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The role of O-GlcNAc transferase in regulating the gene transcription of developing and failing hearts

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Abstract

Heart failure treatment currently centers on symptom management, primarily through reductions in systemic blood pressure and fluid retention. The O-linked attachment of β -N-acetylglucosamine to cardiac proteins is increased in cardiovascular disease and heart failure, and O-GlcNAc transferase (OGT) is the enzyme that catalyzes this addition. Deletion of OGT is embryonically lethal, and cardiomyocyte-specific OGT knockdown causes the exacerbation of heart failure. Stem cell therapy is currently a major focus of heart failure research, and it was recently discovered that OGT is intricately involved with stem cell differentiation. This article focuses on the relationship of OGT with epigenetics and pluripotency, and integrates OGT with several emerging areas of heart failure research, including calcium signaling.

Keywords

CaMKII; cardiac stem cells; epigenetics; heart failure; OGT; pluripotency

Current medical approaches to heart failure treatment primarily center on the management of deleterious sequelae. The reduction of systemic blood pressure and fluid retention are the major goals of patient care in the management of heart failure, as well as the maintenance of cardiac contractile function, and this is accomplished almost exclusively through pharmaceutical approaches. There are currently few therapies that specifically target the changes seen within cardiomyocytes themselves. This could be due to the fact that what is presently understood about the processes underlying cardiomyocyte remodeling, hypertrophy and function, as well as how these processes are changed with the progression to heart failure, remain poorly characterized. However, there are several emerging areas of research that suggest that targeting key regulators of cardiac gene transcription and/or stem cells could be more beneficial than the pharmaceutical treatments that are currently used in

clinical settings. Therefore, the aims of this article are to highlight recent discoveries that are challenging long-held conceptions of cardiomyocyte plasticity and to explore the exciting new therapeutic approaches that have been made possible by these findings.

A primer on O-GlcNAc transferase & protein O-GlcNAcylation

Myriad processes occur within cells via protein–protein interactions and enzyme activity. In the cardiomyocyte, the delicate coordination of protein actions is particularly important as these cells must not only contract continually, but also metabolize substrates, eliminate waste and remodel and adapt unceasingly from before birth until death. An important regulator of protein interactions and enzyme activity is that of post-translational modification (PTM) of proteins. The majority of PTMs are transient, reversible and labile, and they influence protein function, cell signaling, gene silencing and transcription without permanent alterations to the genome. Although many PTMs have been identified, there are an increasing number of studies demonstrating that enzymes that catalyze the attachment and removal of moieties to proteins (kinases, phosphatases, acetylases and ubiquitinases, among others) are themselves often modified, and this has added an increased level of complexity to this field of research. Of specific importance to this article is the interaction of protein phosphorylation and O-GlcNAcylation (reviewed in detail elsewhere [1]), as this is increasingly being reported to be a finely tuned mechanism in cells, but particularly in the cardiomyocyte.

Phosphorylation, the addition of a phosphate group to serine, threonine and tyrosine residues of proteins, is a very well-characterized process that changes the conformation of a protein, thus altering protein–protein interactions, enzyme activities, ion channels, and so on. A similar but relatively new field of study that is increasingly being recognized as an essential regulator of proteins is the O-linkage of a β -N-acetylglucosamine (O-GlcNAc) sugars to proteins; this is highly analogous to phosphorylation, as O-GlcNAc can compete for the same serine and threonine sites as phosphate [1]. O-GlcNAcylation is the final result of glucose being shunted through the hexosamine biosynthesis pathway and has been found to post-translationally modify over 1000 proteins to date, including several histone and histone-associated proteins [2]. Interestingly, however, while there are hundreds of kinases and phosphatases that control phosphate cycling on proteins, only O-GlcNAc transferase (OGT) catalyzes protein O-GlcNAcylation and only O-GlcNAcase removes the moiety. This process became further complicated when it was discovered that OGT, as well as many kinases and phosphatases, can be both O-GlcNAc modified and phosphorylated, sometimes competing for the same protein residue [3,4]. Therefore, while this article will present and discuss the increasing amount of evidence suggesting a pivotal role of OGT and O-GlcNAcylation in both the development and treatment of heart failure, specifically targeting this process for therapeutic purposes does present multiple technical challenges.

