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Key Words: O-GlcNAc | intracellular glycosylation | signal transduction | proteomics | mass spectrometry | diabetes mellitus | infarction | glycoproteins | methods
Defining the Heart and Cardiovascular O-GlcNAcome: A Review of Approaches and Methods
Venkata D.P. Paruchuri and Natasha E. Zachara

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Proteomics

Defining the Heart and Cardiovascular O-GlcNAcome
A Review of Approaches and Methods

Venkata D.P. Paruchuri, BTech, MSE; Natasha E. Zachara, BTech, PhD

Recently, the modification of nuclear, cytoplasmic, and mitochondrial proteins by O-linked β-N-acetylglucosamine (O-GlcNAc) has emerged as a novel regulator of protein function that is relevant to heart and cardiovascular function. Elevated O-GlcNAc levels on several proteins are associated with diabetic cardiomyopathy and the development of atherosclerotic plaques. In contrast, in response to numerous forms of cellular injury, including models of ischemic preconditioning, the O-GlcNAc modification is elevated. Elevating O-GlcNAc levels before, or immediately after, injury is protective in both in vivo and in vitro models of ischemia-reperfusion injury. The proteins modified by O-GlcNAc, and how this alters their function, leading to these phenotypes have yet to be defined at a molecular level. One challenge is identifying proteins that are modified by O-GlcNAc, especially those that are dynamically modified by O-GlcNAc in heart and cardiovascular tissue. In this review, we will assess high-throughput methods available for identifying O-GlcNAc–modified proteins and mapping O-GlcNAc modification sites.

The modification of nuclear, cytoplasmic, and mitochondrial proteins by monosaccharides of O-linked β-N-acetylglucosamine (O-GlcNAc) was first reported in 1984.1,2 Recent proteomic screens suggest that >1000 proteins in the cell are modified by O-GlcNAc3–10 and that these O-GlcNAc–modified proteins fall into many functional groups, including transcription factors and poly-merases, cytoskeletal proteins, kinases and phosphatases, metabolic enzymes, ribosomal proteins, proteasomal pro- teins, and viral proteins.3–10 Not surprisingly, deletion of the O-GlcNAc transferase (OGT), the enzyme that adds O-GlcNAc, is lethal in mice at E4.5 and in isolated embryonic fibroblasts.11,12

O-GlcNAc is thought to regulate protein function in a manner analogous to protein phosphorylation,13 and like protein phosphorylation, levels of the O-GlcNAc protein modification, or O-GlcNAcylation, respond to numerous signals, including cellular damage, such as that caused by ischemia-reperfusion injury.13,14 Importantly, for a subset of proteins, O-GlcNAc and phosphorylation appear to be reciprocals, because the site of O-GlcNAcylation and phosphorylation is the same or, alternatively, the sites are proximal to each other.13 Supporting a model in which proteins are dynamically cycled between O-GlcNAcylated and phosphorylated states, the O-GlcNAc transferase is found in a complex with protein phosphatases.13,15

Unlike protein phosphorylation, the addition and removal of O-GlcNAc is catalyzed by just 2 enzymes, the O-GlcNAcase (removes O-GlcNAc) and the O-GlcNAc transferase (adds O-GlcNAc).13 The high-energy sugar donor used by OGT is UDP-GlcNAc. A fraction (2–5% in adipocytes) of glucose imported into the cell is converted to UDP-GlcNAc through the hexosamine biosynthetic pathway; as such, O-GlcNAc is thought to be an effector of glucose, as well as glutamine and glucosamine (Figure 1).13 These observations, and other studies, have led to the idea that elevated levels of O-GlcNAc are involved in mediating some of the complications associated with type 2 diabetes mellitus.16–18 Several studies have associated increased levels of O-GlcNAc with cardiac dysfunction in diabetes, including reduced mitochondrial function19 and altered calcium cycling by Serc2A.20,21 In a series of elegant studies, Dillmann and coworkers showed that reducing O-GlcNAc levels by overexpression of the O-GlcNAcase reverses some of the cardiac phenotypes associated with diabetes.19–21 Although recent studies22,23 suggest that the role of O-GlcNAc in mediating the complications associated with type 2 diabetes may be more complicated than originally postulated.

