desno: prethodna primjena 5 mM glukozamina tijekom 10 minuta inhibira porast dijastoličkog $[Ca^{2+}]_i$ izazvanog od AngII. B) Promjene baznog $[Ca^{2+}]_i$ nakon primjene AngII u odnosu na kontrole. Glukozamin i PUGNAc, koji je inhibitor O-GlcNAkaze, oboje smanjuju porast $[Ca^{2+}]_i$ dok je aloksan, inhibitor OGT, djelomice poništio učinak glukozamina.

Figure 2. Increased O-GlcNAc levels inhibit AngII-induced $[Ca^{2+}]_i$ rise. A.) Left: AngII (indicated by the arrow) treatment causes rapid increase in diastolic $[Ca^{2+}]_i$ in neonatal rat cardiomyocytes. Right: pretreatment with 5 mM glucosamine for 10 min inhibits the diastolic $[Ca^{2+}]_i$ increase elicited by AngII. B.) Changes in baseline $[Ca^{2+}]_i$ after AngII treatment, relative to controls. Both glucosamine and PUGNAc, an inhibitor of O-GlcNAcase, reduced the increment of $[Ca^{2+}]_i$ whereas alloxan, an inhibitor of OGT, partially reversed the effect of glucosamine.

žaja, a kod druge izloženosti može pomoći u smanjivanju oštećenja izazvanog stresom. Kao i ranije, pretpostavljamo da se taj učinak zbiva pomoću višestrukih mehanizama i meta, a ne modificiranjem jedinstvenog odabranog glasnika. Vjerojatno je da ukupna razina O-GlcNAc u stanici odražava općenitu podnošljivost za stres i stanje prilagodbe u određenom vremenu.

Učinak HBP i O-GlcNAc u šećernoj bolesti i odgovoru na stres vrlo je proturječan. Moguće objašnjenje za to jest to činjenica da je šećerna bolest dugotrajna, kronična bolest kod koje su pridruženoj hiperglikemiji i povećanom O-GlcNAc potrebni mjeseci ili godine da bi razvili komplikacije šećerne bolesti, dok je O-glikozilacija izazvana stresom akutno i vrlo ubrzano stanje (12). To znači da su u šećernoj bolesti i stresu modificirani različiti proteini, ili pak da su uključeni isti proteini no da početna, kratkotrajna aktivacija nije dovoljna za pokretanje aktivacije signalnih slijedova (kaskada) i štetne učinke zapažene kod šećerne bolesti.

the stress-induced damage. As above, we assume that this effect occurs via multiple mechanisms and targets rather than by modifying a single selected messenger. It is plausible that the overall O-GlcNAc level of the cell reflects the general stress tolerance and adaptation state at a given time.

The effect of HBP and O-GlcNAc in diabetes and in stress response is quite contradictory. A possible explanation is that while diabetes is a long term, chronic disease and the associated hyperglycemia and elevated O-GlcNAc need months or years to develop diabetic complications, the stress-induced O-glycosylation is an acute, very rapid condition (12). This means either that in diabetes and in stress different proteins are modified, or that the same proteins are involved, but the initial, short term activation is not sufficient to initiate the activation of signaling cascades and the deleterious effects observed in diabetes.

It is well known that diabetic patients have a significantly increased risk for cardiovascular damage and ischemic

Za oboljele od šećerne bolesti dobro je poznato da imaju značajno povećani rizik za kardiovaskularno oštećenje i epizode ishemije. Za to je potrebna dugotrajna izloženost visokoj koncentraciji glukoze koja nepovratno oštećuje srčanožilni sustav. Tijekom akutnog napadaja ishemije mogući regulacijski porast O-GlcNAc je beznačajan u odnosu na sprječavanje ozljeda izazvanih hipoksijom. S druge strane, bolesnik koji nema šećernu bolest mogao bi imati veću korist od povećane glukoze i HBP koja je već uključena uporabom infuzija. Premda u krajnje teorijskom smislu, primjena glukozamina mogla bi imati iste ili bolje rezultate. Glukozamin je već u širokoj uporabi kao terapijski lijek kod osteoartritisa. On bi mogao imati prodijabetički učinak iako dosadašnje studije nisu to mogle nedvosmisleno dokazati (87). Čini se da je relativno neškodljiv u normalnoj dozi ili kod kratkotrajne primjene.