The gene encoding OGT resides on the X chromosome, has been highly conserved through evolution and is essential for mammalian development [5,6]. There are three distinct OGT protein subunits that are all encoded by the single OGT gene present in mammals, with each subunit serving a discrete function within the cell [7]. The longest OGT subunit is designated ncOGT as it is localized to both the nucleus and cytoplasm; this is the most well

characterized of the three subunits and the specific weight of the protein varies between 110 and 116 kDa in the literature [6,7]. The second subunit contains a mitochondrial targeting sequence, has a weight of approximately 103 kDa and has been designated mOGT. The final OGT subunit is the shortest, with a weight of approximately 78 kDa, and is termed sOGT. All subunits share a common catalytic domain; however, they vary in their N-termini and number of tetratricopeptide repeats, ranging from 2.5 for sOGT to 12.5 for the full-length ncOGT [6,7]. It should also be noted that the functional OGT enzyme is comprised of a trimer consisting of two 110-kDa subunits and one 78-kDa subunit [6].

Recent studies have reported that OGT plays a central role in the regulation of cardiac gene transcription, cell cycling and calcium handling, suggesting that cardiomyocyte OGT has the potential to be a powerful therapeutic target in heart failure. Furthermore, O-GlcNAc modification is both nutrient and stress sensitive and is an alternative pathway for glucose metabolism, while members of the kinase family are heavily influenced by ions such as calcium, as is the case with the CaMK family. Thus, the increasing evidence that O-GlcNAc and phosphate cycling are tightly controlled by enzyme PTMs subsequently led to several recent reports that further highlight the intricacies of calcium and glucose relationships in the muscle, and more specifically in cardiac muscle. Therefore, this article explores the relationships between OGT and calcium in the realm of pathological cardiac signaling, epigenetics and stem cell fate, and will identify key areas in which manipulation of OGT increasingly appears to be a promising new candidate for interventional and/or pharmaceutical therapies.

OGT is essential to the developing & failing heart

Although increased O-GlcNAc levels were originally implicated in the detrimental effects of hyperglycemia, particularly with diabetes, many studies are now demonstrating that O-GlcNAc is increased in settings of both acute and chronic cardiac and metabolic disease. For example, chronically increased protein O-GlcNAcylation has been specifically reported in diabetic cardiac dysfunction [8], western diet consumption [9], cardiac hypertrophy and heart failure [10]. Conversely, acute pharmacologically induced elevations in protein O-GlcNAcylation protect the heart against ischemia–reperfusion injury [11]. Taken together, these findings add a layer of complexity to the question of whether increases in protein O-GlcNAcylation are beneficial or detrimental to stress responses in the heart. Initial *in vitro* approaches utilizing siRNA in neonatal rat cardiomyocytes suggested that OGT was not only essential for cardiomyocyte survival against ischemia–reperfusion injury, but also that OGT was required for the translocation of the antiapoptotic protein Bcl-2 to the mitochondria [12]; however, it has been difficult to answer this question using mutagenesis models. OGT is the only enzyme that catalyzes the attachment of O-GlcNAc to proteins; this makes silencing transcription problematic, as OGT deletion in the germline is embryonically lethal and results in nonviable offspring [13]. Indeed, the specific role of OGT in the intact heart remained poorly understood until Watson and coworkers developed an inducible cardiomyocyte-specific OGT-knockdown mouse and examined the response to surgically induced myocardial infarction [14]. Mice were examined up to 4 weeks postoperation and an exacerbation of heart failure was observed in the OGT-knockdown mice versus their surgical control animals with concomitant postinfarct tissue remodeling (fibrosis and