In contrast to the data previously discussed, O-GlcNAc plays positive roles in heart and cardiovascular models, in particular during ischemia-reperfusion injury (Ngoh et al24 and references therein). In 2004, O-GlcNAcylation was reported to be induced in response to numerous forms of cellular stress and the elevated levels of O-GlcNAc observed after stress were prosurvival.25 These data have been recapitulated in several cardiac models of ischemia-reperfusion injury, including neonatal cardiomyocytes treated with either H2O2 or hypoxia,26–28 or after coronary artery occlusion of hearts in vivo and in vitro.27,29–34 Importantly, elevating the levels of O-GlcNAc during reperfusion alone protects cells, suggesting that elevating O-GlcNAc levels may be clinically relevant.32

Defining the O-GlcNAcome

To fully understand the mechanism(s) by which O-GlcNAc promotes heart and cardiovascular survival and contributes to...
cardiac dysfunction in models of type 2 diabetes, defining the O-GlcNAcome and how this is altered in these conditions is critically important. How do we define the O-GlcNAcome? A proteome is defined as the entire set of proteins expressed by a genome, cell, tissue, or organism at a given time under a defined condition. Thus, an O-GlcNAcome is the set of proteins that are modified by O-GlcNAc at a given time under a defined condition and, more specifically, would also identify at which sites, and to what extent, a protein was modified by O-GlcNAc. Identifying proteins that are modified by O-GlcNAc, the first step in defining an O-GlcNAcome, in any cell or tissue is challenging for several reasons: (1) modification of a protein by O-GlcNAc does not alter the protein's isoelectric point and rarely alters the protein's molecular weight, as assessed by SDS-PAGE; (2) the O-GlcNAc modification is chemically labile and is rapidly released on exposure to a mild base or acid; (3) unless inhibited, the O-GlcNAcase and the lysosomal hexosaminidases, HexA and HexB, rapidly cleave the O-GlcNAc modification during protein extraction; (4) in most mass spectrometers, the O-GlcNAc modification is labile; and (5) peptides modified by O-GlcNAc are suppressed during mass spectrometry (MS) (ie, even if the O-GlcNAc–modified peptide is the major species in the spectrum, the signal will be much lower than that of unmodified peptides).

The major challenge in mapping sites of O-GlcNAc modification by MS is the O-glycosidic bond, which is broken during collision-induced dissociation (CID) or collision-activated dissociation (CAD), the step at which peptides are fragmented to provide sequence information. Orbitraps, which facilitate MS^n, became popular because peptides could be fragmented after O-GlcNAc has been ionized, providing sequence information and, at low efficiency, the O-GlcNAc modification sites. An alternative to these techniques has emerged in the past 5 years; both electron-capture dissociation (ECD) MS and electron-transfer dissociation (ETD) MS appear well suited to mapping posttranslational modifications, including O-GlcNAc.

Mass spectrometry is not inherently quantitative because of this multiple techniques have been developed that allow researchers to probe the differences in protein expression or the stoichiometry of posttranslational modifications in different samples. The advantage of these techniques is that they allow a researcher to ask the following questions: Which proteins, peptides, or sites are O-GlcNAc modified in response to ischemic preconditioning? On which proteins is O-GlcNAc differentially regulated in models of type 2 diabetes? The readers are referred to recent reviews that discuss these techniques in detail. Some of the most common techniques, which are compatible with enriching O-GlcNAc–modified proteins/peptides, include the following: (1) Multiple-/single-reaction monitoring: deuterium/chemically labeled standard peptides/glycopeptides are used to quantify the absolute concentration of peptides/glycopeptides in test samples. Thus, it is necessary to have previously identified glycopeptides/peptides of interest. (2) Stable isotope labeling of amino acids in cell culture (SILAC): different nonradioactive isotopes (13C, 15N, 2H) of amino acids are used to label different cell populations, allowing proteins/peptides from different treatments to be differentiated and quantified in mass spectra. This technique is ideal for cell culture, although it requires 6 passages of cells for complete incorporation of
the isotope. Recently, this method has been adapted to whole animals, although the cost is prohibitive. (3) Isobaric tags for relative and absolute quantification (iTRAQ): tags react with either the N-terminus of peptide or lysine residues and contain a reporter ion group and a mass balance region. During CID/CAD, the reporter ion is released, providing quantification. Currently, iTRAQ is the most amenable technique for labeling peptides released from tissues, such as the heart. In addition, iTRAQ can be used to quantify up to 8 samples concurrently; as such, it allows

the most flexibility as several replicates or treatments can be tested.