Upala

Uloga O-GlcNAc u upali je prijeporna. U šećernoj bolesti je aktiviran NF-κB, a i posrednici upale povećavaju svoj izražaj, primjerice TGF-β1 ili PAI-1 (62,77). Pretjerani izražaj TGF-β1 je vjerojatno povezan s aktivacijom PKC (78) koji sudjeluje u inzulinskoj rezistenciji i komplikacijama, dok promotor PAI-1 aktivira Sp1, jedan od prvih čimbenika prijepisa za kojega je utvrđeno da je O-glikoziliran.
S druge strane, nekoliko je studija opisalo da primjena glukozamina inhibira NF-κB u konjuktivnim stanicama (88) ili u hondrocitima (13). Ta inhibicija očigledno može objasniti blagotvorne učinke glukozamina kod osteoartritisa. Također je u studijama izviješteno da glukozamin sprječava proliferaciju T-stanica izazvanu CD3 (89) te utvrđeno da produžuje preživljenje kardijalnog alo-presatka u miševa (90). Autori druge publikacije predlažu da bi protuupalni i imunosupresijski učinak glukozamina mogao biti povezan s prolaznom izloženošću, dok je za inzulinsku rezistenciju nužna stalna prisutnost glukozamina.

Zloćudne bolesti

Postoji relativno malo dostupnih podataka o ulozi O-GlcNAc u zloćudnim bolestima. O-GlcNAc, međutim, ima značajnu ulogu u staničnom ciklusu, a brojni su čimbenici prijepisa podložni O-glikozilaciji. Primjerice, za protoonkogen c-myc je utvrđeno da je O-glikoziliran, a mjesto(a) O-GlcNAc je smješteno unutar ili blizu N-terminalne domene aktivacije prijepisa/zloćudne transformacije, područja gdje se mutacije često nalaze u Burkittovim i s AIDS-om povezanim limfomima (91). Za supresor tumora p53 također je utvrđeno da je O-glikoziliran; čini se da O-GlcNAc modulira njegovu sposobnost vezanja DNA (8) ili blokira fosforilaciju koja odgađa proteolitsku razgradnju p53 (92).
Čimbenik prijepisa Sp1 također se često povezuje s karcinomima (93). Sp1 je povezan sa stanjem hipoglikozilacije i ubrzano ga razgrađuje proteazom, no ta se razgradnja

episodes. This needs a long time exposure to a high level of glucose which irreparably damages the cardiovascular system. During an acute ischemic attack, the possible regulatory O-GlcNAc elevation is insignificant in terms of preventing hypoxia-induced injuries. On the other hand, a non-diabetic patient could better benefit from elevated glucose and HBP, as it has already been implicated by the use of GIK infusions. Although it is highly theoretical, glucosamine treatment could grant the same or better results. Glucosamine is already a widely used therapeutic drug in osteoarthritis. It may have a pro-diabetic effect, although the studies so far could not prove this unequivocally (87). It seems that it is relatively harmless at normal dose or at short term application.

Inflammation

The role of O-GlcNAc in inflammation is controversial. In diabetes, NF-κB is activated, and inflammation mediators also increase their expression, such as TGF-β1 or PAI-1 (62,77). TGF-β1 overexpression is probably related to PKC activation (78), which is implicated in insulin resistance and diabetic complications, whereas PAI-1 promoter is activated by Sp1, one of the first transcriptional factors that has been found to be O-glycosylated.
On the other hand, a couple of studies described that glucosamine treatment inhibits NF-κB in conjunctival cells (88) or in chondrocytes (13). Apparently, this inhibition could explain the beneficial effects of glucosamine in osteoarthritis. It has been also reported that glucosamine prevents CD3-induced T cell proliferation (89), and found that it prolongs cardiac allograft survival in mice (90). The authors of the latter publication propose that the anti-inflammatory and immunosuppressant effect of glucosamine might be connected to transient exposures whereas insulin resistance requires the presence of glucosamine continuously.

Malignant diseases

There is relatively scarce information available about the role of O-GlcNAc in malignant diseases. However, O-GlcNAc plays a significant role in cell cycle, and numerous transcriptional factors are subject to O-glycosylation. For example, c-myc proto-oncogene was found to be O-glycosylated and the O-GlcNAc site(s) are located within or near the N-terminal transcription activation/malignant transformation domain, a region where mutations are frequently found in Burkitt and AIDS-related lymphomas (91). The tumor suppressor p53 was also found to be O-glycosylated; O-GlcNAc seems to modulate its DNA binding capacity (8) or to block phosphorylation which delays proteolytic degradation of p53 (92).
The transcriptional factor Sp1 is also often associated with cancers (93). Correlating with hypoglycosylated state, Sp1 is rapidly degraded by the proteasome and this deg-

može spriječiti primjenom glukoze ili glukozamina (41). Drugo, Sp1 sadrži jedinstveni ostatak O-GlcNAc čija modifikacija inhibira hidrofobne interakcije između Sp1 i dva spoja: proteinski povezanog čimbenika koji veže TATA (TAFII110) te holo-Sp1 (61). Roos i sur. predlažu da Sp1 nakon vezanja DNA mora izgubiti svoj ostatak O-GlcNAc pomoću nestalnog mehanizma uz fosforilaciju kako bi vezao TAFII110, holo-Sp1 i pokrenuo prijepis.