apoptosis, among others). The importance of OGT in cardiac development became especially clear with subsequent studies from the same laboratory, which utilized a constitutive cardiomyocyte-specific OGT-knockdown mouse model. When cardiac OGT is absent in the embryonic and developing heart, a marginally better survival rate than the earlier germline studies is observed, although only a 12% survival rate up to 4 weeks of age occurs in this model [15]. Surviving animals are significantly smaller than wild-type littermates and have dilated hearts, overt cardiac dysfunction and ECG abnormalities. Therefore, it is clear that while cardiac O-GlcNAc is elevated in pathological conditions, OGT is essential to both cardiomyocyte development and the *in vivo* cardiac stress response. This is perhaps not surprising, as these findings are consistent with an earlier report that O-GlcNAc is required for noncardiac cell survival in response to various stressors [16].

As mentioned above, the cardiomyocyte is a unique contractile cell in that it must continually contract and relax and must also adapt its metabolic profile according to the available fuel source. This flexibility is essential during the switch from fetal development, in which the primary cardiac fuel is glucose oxidation, to postnatal cardiac maturity, in which the heart relies primarily on fatty acid oxidation; this topic is reviewed in depth by Bernardo and colleagues [17]. However, the failing heart reverts to the fetal phenotype both structurally (i.e., β -MHC to β -MHC and cardiac β -actin to skeletal β -actin) and metabolically (from fatty acid oxidation back to glucose oxidation); this switch is associated with decreased contractility, impaired cardiac function and an increase in mortality. Given the multitude of interactions and processes required in order to maintain consistent heart function, it is not surprising that cardiac OGT, a nutrient- and stress-responsive enzyme, is essential for the mammalian heart to reach maturation. Furthermore, the recently established relationship between OGT and the CaMK family, as well as the contractile dysfunction observed in cardiomyocytes lacking OGT, introduces the possibility of cardiac electrolyte (calcium, sodium and potassium) disturbances. Alterations in the cardiac cycle action potential ion exchange system are associated with ECG abnormalities, and it is reasonable to suspect the involvement of CaMKII in this process as well.

OGT & cardiac glucose & calcium

Until recently, the majority of O-GlcNAc research in the heart has been in the realm of Type 2 diabetes and hyperglycemia, primarily because UDP-GlcNAc, the substrate for O-GlcNAc, is a byproduct of glucose metabolism. For example, increases in glucose and protein O-GlcNAcylation are associated with cardiomyocyte dysfunction, excitation–contraction coupling abnormalities [18] and prolonged calcium transients [19]; however, blocking protein O-GlcNAcylation while preserving high-glucose conditions normalizes cardiomyocyte function [19]. Classical reasoning continued to dictate that any increases in protein O-GlcNAcylation must be secondary to increased flux through the hexosamine biosynthesis pathway. This logic persisted for several years, until Taylor and colleagues reported that O-GlcNAc attachment to proteins occurs in the complete absence of extracellular substrate, and that this mechanism is through an increase in OGT protein and pre-existing intracellular substrate [20]. While surprising at the time, these data actually compliment an earlier study that elegantly demonstrated upregulation of O-GlcNAc levels in

response to a variety of cellular stress conditions, such as exposure to heat and ultraviolet light radiation, independent of any changes in substrate availability [16].

Of specific interest to the O-GlcNAc field is the 2008 report by Song and colleagues, which identified phosphorylation of OGT by CaMKIV in brain somatic cells [4]. The following year, Dias and coworkers confirmed these findings and expanded upon this relationship by reporting that O-GlcNAc modification connects the hexosamine biosynthesis pathway and calcium signaling pathways via CaMKIV O-GlcNAcylation in HEK293A cells [21]. Although CaMKIV is not a predominant cardiac isoform, these were the first studies to link the hexosamine biosynthesis pathway with calcium signaling via CaMK, and since 2008, several other reports have linked hexosamine biosynthesis pathway flux to calcium signaling. For example, CaMKII, another CaMK isoform that is abundantly present in cardiac tissue, regulates cardiac action potential cycles through involvement with sodium, potassium and calcium channels (reviewed by [22]). CaMKII is not only O-GlcNAcylated, but the enzymatic activity of CaMKII has recently been linked to the extent of its O-GlcNAcylation [23].