### Enrichment of O-GlcNAc–Modified Proteins and Peptides

The main challenge in studying the O-GlcNAc modification is enriching proteins or peptides that are O-GlcNAc modified for analysis. If the focus is on O-GlcNAc modification sites, then it is ideal to enrich glycopeptides because of the

---

**Table 1. Strategies for Enriching O-GlcNAc–Modified Proteins and Peptides**

<table>
<thead>
<tr>
<th>Method</th>
<th>Brief Description</th>
<th>Reagents Commercially Available</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Quantitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEMAD₉,₁₀</td>
<td>β-Elimination of amino acids (serine/threonine), followed by the addition of DTT; thiol affinity chromatography is used to enrich peptides</td>
<td>Yes</td>
<td>Can be used to map both phosphorylation and glycosylation sites; replaces the β-O-glycosidic with a more stable bond</td>
<td>Inherently destructive; requires extensive controls to differentiate phosphorylation and O-GlcNAcylolation; serines are more susceptible to β-elimination than threonine</td>
<td>Yes; deuterated DTT</td>
</tr>
<tr>
<td>Staudinger ligation⁵⁵</td>
<td>Metabolic labeling with N-acetylglucosamine; azide group can be derivatized with a variety of chemical tags (eg, biotin)</td>
<td>Yes (Invitrogen)</td>
<td>Low chance of nonspecific labeling; ideal for identifying proteins/peptides modified by O-GlcNAc</td>
<td>Site mapping is challenging; reagents are expensive; effect of azido sugars on cellular metabolism has not been fully studied</td>
<td>Possible; SILAC</td>
</tr>
<tr>
<td>Mutated GalT₂⁸⁹,₆–₄,₅₄</td>
<td>A mutant GalT is used to add an unnatural UDP-GalNAc, UDP-GalNAz, which can be derivatized with biotin</td>
<td>Some</td>
<td>Provides direct evidence of O-GlcNAc modification; nondestructive technique; can be used to study the interplay between phosphorylation and glycosylation</td>
<td>Limited by the ability of GalT to modify all terminal GlcNAc residues; possibly to modify N-linked glycans, in particular single GlcNAc residues bound to peptides through an amide bond</td>
<td>Yes; SILAC, iTRAQ, other deuterated labels</td>
</tr>
<tr>
<td>Galactosyltransferase¹,₅₀,₅¹</td>
<td>GaIT is used to transfer galactose to terminal GlcNAc residues; proteins and peptides are enriched by RCA1 affinity chromatography</td>
<td>Yes</td>
<td>Provides direct evidence of O-GlcNAc modification; useful for other biochemical analyses of O-GlcNAc</td>
<td>Limited by the ability of GaIT to modify all terminal GlcNAc residues; possible to modify N-linked glycans, in particular single GlcNAc residues bound to peptides through an amide bond</td>
<td>Possible; SILAC, iTRAQ</td>
</tr>
<tr>
<td>Lectins³,₅₁,₅₆</td>
<td>Binding of O-GlcNAc moeity to lectins is exploited</td>
<td>Yes</td>
<td>Inexpensive technique that can be readily coupled with Western blotting and 2D gel electrophoresis for preliminary testing</td>
<td>False positives possible; not all O-GlcNAc–modified proteins/peptides may be enriched; affinity to O-GlcNAc–modified peptides is weak</td>
<td>Possible; SILAC</td>
</tr>
<tr>
<td>Antibodies⁴⁶,₄₇,₅⁷,₅₈</td>
<td>Binding to O-GlcNAc–modified proteins is exploited</td>
<td>Most (see Table 2)</td>
<td>Inexpensive technique that can be readily coupled with Western blotting</td>
<td>False positives may occur because of protein-protein interactions; not all O-GlcNAc–modified proteins/peptides may be enriched</td>
<td>Yes; SILAC, iTRAQ</td>
</tr>
<tr>
<td>Periodate⁵⁹</td>
<td>The O-GlcNAc moeity is periodate oxidized and then captured by hydrazine resin; modified peptides are released by hydroxylamine cleavage</td>
<td>Yes</td>
<td>Might help in identification of a different peptide population, thereby complimenting other techniques</td>
<td>N-terminal serine and threonine are also oxidized by periodate treatment; this method also enriches other glycans</td>
<td>Possible, SILAC</td>
</tr>
<tr>
<td>Lectin weak-affinity chromatography⁷</td>
<td>Exploits the weak affinity between GlcNAc and WGA</td>
<td>Yes</td>
<td>Enriches O-GlcNAc–modified peptides directly without chemical derivatization</td>
<td>Specificity is an issue; complex carbohydrates with terminal GlcNAc or sialic acid can also be bound</td>
<td>Yes; SILAC, iTRAQ</td>
</tr>
</tbody>
</table>