Hipermetilirani gen u karcinomu 1 (HIC1) je kandidat za gen supresora tumora kojega O-GlcNAc modificira u nekoliko zloćudnih staničnih linija; čini se, međutim, da O-glikozilacija utječe na stabilnost, a ne afinitet za vezanje DNA (94). Konačno, O-GlcNAc može modificirati i RNA polimerazu II. Jedna studija izvještava da OGT međusobno djeluje s kompleksom histonske deacetilaze vezanjem na korepresor mSin3A te potiskuje prijepis usporedo s deacetilacijom histona. mSin3A usmjerava OGT na promotore kako bi inaktivirao čimbenike prijepisa i RNA polimerazu II modifikacijom O-GlcNAc (95).

Način na koji O-GlcNAc utječe na razvoj zloćudnih poremećaja još uvijek je sporan zato jer O-GlcNAc može spriječiti razgradnju čimbenika prijepisa, no čini se da također izravno blokira ili aktivira te iste čimbenike. Za razumijevanje složenog ponašanja O-glikozilacije biti će potrebno uzeti u obzir lokalizaciju, prostornu organizaciju OGT te također kartiranje pojedinačnih (a moguće i višestrukih) mjesta za O-GlcNAc na čimbenicima prijepisa.

Zaključci

Dokazi utvrđeni tijekom posljednja dva desetljeća ukazuju na O-GlcNAc kao jedinstven no važan unutarstanični signalni mehanizam koji obuhvaća i sudjeluje u skoro svakom staničnom događaju, bilo fiziološkom ili patološkom. Premda je O-glikozilacija općenit, uobičajen proces u stanici kojega regulira jedan enzim - OGT, mehanizam, učinci i proteini podložni modifikaciji O-GlcNAc su visokospecifični. To se postiže prostornom i vremenskom organizacijom te usklađenošću s fosforilacijom. Modulacija nekoliko signalnih događaja u šećernoj bolesti, stresu, zloćudnim bolestima ili u upali nesumnjivo zaslužuje pozornost u daljnjem istraživanju. Premda su povećane koncentracije O-GlcNAc očigledno štetne u šećernoj bolesti, razjašnjenje njegove uloge u odgovoru na akutni stres moglo bi predstavljati veliki korak naprijed prema poboljšanoj prevenciji ishemijskih/reperfuzijskih ozljeda. Možemo se nadati da će bolje razumijevanje O-GlcNAc u budućnosti pomoći kako u smanjenju komplikacija šećerne bolesti, tako i u povećanju životnog vijeka bolesnika s ishemijom.

radation can be prevented by glucose or glucosamine treatment (41). Second, Sp1 contains a single O-GlcNAc residue whose modification inhibits hydrophobic interactions between Sp1 and two partners, the TATA binding protein-associated factor (TAFII110) and holo-Sp1 (61). Roos et al. propose that, upon DNA binding, Sp1 has to lose its O-GlcNAc residue by a flip-flop mechanism with phosphorylation in order to bind TAFII110, holo-Sp1 and induce transcription.

The 'hypermethylated in cancer 1' gene (HIC1) is a candidate tumor suppressor gene, and is modified by O-GlcNAc in a number of malignant cell lines; however, the O-glycosylation seems to affect stability and not DNA binding affinity (94). Finally, RNA polymerase II can be modified by O-GlcNAc. It has been reported that OGT interacts with a histone deacetylase complex by binding to the corepressor mSin3A, and represses transcription in parallel with histone deacetylation. mSin3A targets OGT to promoters to inactivate transcriptional factors and RNA polymerase II by O-GlcNAc modification (95).

The mode in which O-GlcNAc influences the development of malignancies is still controversial, since it can prevent the degradation of transcriptional factors, but also seems to directly block or activate the same factors. Understanding the complex behavior of O-glycosylation will require taking into account the localization, spatial organization of OGT and also the mapping of the individual (and possibly multiple) O-GlcNAc sites on transcriptional factors.