Recent work in this area has demonstrated that CaMKII attenuates the increased protein O-GlcNAcylation observed with glucose deprivation [24]. This was not due to energy deficiency, because cell culture media were supplemented with pyruvate, a readily oxidized energy source for cardiomyocytes. It was further determined that O-GlcNAc modification of CaMKII is essential to the molecular memory process observed with CaMKII activation, which allows the enzyme to retain activity once intracellular calcium stores have been depleted [23]. This finding was strengthened by the concomitant discovery that CaMKII activity is increased in the presence of high glucose [23]. CaMK is not the only calcium-sensitive cell signaling process associated with cardiac O-GlcNAc levels; O-GlcNAc has also recently been identified to be a mediator of store-operated calcium entry (SOCE) in cardiac tissue [25]. This is an exciting finding and suggests that SOCE is inhibited in the presence of O-GlcNAcylated STIM1 in a dose-dependent manner. Collins and colleagues provide a detailed description of STIM1-mediated SOCE in a recent review [26], and we hypothesize that this may be a mechanism by which O-GlcNAc mediates calcium transients in cardiac tissue. Collectively, these findings confirm that cardiac O-GlcNAc and calcium signaling are heavily integrated and possibly interdependent (**Figure 1**). Given the complex nature of cardiomyocyte signaling, metabolism, contraction and remodeling, further elucidation of the connections between O-GlcNAc, glucose and calcium in the heart, as well as the impacts of these interactions, will be fundamental to the development of more effective therapies in clinical patients.

OGT & epigenetics/transcription

While PTMs have long been known to regulate cell signaling, it has only recently been determined that phosphorylation, methylation, acetylation and sumoylation modify histone proteins and impact transcription through regulating access of the transcriptional machinery to DNA [27,28]. In 1993, RNA polymerase II was identified to be a glycoprotein [29]; however, it took nearly 20 additional years to determine that O-GlcNAc modification of RNA polymerase II is involved with the initiation of transcription [30]. In the interim, a

groundbreaking study linking OGT with histone regulation was reported by Yang and coworkers in 2002, which clearly demonstrates that OGT interacts with mSin3A, a key chromatin repressor that modulates DNA compaction [31]. The authors further proposed that this targeting of OGT to mSin3A may trigger the disassembly of activation complexes in concert with histone deacetylases (HDACs), and that this may be a prime regulator of gene silencing. Interestingly, however, few studies emerged after these exciting data were published to further examine the role of OGT or O-GlcNAc in gene transcription. Indeed, it is only within the past few years that O-GlcNAc modification of histones has been investigated and recognized. In 2010, Sakabe and coworkers identified sites on histones H2A, H2B and H4 that are subject to O-GlcNAcylation [2], thus providing evidence of a direct role for OGT in the transcription process beyond the previously identified relationship between OGT and the mSin3A/HDAC complex. In addition, O-GlcNAc may play an important role in DNA repair, as a recent study reported that various proteins involved in DNA damage, nuclear transport and transcriptional regulation are O-GlcNAc modified [32]. Given the previously described relationship between protein phosphorylation and O-GlcNAcylation, as well as the role of OGT and O-GlcNAc in cytoprotection, it is perhaps not entirely surprising that Zachara and colleagues found that the methylation and acetylation state of histones may be regulated by O-GlcNAcylation following stress, and that O-GlcNAc assists in stabilizing DNA in response to toxic agents such as Bleocin and doxorubicin [32].