DTT indicates Dithiothreitol.
suppression issues previously discussed. There are numerous techniques, and the choice is dependent on the equipment that is readily available to the researcher and on the goals of the project. Table 1 summarizes the techniques available and highlights their advantages and disadvantages, and Figure 2 highlights the general strategy and possible points of labeling with iTRAQ, SILAC, or deuterated labels (2H label, BEMAD, GaIT), are highlighted by white arrows. A. The strategy when enriching O-GlcNAc-modified proteins before identification and site-mapping. B. The strategy when enriching O-GlcNAc-modified peptides before identification and site-mapping.

Enrichment of O-GlcNAc–Modified Proteins Using Lectins and Antibodies

There are numerous antibodies and lectins that recognize either the O-GlcNAc modification, or terminal GlcNAc residues, that can be used to enrich O-GlcNAc–modified proteins by traditional immunoprecipitation (IP) methods (Table 2). However, only wheat germ agglutinin has been used successfully to purify glycopeptides. Any technique that uses antibodies, or lectins, that are only specific for terminal GlcNAc residues will encounter issues from pep-

tides/proteins bearing prototypical glycans; as such, it is advantageous to use proteins isolated from nuclear/cytoplasmic preparations. The main disadvantage of this technique is that proteins that interact with O-GlcNAc–modified proteins are also pulled down; as a result, the O-GlcNAc modification status of proteins of interest must be confirmed by independent techniques.

The low affinity of many O-GlcNAc–specific antibodies and lectins, as well as flexibility in peptide structure, precludes the broad application of lectins/antibodies for the purification of most O-GlcNAc–modified peptides by immunoprecipitation. To overcome these obstacles, Vosseller and coworkers hypothesized that using weak lectin affinity chromatography may be useful. Herein, glycopeptides are retarded, rather than retained, on a long column on which the GlcNAc-specific wheat germ agglutinin is immobilized. Vosseller and coworkers identified >145 O-GlcNAc–modified peptides using this technique; when combined with ECD-MS/MS, they mapped several novel O-GlcNAc modification sites. The main disadvantage is the size of the column required (12 m) that is currently not commercially available.

BEMAD

O-glycans can be removed from proteins by β-elimination, which typically results in serine or threonine being converted into their dehydrated equivalents (dehydroalanine and α-amino butyric acid, respectively). Wells and coworkers used the labile nature of O-GlcNAc to develop conditions in which O-GlcNAc could be β-eliminated preferentially to complex glycans and protein phosphorylation. To mark the site of O-GlcNAc modification, the α/β-unsaturated carbonyl is derivatized with either DTT or biotin pentylamine. This results in a more stable chemical bond and can be used to enrich the peptides by thiol or streptavidin affinity chromatography, respectively. The β-Elimination, followed by Michael Addition, of DTT has been termed BEMAD. One advantage of this technique is that it can be adapted to map phosphorylation sites, allowing researchers to probe the complex interplay between O-GlcNAc and phosphorylation. Moreover, by using density-labeled DTT, it is possible to perform quantitative proteomics. Last, 1 significant advantage of this technique is that it can be performed on most mass spectrometers, rather than relying on ETD/ECD technology. Limitations include the following: (1) glycosylation sites flanked by prolines are particularly problematic because of peptide cleavage; (2) phosphopeptide and glycopeptide controls are crucial using this technique, because both O-GlcNAcylation and phosphorylation β-eliminate; (3) the technique appears to work best on enriched glycoprotein populations; and (4) alkylated cysteine residues are modified by BEMAD and, as such, it is possible to enrich peptides that are not phosphorylated or glycosylated.