Conclusions

The evidence established in the last two decades shows that O-GlcNAc is a unique, but important intracellular signaling mechanism, covering and participating in almost every cellular event, being either physiological or patho-physiological. Although O-glycosylation is a general, common process in the cell regulated by a single enzyme - OGT -, the mechanism, the effects of, and the proteins subjected to O-GlcNAc modifications are highly specific. This is achieved by spatial and timed organization, and by a harmonized coordination with phosphorylation. The modulation of a number of signaling events in diabetes, stress, malignant diseases or in inflammation undoubtedly deserves the attention in further research. Although elevated levels of O-GlcNAc are clearly detrimental in diabetes, clarifying its role in acute stress response could be a great step forward to improve the prevention of ischemia/reperfusion injuries. Hopefully the better understanding of O-GlcNAc in the future will help to both reduce diabetic complications and to increase the life expectancy of ischemic patients.

Adresa za dopisivanje:

Gabor L. Kovacs
Institute of Laboratory Medicine
Faculty of Medicine
University of Pecs
Ifjusag str. 13
H-7624 Pecs
Hungary
e-pošta: gabor.l.kovacs@aok.pte.hu
tel: +36 72 536 120
faks: +36 72 536 121

Corresponding author:

Gabor L. Kovacs
Institute of Laboratory Medicine
Faculty of Medicine
University of Pecs
Ifjusag str. 13
H-7624 Pecs
Hungary
e-mail: gabor.l.kovacs@aok.pte.hu
phone: +36 72 536 120
fax: +36 72 536 121

Literatura/References

1 *Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! Biochim Biophys Acta 2006;1761:599-617.*

2 *Torres CR, Hart GW. Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for O-linked GlcNAc. J Biol Chem 1984;259:3308-17.*

3 *Wells L, Vosseller K, Hart GW. A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. Cell Mol Life Sci 2003;60:222-8.*

4 *Slawson C, Zachara NE, Vosseller K, Cheung WD, Lane MD, Hart GW. Perturbations in O-linked beta-N-acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. J Biol Chem 2005;280:32944-56.*

5 *Lamarre-Vincent N, Hsieh-Wilson LC. Dynamic glycosylation of the transcription factor CREB: a potential role in gene regulation. J Am Chem Soc 2003;125:6612–3.*

6 *Jackson SP, Tjian R. O-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. Cell 1988; 55:125–33.*

7 *James LR, Tang D, Ingram A, Ly H, Thai K, Cai L, Scholey JW. Flux through the hexosamine pathway is a determinant of nuclear factor κB-dependent promoter activation. Diabetes 2002;51:1146–56.*

8 *Shaw P, Freeman J, Bovey R, Iggo R. Regulation of specific DNA binding by p53: evidence for a role for O-glycosylation and charged residues at the carboxy-terminus. Oncogene 1996;12:921–30.*

9 *Hiromura M, Choi CH, Sabourin NA, Jones H, Bachvarov D, Usheva A. YY1 is regulated by O-linked N-acetylglucosaminylation (O-GlcNAcylation). J Biol Chem 2003;278:14046–52.*

10 *Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: current status. Am J Physiol Endocrinol Metab 2006;290:E1-E8.*

11 *Zachara NE, O'Donnell N, Cheung WD, Mercer JJ, Marth JD, Hart GW. Dynamic O-GlcNAc modification of nucleocytoplasmic proteins in response to stress. A survival response of mammalian cells. J Biol Chem 2004;279:30133-42.*

12 *Fulop N, Marchase RB, Chatham JC. Role of protein O-linked N-acetylglucosamine in mediating cell function and survival in the cardiovascular system. Cardiovasc Res 2007;73:288-97.*

13 *Largo R, Alvarez-Soria MA, Diez-Ortego I, Calvo E, Sanchez-Pernaute O, Egido J, Herrero-Beaumont G. Glucosamine inhibits IL-1beta-induced NFkappaB activation in human osteoarthritic chondrocytes. Osteoarthritis Cartilage 2003;11:290-8.*

14 *Liu J, Pang Y, Chang T, Bounelis P, Chatham JC, Marchase RB. Increased hexosamine biosynthesis and protein O-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. J Mol Cell Cardiol 2006;40:303-12.*

15 *Snow DM, Hart GW. Nuclear and cytoplasmic glycosylation. Int Rev Cytol 1998;181:43-74.*

16 *Nagy T, Champattanachai V, Marchase RB, Chatham JC. Glucosamine inhibits angiotensin II-induced cytoplasmic Ca2+ elevation in neonatal cardiomyocytes via protein-associated O-linked N-acetylglucosamine. Am J Physiol Cell Physiol 2006;290:C57-65.*