Contrary to its positive effect on gene transcription, O-GlcNAc has also recently been shown to negatively regulate the terminal differentiation of skeletal muscle through glycosylation of Mef2D, a transcriptional activator of myogenin [33]. Furthermore, we recently reported that increased O-GlcNAc levels are associated with a blunting of the cardiac hypertrophic responses [8]. Although this may at first appear to be counterintuitive, this supports previous findings that the class II HDAC4 represses hypertrophy in cardiac muscle through complexing with Mef2 target genes (reviewed in [34]), and CaMKII accomplishes the inverse to this signal through HDAC4 phosphorylation. As we have recently demonstrated O-GlcNAcylation of cardiac HDAC4 [35,36], it is not beyond the scope of possibility that the O-GlcNAcylation state of HDAC4 influences its association with transcriptional activators and serves to repress hypertrophy through this complex. Taken together, these findings are an extension of the process of reversion to fetal cardiac gene morphology and metabolism described earlier in this article, and these data implicate a deeper involvement of O-GlcNAc in the mediation of this process than previously appreciated. Further studies are required in order to further elucidate this mechanism; however, a large body of literature favorably supports this hypothesis (described in detail by Xie and Hill [37]).

Acetylation and methylation of histones confers an epigenetic signature that regulates the gene expression of cardiac hypertrophy by altering activity in promoter regions [38]. In light of recently published studies, O-GlcNAc has now been added to this epigenetic regulatory cascade, and has further been linked to the regulation of specific transcriptional activators and repressors, such as the previously described relationship with the transcriptional regulatory protein mSin3A. In addition to transcriptional activity, OGT has been identified as being crucial to cell cycle regulation as controlled by HCF1: this has been reviewed in

depth by Hanover and colleagues [39]. Despite these advances in our understanding, the relationships between transcriptional activation and repression are proving to be much more complex than previously appreciated. However, a 2009 study by Sinclair and coworkers made great strides in the world of OGT and epigenetics when it was found that OGT may play a direct role in developmental regulation and stem cell maintenance through mediation of the super sex combs gene of the polycomb group [40].

Indeed, intriguing recent studies suggest that OGT is indeed pivotal to transcription when examined in conjunction with TET enzymes. The TET family includes TET1, TET2 and TET3 and regulates gene transcription through the conversion of 5-methylcytosine to 5-hydroxymethyl-cytosine (reviewed by [41]). Each TET enzyme is believed to have discrete functions and to coordinate different processes of gene transcription, and it is interesting that OGT interacts directly with TET2 [42]. TET1 and OGT also interact at transcription start sites (TSSs), suggesting that 5-hydroxymethylcytosine levels are regulated through the stabilizing interaction of OGT with TET1 and that the recruitment of OGT to chromatin is mediated via TET1 [43]. Recent work by Vella and coworkers confirms the existence of stable complexes containing TET1/OGT and TET2/OGT, and that these complexes also include mSin3A and HDAC1 [44]. This study also demonstrates that, while OGT recruitment preferentially occurs at the TSSs of upregulated genes and would theoretically inhibit transcription, several other genes are downregulated upon loss of OGT at TSSs. These seemingly paradoxical data suggest that OGT may both negatively and positively influence transcription. Nevertheless, these recent studies are very exciting as they not only complement the initial report by Yang and colleagues [31], they also provide further evidence that OGT is pivotal in the regulation of histone compaction and gene transcription.