Enrichment Technologies Based on Galtransferase

Bovine milk UDP-Gal:GlcNAc β1 to 4 galactosyltransferase (GalT) will transfer a galactose residue from UDP-galactose to any terminal GlcNAc residue, resulting in LacNAc (Gal β1–4-GlcNAc) and has been used extensively for the detec-
diagnostic ion are released are subjected to further analysis. This method is ideal for identifying modified peptides at subfemtomole concentrations from complex mixtures.50

**GalT** and Ketones

Khidekel and coworkers used a mutant GalTY289L, with an enlarged binding pocket,52 to derivatize O-GlcNAc with an unnatural UDP-galactose containing a ketone moiety.53 The ketone can be derivatized with an aminooxy-biotin that can be detected by streptavidin-Horse Radish Peroxidase (HRP)53 or enriched by streptavidin affinity chromatography.7 The latter facilitates the enrichment of O-GlcNAc–modified peptides and has been successfully used to identify proteins from both cell and tissue lysates, including low-abundance transcription factors.6,7 Although the ketone-biotin tag facilitates the identification of glycopeptides, because of the unique fragmentation pattern, mapping glycosylation sites appears more challenging because of the lability of the β-O-glycosidic bond. To overcome these limitations, this technique has been successfully combined with BEMAD and ETD-MS to map O-GlcNAc modification sites.6,7 More important, in a recent study,7 the addition of deuterated NaCNBH3 (NaCNBD3) has been incorporated to facilitate quantification of O-GlcNAc levels from 2 different tissue populations. The mutant GalT also tolerates UDP-GalNAz, which can be derivatized using Click-it chemistry (Invitrogen). This facilitates both the detection and enrichment of O-GlcNAc–modified proteins and peptides.

**Chemienzymatic Tagging With Photocleavable Linkers**

Peptides are labeled with GalNAz using GalTY289L and UDP-GalNAz. The GalNAz is derivatized with an alkene

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**Table 2. Antibodies and Lectins That Recognize O-GlcNAc or GlcNAc**

<table>
<thead>
<tr>
<th>Name</th>
<th>Antibody/Lectin*</th>
<th>Recognizes</th>
<th>Commercially Available</th>
<th>IP</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTD110.6</td>
<td>Antibody (IgM)</td>
<td>O-GlcNAc</td>
<td>Pierce, Sigma-Aldrich, Covance</td>
<td>Yes†</td>
<td>47</td>
</tr>
<tr>
<td>RL2</td>
<td>Antibody (IgG)</td>
<td>O-GlcNAc</td>
<td>Abcam, Affinity Bioreagents, Santa Cruz Biotechnology</td>
<td>Yes</td>
<td>60</td>
</tr>
<tr>
<td>MY95</td>
<td>Antibody (IgG)</td>
<td>O-GlcNAc</td>
<td>?</td>
<td>?</td>
<td>57</td>
</tr>
<tr>
<td>18B10.C7(3)</td>
<td>Antibody (IgG)</td>
<td>O-GlcNAc</td>
<td>Millipore</td>
<td>Yes</td>
<td>46</td>
</tr>
<tr>
<td>9D1.E4(10)</td>
<td>Antibody (IgG)</td>
<td>O-GlcNAc</td>
<td>Millipore</td>
<td>Yes</td>
<td>46</td>
</tr>
<tr>
<td>1F5.D6(14)</td>
<td>Antibody (IgG)</td>
<td>O-GlcNAc</td>
<td>Millipore</td>
<td>Yes</td>
<td>46</td>
</tr>
<tr>
<td>6D93</td>
<td>Antibody (IgG)</td>
<td>O-GlcNAc</td>
<td>Santa Cruz Biotechnology</td>
<td>?</td>
<td>SantaCruz</td>
</tr>
<tr>
<td>10D8</td>
<td>Antibody (IgM)</td>
<td>O-GlcNAc</td>
<td>Santa Cruz Biotechnology</td>
<td>?</td>
<td>SantaCruz</td>
</tr>
<tr>
<td>HGAC 85</td>
<td>Antibody (IgG)</td>
<td>GlcNAc</td>
<td>Abcam, Pierce, Enzo Life Sciences, Affinity Bioreagents</td>
<td>Yes</td>
<td>58</td>
</tr>
<tr>
<td>HGAC 49</td>
<td>Antibody (IgM)</td>
<td>GlcNAc</td>
<td>?</td>
<td>Yes</td>
<td>58</td>
</tr>
<tr>
<td>HGAC 39</td>
<td>Antibody (IgG)</td>
<td>GlcNAc</td>
<td>?</td>
<td>Yes</td>
<td>58</td>
</tr>
<tr>
<td>WGA‡</td>
<td>Lectin</td>
<td>GlcNAc/sialic acid</td>
<td>EY Labs, Vector labs</td>
<td>Yes</td>
<td>…</td>
</tr>
<tr>
<td>sWGA</td>
<td>Lectin</td>
<td>GlcNAc</td>
<td>EY Labs, Vector labs</td>
<td>Yes</td>
<td>…</td>
</tr>
<tr>
<td>GS II</td>
<td>Lectin</td>
<td>GlcNAc</td>
<td>EY Labs, Vector labs</td>
<td>Yes</td>
<td>…</td>
</tr>
</tbody>
</table>