17 *Lefebvre T, Ferreira S, Dupont-Wallois L, Bussiere T, Dupire MJ, Delacourte A, et al. Evidence of a balance between phosphorylation and O-GlcNAc glycosylation of Tau proteins–a role in nuclear localization. Biochim Biophys Acta 2003;1619:167–76.*

18 *Kelly WG, Dahmus ME, Hart GW. RNA polymerase II is a glycoprotein. Modification of the COOH-terminal domain by O-GlcNAc. J Biol Chem 1993;268:10416–24.*

19 *Federici M, Menghini R, Mauriello A, Hribal ML, Ferrelli F, Lauro D, et al. Insulin-dependent activation of endothelial nitric oxide synthase is impaired by O-linked glycosylation modification of signaling proteins in human coronary endothelial cells. Circulation 2002;106:466–72.*

20 *Reason AJ, Morris HR, Panico M, Marais R, Treisman RH, Haltiwanger RS, et al. Localization of O-GlcNAc modification on the serum response transcription factor. J Biol Chem 1992;267:16911–21.*

21 *Parker GJ, Lund KC, Taylor RP, McClain DA. Insulin resistance of glycogen synthase mediated by o-linked N-acetylglucosamine. J Biol Chem 2003;278:10022–7.*

22 *Shafi R, Lyer SP, Ellies LG, O'Donnell N, Marek KW, Chui D, et al. The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc Natl Acad Sci USA 2000;97:5735–9.*

23 *Kreppel LK, Blomberg MA, Hart GW. Dynamic glycosylation of nuclear and cytosolic proteins: cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. J Biol Chem 1997;272:9308–15.*

24 *Wells L, Gao Y, Mahoney JA, Vosseller K, Chen C, Rosen A, Hart GW Dynamic O-glycosylation of nuclear and cytosolic proteins: further characterization of the nucleocytoplasmic beta-N-acetylglucosaminidase, O-GlcNAcase. J Biol Chem 2002;277:1755–61.*

25 *Comer FI, Vosseller K, Wells L, Accavitti MA, Hart GW. Characterization of a mouse monoclonal antibody specific for O-linked N-acetylglucosamine. Anal Biochem 2001;293:169-77.*

26 *Snow CM, Senior A, Gerace L. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. J Cell Biol 1987;104:1143-56.*

27 *James LR, Fantus IG, Goldberg H, Ly H, Scholey JW. Overexpression of GFAT activates PAI-1 promoter in mesangial cells. Am J Physiol Renal Physiol 2000;279:F718-27.*

28 *McClain DA, Lubas WA, Cooksey RC, Hazel M, Parker GJ, Love DC, Hanover JA. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proc Natl Acad Sci USA 2002;99:10695–9.*

29 *Clark RJ, McDonough PM, Swanson E, Trost SU, Suzuki M, Fukuda M, Dillmann WH. Diabetes and the accompanying hyperglycemia impairs cardiomyocyte calcium cycling through increased nuclear O-GlcNAcylation. J Biol Chem 2003;278:44230-7.*