OGT & pluripotency/embryonic stem cells

Contrary to the long-held notion that adult cardiomyocytes are terminally differentiated and incapable of extensive remodeling, postnatal cardiomyocytes of mammals are actually capable of substantial plasticity [45]. Following cardiac injury, such as that induced by hypertension or pressure overload, cardiomyocytes return to an embryonic gene expression or fetal gene program [46]; this is particularly evident in the pressure-overloaded heart, such as that seen with hypertension [17]. Interestingly, these 'de-differentiated' myocytes are not apoptotic in continuous culture, in spite of significant morphological changes [45]; in fact, reactivation of the fetal gene program triggers compensatory remodeling and growth in the cell. A potential regulator of this process is CaMKII, as knockdown of CaMKII protects the heart against pressure overload-induced hypertrophy, allows HDAC4 to remain in nuclear complex (thereby preventing Mef2-induced transcription) and also suppresses apoptosis of cardiomyocytes [47,48]. While this has not been conclusively demonstrated, **Figure 1** proposes a mechanism by which CaMKII signaling and O-GlcNAc modification may regulate the already well-characterized HDAC4 cardiac hypertrophic signaling cascade [49–52]. Another potential contributor is the progenitor cell marker c-kit, as re-expression is evident with more extensive de-differentiation of mammalian cardiomyocytes, beyond that of the previously described zebrafish de-differentiation process [53]. Furthermore, rodent cardiomyocyte de-differentiation is characterized by losses of cardiomyocyte morphology, cardiac filament protein and transcripts and electrophysiological properties [45]. De-

differentiated cells become antigenically and morphologically similar to cardiac progenitor cells, which is due, at least in part, to upregulation of inflammatory oncostatin M (OSM). A member of the IL-6 family of proinflammatory cytokines [54], OSM has been independently implicated in the return to the cardiac fetal gene program. In addition, OSM is associated with irregular arrangements of contractile cardiac proteins, a significant reduction in cardiac myofilaments and a nearly complete loss of striated muscle patterns [54]. Finally, *in silico* modeling using the YinOYang server [55,56] suggests that OSM can be reciprocally modified by both O-GlcNAcylation and phosphorylation at multiple sites. Taken together, these findings suggest a potentially deeper control of de-differentiation by PTM cycling than we currently know.

Embryonic stem (ES) cells have become increasingly popular in recent years as potential therapeutic treatments for damaged heart tissue, due to the fact that human cardiomyocytes only have an annual renewal rate of between 0.45 and 1.00% [57]. However, the roles of PTMs in ES cell differentiation and development, and the potential regulation of these cells via these processes, have only recently begun to be examined. In terms of the potential role of O-GlcNAc in cardiac ES cell applications, it is interesting to note that protein O-GlcNAc modification decreases in ES cells as they advance through the differentiation process [58]. This appears to be primarily due to a decrease in OGT expression to approximately 60% of the baseline of OGT by day 7 of differentiation. This corresponds to the time at which cardiac embryoid bodies begin to contract spontaneously and is also associated with concomitant expression of β -MHC protein in embryoid bodies. Interestingly, a cardiac-specific isoform of CaMKII is detected in ES cells only after 7–10 days of differentiation [59] and constitutively active CaMKII rescues dysfunctional cardiac stem cell (CSC) differentiation in a mutated myosin light-chain cell model [60]. While these separate findings indicate a time-dependent linkage between OGT and CaMKII expression during differentiation, no causative links have been identified. **Figure 1** illustrates what is currently understood regarding OGT and CaMKII signaling in HDAC4-dependent cardiac hypertrophy signaling, while **Figure 2** is a proposed schematic of a balance between OGT and CaMKII. However, to our knowledge, these enzymes have not yet been concurrently examined, and further investigation is required in order to confirm this mechanism. Due to the requirement for calcium cycling in order to regulate cardiomyocyte contractions (recently reviewed in depth elsewhere [61]), an important extension of this study would be to examine protein levels of CaMKII and SOCE/STIM1 at the time of OGT loss in ES cells, as this may further explain the loss of OGT protein at ES cell differentiation day 7 in cardiomyocytes [58].