*Several antibodies directed against the O-GlcNAc modification at specific sites on specific proteins have been generated. These site-specific antibodies have not been discussed. IP indicates immunoprecipitate.
†In our hands, CTD110.6 will not immunoprecipitate O-GlcNAc–modified proteins efficiently at NP-40/Triton X-100 concentrations 0.1% v/v. However, RL2, HGAC85, HGAC49, and HGAC35 appear more tolerant to detergent; we have used them to successfully immunoprecipitate O-GlcNAc–modified proteins at 0.5% v/v NP-40.
‡Succinylation of WGA (sWGA) reduces its affinity for sialic acid. Notably, succinylation also reduces the affinity of sWGA for GlcNAc. As such, typically WGA is used for purification and sWGA is used for immunoblotting.
linked to biotin through a photocleavable linker. This allows the enrichment of peptides labeled with GalNAz, followed by the efficient elution of the peptides from resin by exposure to UV irradiation. This is a significant advantage, and it is often difficult to elute proteins from streptavidin without the addition of acid. The released peptides also contain a basic aminomethyltriazoyl acetylgalactosamine, and it is often difficult to elute proteins from streptavidin exposure to UV irradiation. This is a significant advantage, followed by the efficient elution of the peptides from resin by standing of the mechanisms by which O-GlcNAc regulates dynamically modified by O-GlcNAc and foster an understanding of the network in which we understand what sites are O-GlcNAc modified, how O-GlcNAcylation of these sites alters protein function, and how the O-GlcNAc modification is regulated by signaling pathways or is misregulated in disease. Recent developments, from many laboratories, have provided us with a suite of technologies (Table 1) that can be applied to answering these questions, in combination with quantitative strategies, such as SILAC or iTRAQ. Although these technologies have yet to be applied widely to heart and cardiovascular models, several studies have used a combination of these techniques to identify proteins that are differentially O-GlcNAc modified during stress,46,49 during diabetes,54 or in tissues,6 demonstrating their utility. Given the limitations of isolated cardiomyocytes, or experiments performed on hearts in vivo or in vitro, it is likely that approaches that combine well with iTRAQ, or that use deuterated labels, will have the most utility in heart and cardiovascular models (Table 1). The application of these technologies to different heart and cardiovascular models (ischemia-reperfusion, heart failure, and diabetes) will likely identify thousands of proteins dynamically modified by O-GlcNAc and foster an understanding of the mechanisms by which O-GlcNAc regulates protein and cellular function during injury and disease.

Conclusions and Future Directions
To understand how the O-GlcNAc modification regulates heart and cardiovascular function, we must build a molecular network in which we understand what sites are O-GlcNAc modified, how O-GlcNAcylation of these sites alters protein function, and how the O-GlcNAc modification is regulated by signaling pathways or is misregulated in disease. Recent developments, from many laboratories, have provided us with a suite of technologies (Table 1) that can be applied to answering these questions, in combination with quantitative strategies, such as SILAC or iTRAQ. Although these technologies have yet to be applied widely to heart and cardiovascular models, several studies have used a combination of these techniques to identify proteins that are differentially O-GlcNAc modified during stress,46,49 during diabetes,54 or in tissues,6 demonstrating their utility. Given the limitations of isolated cardiomyocytes, or experiments performed on hearts in vivo or in vitro, it is likely that approaches that combine well with iTRAQ, or that use deuterated labels, will have the most utility in heart and cardiovascular models (Table 1). The application of these technologies to different heart and cardiovascular models (ischemia-reperfusion, heart failure, and diabetes) will likely identify thousands of proteins dynamically modified by O-GlcNAc and foster an understanding of the mechanisms by which O-GlcNAc regulates protein and cellular function during injury and disease.

Acknowledgments
We apologize to our colleagues whose work was not discussed; it did not fall under the purview of this review or was omitted because of space limitations.

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Disclosures
None.

References
Paruchuri and Zachara Methods for Defining the O-GlcNAcome

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58. Paruchuri and Zachara Methods for Defining the O-GlcNAcome

Key Words: O-GlcNAc, intracellular glycosylation, signal transduction, proteomics, mass spectrometry, diabetes mellitus, infarction, glycoproteins, methods

AQ: 18