30 Hresko RC, Heimberg H, Chi MM, Mueckler M. Glucosamine-induced insulin resistance in 3T3-L1 adipocytes is caused by depletion of intracellular ATP. J Biol Chem 1998;273:20658-68.
31 Liu J, Marchase RB, Chatham JC. Glutamine-induced protection of isolated rat heart from ischemia/reperfusion injury is mediated via the hexosamine biosynthesis pathway and increased protein O-GlcNAc levels. J Mol Cell Cardiol 2007;42:177-85.
32 Konrad RJ, Zhang F, Hale JE, Knierman MD, Becker GW, Kudlow JE. Alloxan is an inhibitor of the enzyme O-linked N-acetylglucosamine transferase. Biochem Biophys Res Commun. 2002;293:207-12.
33 Haltiwanger RS, Grove K, Philipsberg GA. Modulation of O-linked N-acetylglucosamine levels on nuclear and cytoplasmic proteins in vivo using the peptide O-GlcNAc-beta-N-acetylglucosaminidase inhibitor O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate. J Biol Chem 1998;273:3611-7.
34 Konrad RJ, Mikolaenko I, Tolar JF, Liu K, Kudlow JE. The potential mechanism of the diabetogenic action of streptozotocin: inhibition of pancreatic β-cell O-GlcNAc-selective N-acetyl-β-D-glucosaminidase. Biochem J 2001;356:31–41.
35 Griffith LS, Schmitz B. O-linked N-acetylglucosamine levels in cerebellar neurons respond reciprocally to pertubations of phosphorylation. Eur J Biochem 1999;262:824–31.
36 Vosseller K, Wells L, Lane MD, Hart GW. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. Proc Natl Acad Sci USA 2002;99:5313–8.
37 Wells L, Kreppel LK, Comer FI, Wadzinski BE, Hart GW. OglcNAc transferase is in a functional complex with protein phosphatase 1 catalytic subunits. J Biol Chem 2004;279:38466–70.
38 Robertson LA, Moya KL, Breen KC. The potential role of tau protein Oglycosylation in Alzheimer's disease. J Alzheimers Dis 2004;6:489–95.
39 Fulop N, Zhang Z, Marchase RB, Chatham JC. Glucosamine cardioprotection in perfused rat heart associated with increased O-Linked N-acetylglucosamine protein modification and altered p38 activation. Am J Physiol Heart Circ Physiol 2007;292:H2227-36.
40 Cheng X, Hart GW. Alternative O-glycosylation/O-phosphorylation of serine-16 in murine estrogen receptor beta: posttranslational regulation of turnover and transactivation activity. J Biol Chem 2001;276:10570–5.
41 Han I, Kudlow JE. Reduced O-glycosylation of Sp1 is associated with increased proteasome susceptibility. Mol Cell Biol 1997;17:2550–8.
42 Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE. OglcNAc modification is an endogenous inhibitor of the proteasome. Cell 2003;115:715–25.
43 Kamemura K, Hart GW. Dynamic interplay between O-glycosylation and O-phosphorylation of nucleocytoplasmic proteins: a new paradigm for metabolic control of signal transduction and transcription. Prog Nucleic Acid Res Mol Biol 2003;73:107-36.
44 Hawkins M, Barzilai N, Liu R, Hu M, Chen W, Rossetti L. Role of the glucosamine pathway in fat-induced insulin resistance. J Clin Invest 1997;99:2173–82.
45 Marshall S, Bacote V, Traxinger RR. Complete inhibition of glucose-induced desensitization of the glucose transport system by inhibitors of mRNA synthesis. Evidence for rapid turnover of glutamine:fructose-6-phosphate amidotransferase. J Biol Chem 1991;266: 10155–61.
46 Rossetti L, Hawkins M, Chen W, Gindi J, Barzilai N. In vivo glucosamine infusion induces insulin resistance in normoglycemic but not in hyperglycemic conscious rats. J Clin Invest 1995;96:132–40.
47 Robinson KA, Sens DA, Buse MG. Pre-exposure to glucosamine induces insulin resistance of glucose transport and glycogen synthesis in isolated rat skeletal muscles. Study of mechanisms in muscle and in rat-1 fibroblasts overexpressing the human insulin receptor. Diabetes 1993;42:1333–46.
48 Ross SA, Chen X, Hope HR, Sun S, McMahon EG, Broschat K et al. Development and comparison of two 3T3-L1 adipocyte models of insulin resistance: increased glucose flux vs glucosamine treatment. Biochem Biophys Res Commun 2000;273: 1033–41.