O-GlcNAc has also been implicated in the regulation of the endogenous transcription factors involved in the coordination of the pluripotency network. Partial knockdown of OGT reduces the self-renewing capacity of ES cells, whereas pharmacological upregulation of O-GlcNAc inhibits the normal differentiation process [43]. This appears to be via O-GlcNAc-mediated activation of Oct4 and Sox2, two core components of pluripotency. Briefly, in undifferentiated ES cells, Oct4 is O-GlcNAc modified and serves to upregulate several genes that maintain ES cell identity. However, once cells differentiate, O-GlcNAc detaches and the expression of pluripotency-related genes targeted by Oct4 decreases. Therefore, this

suggests a possible mechanism for the reported observation that O-GlcNAc and OGT levels decrease upon differentiation [58]. Despite these findings, it is not known whether OGT and O-GlcNAc are actual mediators of ES cell development and pluripotency, nor whether the changes that are reported are downstream products of these processes.

As described above, several research groups are currently investigating the influence of OGT on ES cells and transcription via pharmacological up/downregulation of OGT and/or O-GlcNAcase, or via siRNA. While these studies are reporting interesting data with regards to the effects of O-GlcNAcylation on transcription, a recent report has shown that site-directed mutagenesis of a TET protein can inhibit O-GlcNAcylation [62]. Specifically, site-directed mutagenesis of TET1 T535A in ES cells results in decreases in not only TET1 protein O-GlcNAcylation, but also decreases in total TET1 protein. These findings are important as they indicate that not only are TET1 protein levels regulated by the O-GlcNAcylation status of TET1, but also that 5-hydroxymethylcytosine modification and repression of TET1 are controlled by OGT. Furthermore, two separate OGT siRNAs decreased total TET1 protein levels by at least 70% in ES cells, and this was almost as effective as TET1 knockdown; these data support previous findings regarding OGT's involvement in TET1 protein stability. It is also important to note that OGT depletion induced the differentiation of ES cells, and this is accompanied by reduced enrichment of 5-hydroxymethylcytosine on TET1 target genes [62]. There is also recent evidence that limiting O-GlcNAc modification of CSCs increased cell death following exposure to hypoxia, and that pharmacologically increased O-GlcNAcylation was protective; of note is the fact that protein O-GlcNAcylation increases in CSCs in a time-dependent manner following hypoxia-reoxygenation [63]. This demonstrates that adult mammalian CSCs require O-GlcNAc PTM for their recovery from acute hypoxic injury and suggests potential for pharmacologic O-GlcNAc modulation as a clinical therapeutic agent following acute cardiac injury; **Figure 3** is a proposed schematic of how this process may occur from a macroscopic level.

Conclusion

The involvement of O-GlcNAc modification of cardiac proteins via OGT has been clearly demonstrated in the realms of pathology, cell survival, epigenetics and gene transcription. As most of these findings have only recently been reported, this article is timely in that we have clearly described a deeper role for OGT in these processes than previously appreciated. Future research in the field of protein-protein interactions is essential, as the current body of literature suggests that no single cell signaling pathway or PTM is able to induce disease or confer health. In addition, over 1000 proteins have been identified to be O-GlcNAc modified to date; however, we have specifically focused on the relationship between OGT and CaMKII in the heart for the present article. Multiple interactions have been correlated here, but further studies are essential for the identification of causative relationships. We do anticipate, however, that further investigation of OGT and CaMKII will provide important insights into the field of cardiac pathology, and that pharmacological or gene therapies targeting these enzymes could provide groundbreaking progress in the treatment of heart failure.

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EXECUTIVE SUMMARY

A primer on O-GlcNAc transferase & protein O-GlcNAcylation

- O-GlcNAc transferase (OGT) attaches GlcNAc, the final product of the hexosamine biosynthesis pathway, to serine and threonine residues of proteins via an O-linkage.
- OGT and phosphokinases have been linked with cardiac signaling, epigenetics and stem cell differentiation.

OGT is essential to the developing & failing heart

- OGT and the CaMK family of proteins are post-translationally modified by O-GlcNAc and phosphate.
- OGT deletion in the germline is embryonically lethal.
- OGT and CaMKII may cooperate in order to control cardiac cycle action potentials and therefore cardiac contractility.

OGT & cardiac glucose & calcium

- O-GlcNAc modification controls the activity of CaMKII.
- CaMKII attenuates the increases in protein O-GlcNAcylation observed in glucose deprivation and may be involved in the cellular stress response.
- Cardiac O-GlcNAc and calcium signaling are heavily integrated and possibly interdependent.

OGT & epigenetics/transcription

- OGT is involved with epigenetics and transcription at multiple levels, including histone compaction and DNA repair.

OGT & pluripotency/embryonic stem cells

- Postnatal cardiomyocytes are capable of substantial plasticity.
- De-differentiated cardiomyocytes are not apoptotic.
- OGT expression decreases in differentiating cardiac stem cells (CSC) at approximately day 7, corresponding to the time at which embryoid bodies begin to contract spontaneously in culture.
- CaMKII is detected starting at day 7 of CSC differentiation.
- OGT and CaMKII may play opposing roles in the progression of CSC differentiation.

Conclusion

- O-GlcNAc modification of cardiac proteins via OGT has been clearly demonstrated in the realms of pathology, cell survival, epigenetics and gene transcription.

- Investigation of enzyme interactions will be pivotal to moving the field of heart failure research into the next stages.
- Pharmacological or gene therapies targeting these enzymes could provide groundbreaking progress in the treatment of heart failure.

Future perspective

Within the next 5–10 years, we anticipate the following advancements in the field of treating cardiac pathologies:

- Pharmacologic therapies will become individualized to specific organs, such as the heart, through cell- and isoform-specific drug development;
- The efficacy of CSC therapies will be improved via preimplantation treatment of embryoid bodies with the induction of OGT up to day 7 and subsequent triggering of CaMKII activity;
- Heart failure may become treatable and reversible through the specific targeting of processes within the cardiomyocytes, rather than managing a collection of symptoms.

When examined in the realm of both pathological and physiological cardiac remodeling, CaMKII and OGT appear to be important targets; we, and others, are further investigating this from a molecular, genetic and mechanistic perspective [23,35,36].

Figure 1. The role of O-GlcNAc in the activation of CaMKII and subsequent cardiac hypertrophy

(A) O-GlcNAcylation of CaMKII stabilizes CaMKII in an open conformation, induces the molecular memory that permits autophosphorylation of CaMKII and allows for activity to continue after the depletion of calcium stores. (B) Once O-GlcNAcylated, CaMKII phosphorylates itself as well as transcriptional proteins, including HDAC4. (C) Phosphorylated HDAC4 dissociates from nuclear complex-containing transcription factors and hypertrophy occurs.

Figure 2. Regulation of cardiac stem cell differentiation by O-GlcNAc transferase and CaMKII
(A) Approximately 7 days into the differentiation of CSCs, the high baseline levels of OGT protein decrease and CaMKII levels become detectible; this coincides with the spontaneous contraction of embryoid bodies and the organization of contractile proteins. **(B)** Therefore, the progression of differentiation appears to be regulated by the precise coordination between OGT and CaMKII [58,59]. OGT: O-GlcNAc transferase.

Figure 3. Proposed schematic of the process by which O-GlcNAc transferase appears to inhibit cancer stem cell differentiation

OGT forms a complex with TET1 at the transcriptional start sites of genes, which catalyzes the hydrolysis of 5mC to 5hmC, induces DNA methylation, decreases transcription, causes gene silencing and decreases the differentiation of cancer stem cells. Downregulation or a decrease in the protein levels of OGT and the subsequent decrease in the formation of the OGT-TET1 complex therefore blocks this process, which allows for transcription to remain active and cancer stem cells to continue to differentiate.

5hmC: 5-hydroxymethylcytosine; 5mC: 5-methylcytosine; ES: Embryonic stem; OGT: O-GlcNAc transferase.