49 Parker G, Taylor R, Jones D, McClain D. Hyperglycemia and inhibition of glycogen synthase in streptozotocin-treated mice: role of O-linked N-acetylglucosamine. J Biol Chem 2004;279:20636–42.
50 Oriente F, Formisano P, Miele C, Fiory F, Maitan MA, Vigliotta G, et al. Insulin receptor substrate-2 phosphorylation is necessary for protein kinase C zeta activation by insulin in L6hIR cells. J Biol Chem 2001;276:37109-19.
51 Kamemura K, Hayes BK, Comer FI, Hart GW. Dynamic interplay between O-glycosylation and O-phosphorylation of nucleocytoplasmic proteins: alternative glycosylation/phosphorylation of THR-58, a known mutational hot spot of c-Myc in lymphomas, is regulated by mitogens. J Biol Chem 2002;277:19229–35.
52 Walgren JL, Vincent TS, Schey KL, Buse MG. High glucose and insulin promote O-GlcNAc modification of proteins, including α-tubulin. Am J Physiol Endocrinol Metab 2003;284:424–34.
53 King IA, Hounsell EF. Cytokeratin 13 contains O-glycosidically linked N-acetylglucosamine residues. J Biol Chem 1989;264:14022–8.
54 Chou CF, Smith AJ, Omary MB. Characterization and dynamics of O-linked glycosylation of human cytokeratin 8 and 18. J Biol Chem 1992;267:3901-6.
55 Hiromura M, Choi CH, Sabourin NA, Jones H, Bachvarov D, Usheva A. YY1 is regulated by O-linked N-acetylglucosaminylation (O-GlcNAcylation). J Biol Chem 2003;278:14046–52.
56 Boehmelt G, Wakeham A, Elia A, Sasaki T, Plyte S, Potter J, et al. Decreased UDP-GlcNAc levels abrogate proliferation control in EMeg32-deficient cells. EMBO J 2000;19:5092-104.
57 Kelly WG, Hart GW. Glycosylation of chromosomal proteins: localization of O-linked N-acetylglucosamine in Drosophila chromatin. Cell 1989;57:243–51.
58 Hanover JA, Cohen CK, Willingham MC, Park MK. O-linked N-acetylglucosamine is attached to proteins of the nuclear pore. Evidence for cytoplasmic and nucleoplasmic glycoproteins. J Biol Chem 1987;262:9887–94.
59 Miller MW, Hanover JA. Functional nuclear pores reconstituted with beta 1-4 galactose-modified O-linked N-acetylglucosamine glycoproteins. J Biol Chem 1994;269:9289–97.
60 Whelan SA, Hart GW. Proteomic approaches to analyze the dynamic relationships between nucleocytoplasmic protein glycosylation and phosphorylation. Circ Res 2003;93:1047-58.
61 Roos MD, Su K, Baker JR, Kudlow JE. O-Glycosylation of an Sp1-derived peptide blocks known Sp1 protein interactions. Mol Cell Biol 1997;17:6472–80.
62 Goldberg HJ, Whiteside CI, Hart GW, Fantus IG. Posttranslational, reversible O-glycosylation is stimulated by high glucose and mediates plasminogen activator inhibitor-1 gene expression and Sp1 transcriptional activity in glomerular mesangial cells. Endocrinology 2006;147:222-31 Erratum in: Endocrinology 2006;147:5490.
63 Freund C, Schmidt-Ullrich R, Baurand A, Dunger S, Schneider W, Loser P, et al. Requirement of nuclear factor-kappaB in angiotensin II- and isoproterenol-induced cardiac hypertrophy in vivo. Circulation 2005;111:2319-25.
64 Umezawa K. Inhibition of tumor growth by NF-kappaB inhibitors. Cancer Sci 2006;97:990-5.
65 Kim YH, Song M, Oh YS, Heo K, Choi JW, Park JM, et al. Inhibition of phospholipase C-beta1-mediated signaling by O-GlcNAc modification. J Cell Physiol 2006;207:689-96.
66 Schipke JD, Friebe R, Gams E. Forty years of glucose-insulin-potassium (GIK) in cardiac surgery: a review of randomized, controlled trials. Eur J Cardiothorac Surg 2006;29:479-85.
67 Schaffer SW, Croft CB, Solodushko V. Cardioprotective effect of chronic hyperglycemia: effect on hypoxia-induced apoptosis and necrosis. Am J Physiol Heart Circ Physiol 2000;278:H1948-54.
68 Hunton DL, Lucchesi PA, Pang Y, Cheng X, Dell'Italia LJ, Marchase RB. Capacitative calcium entry contributes to nuclear factor of activated T-cells nuclear translocation and hypertrophy in cardiomyocytes. J Biol Chem 2002;277:14266-73.

69 Bush EW, Hood DB, Papst PJ, Chapo JA, Minobe W, Bristow MR, et al. Canonical transient receptor potential channels promote cardiomyocyte hypertrophy through activation of calcineurin signaling. J Biol Chem 2006;281:33487-96.

70 Buse MG, Robinson KA, Marshall BA, Hresko RC, Mueckler MM. Enhanced O-GlcNAc protein modification is associated with insulin resistance in GLUT1-overexpressing muscles. Am J Physiol Endocrinol Metab 2002;283:241–50.

71 Andreozzi F, D'Alessandris C, Federici M, Laratta E, Del Guerra S, Del Prato S, et al. Activation of the hexosamine pathway leads to phosphorylation of insulin receptor substrate-1 on Ser307 and Ser612 and impairs the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin insulin biosynthetic pathway in RIN pancreatic beta-cells. Endocrinology 2004;145:2845–57.

72 Park SY, Ryu J, Lee W. O-GlcNAc modification on IRS-1 and Akt2 by PUGNAc inhibits their phosphorylation and induces insulin resistance in rat primary adipocytes. Exp Mol Med 2005;37:220-9.

73 Chen G, Liu P, Thurmond DC, Elmendorf JS. Glucosamine-induced insulin resistance is coupled to O-linked glycosylation of Munc18c. FEBS Lett 2003;534:54–60.

74 Robinson KA, Ball LE, Buse MG. Reduction of O-GlcNAc protein modification does not prevent insulin resistance in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 2007;292:E884-90.

75 Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.

76 Daniels MC, Kansal P, Smith TM, Paterson AJ, Kudlow JE, McClain DA. Glucose regulation of transforming growth factor-alpha expression is mediated by products of the hexosamine biosynthesis pathway. Mol Endocrinol 1993;7:1041–8.

77 Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED. High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in procine glomerular mesangial cells. J Clin Invest 1998;101:160–9.

78 Weigert C, Brodbeck K, Sawadogo M, Haring HU, Schleicher ED. Upstream stimulatory factor (USF) proteins induce human TGF-beta1 gene activation via the glucose-response element-1013/-1002 in mesangial cells: up-regulation of USF activity by the hexosamine biosynthetic pathway. J Biol Chem 2004;279:15908–15.

79 Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest 2001;108:1341–8.

80 Matthews JA, Acevedo-Duncan M, Potter RL. Selective decrease of membrane-associated PKC-alpha and PKC-epsilon in response to elevated intracellular O-GlcNAc levels in transformed human glial cells. Biochim Biophys Acta 2005;1743:305-15.

81 Yang S, Zou LY, Bounelis P, Chaudry I, Chatham JC, Marchase RB. Glucosamine administration during resuscitation improves organ function after trauma hemorrhage. Shock 2006;25:600-7.

82 Champattanachai V, Marchase RB, Chatham JC. Glucosamine protects neonatal cardiomyocytes from ischemia-reperfusion injury via increased protein-associated O-GlcNAc. Am J Physiol Cell Physiol 2007;292: C178-87.

83 Roquemore EP, Chevrier MR, Cotter RJ, Hart GW. Dynamic O-GlcNAcylation of the small heat shock protein αB-crystallin. Biochemistry 1996;35:3578–86.

84 Wright DC, Geiger PC, Holloszy JO, Han DH. Contraction- and hypoxia-stimulated glucose transport is mediated by a Ca^{2+}-dependent mechanism in slow-twitch rat soleus muscle. Am J Physiol Endocrinol Metab 2005;288:E1062-6.

85 Bolli R. Preconditioning: a paradigm shift in the biology of myocardial ischemia. Am J Physiol Heart Circ Physiol 2007;292:H19-27.

86 Hausenloy DJ, Yellon DM. Survival kinases in ischemic preconditioning and postconditioning. Cardiovasc Res 2006;70:240-53.

87 Marshall PD, Poddar S, Tweed EM, Brandes L. Clinical inquiries: Do glucosamine and chondroitin worsen blood sugar control in diabetes? J Fam Pract 2006;55:1091-3.

88 Chen JT, Chen CH, Horng CT, Chien MW, Lu DW, Liang JB, et al. Glucosamine sulfate inhibits proinflammatory cytokine-induced ICAM-1 production in human conjunctival cells in vitro. J Ocul Pharmacol Ther 2006;22:402-16.

89 Forchhammer L, Thorn M, Met O, Gad M, Weidner MS, Claesson MH. Immunobiological effects of glucosamine in vitro. Scand J Immunol 2003;58:404-11.

90 Ma L, Rudert WA, Harnaha J, Wright M, Machen J, Lakomy R, et al. Immunosuppressive effects of glucosamine. J Biol Chem 2002;277:39343-9.

91 Chou TY, Hart GW. O-linked N-acetylglucosamine and cancer: messages from the glycosylation of c-Myc. Adv Exp Med Biol 2001;491:413-8.

92 Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, Kim YS, Cho JW. Modification of p53 with O-linked N-acetylglucosamine regulates p53 activity and stability. Nat Cell Biol 2006;8:1074-83.

93 Kanai M, Wei D, Li Q, Jia Z, Ajani J, Le X, et al. Loss of Kruppel-like factor 4 expression contributes to Sp1 overexpression and human gastric cancer development and progression. Clin Cancer Res 2006;12:6395-402.

94 Lefebvre T, Pinte S, Guerardel C, Deltour S, Martin-Soudant N, Slomianny MC, et al. The tumor suppressor HIC1 (hypermethylated in cancer 1) is O-GlcNAc glycosylated. Eur J Biochem 2004;271:3843-54.

95 Yang X, Zhang F, Kudlow JE. Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein O-GlcNAcylation to transcriptional repression. Cell 2002;110:69-80.