O-GlcNAcAtlas: A Database of Experimentally Identified O-GlcNAc

Sites and Proteins

Junfeng Ma^{1,2}, Yaoxiang Li², Chunyan Hou³, Ci Wu²

²Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington DC, USA.

³Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, Liaoning, China.

¹To whom correspondence should be addressed: Tel: +1-202-6873802; e-mail: junfeng.ma@georgetown.edu

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ABSTRACT

O-linked β -N-acetylglucosamine (O-GlcNAc) is a post-translational modification (i.e., O-GlcNAcylation) on serine/threonine residues of proteins. As a unique intracellular monosaccharide modification, protein O-GlcNAcylation plays important roles in almost all biochemical processes examined. Aberrant O-GlcNAcylation underlies the etiologies of a number of chronic diseases (including cancer, diabetes, and neurodegenerative disease). With the tremendous improvement of techniques, thousands of proteins along with their O-GlcNAc sites have been reported. However, until now there is no database dedicated to accommodate the rapid accumulation of such information. Thus, O-GlcNAcAtlas is created to integrate all experimentally identified O-GlcNAc sites and proteins from 1984 to Dec, 2019. O-GlcNAcAtlas consists of two datasets (Dataset-I and Dataset-II, for unambiguously identified sites and ambiguously identified sites, respectively), representing a total number of 4571 O-GlcNAc modified proteins. For each protein, comprehensive information (including gene name, organism, modification sites, site mapping methods and literature references) is provided. To solve the heterogeneity among the data collected from different sources, the sequence identity of these reported O-GlcNAc peptides are mapped to the UniProtKB protein entries. To our knowledge, O-GlcNAcAtlas is the comprehensive and curated database encapsulating all O-GlcNAc sites and proteins identified in the past 35 years. We expect that O-GlcNAcAtlas will be a useful resource which will facilitate site-specific O-GlcNAc functional studies and computational analyses of protein O-GlcNAcylation. The public version of the web interface to the O-GlcNAcAtlas can be found at <https://oglcnac.org>.

1. Introduction

O-linked β -N-acetylglucosamine (O-GlcNAc), which was discovered in early 1980s, is a post-translational modification (i.e., O-GlcNAcylation) on serine/threonine residues of proteins (Torres et al. 1984; Holt et al. 1986). Distinct from the traditional glycosylation (i.e., N-glycosylation, O-glycosylation, and GPI-anchored glycosylation), O-GlcNAcylation is a unique intracellular monosaccharide modification without being further elongated into complex sugar structures (Wells et al. 2001; Hart et al. 2007). By modulating various aspects of target proteins (e.g., activity, localization, stability and others), O-GlcNAcylation exerts diverse functional roles (Hart et al. 2011; Bond et al. 2013; Hart 2019). After several decades' endeavor, it has been revealed that O-GlcNAcylation exists in all metazoans (including animals, insects, and plants), some bacteria, fungi and virus. For example, mounting evidence has demonstrated that deregulated protein O-GlcNAcylation underlies multiple human diseases, especially in diabetes (Ma et al., 2013; Vaidyanathan et al. 2014), cancer (Slawson et al. 2011; Ma et al. 2014; Ferrer et al. 2016), and neurodegenerative diseases (Yuzwa et al. 2014; Wani et al. 2017). Moreover, targeting protein O-GlcNAcylation holds great promise for biomedical applications (e.g., as therapeutic targets and biomarkers) (Zhu et al. 2020).

Although great progress has been made towards the understanding of myriads roles of protein O-GlcNAcylation, site-specific O-GlcNAc studies have been lagged behind, largely due to lack of powerful site mapping methods. Indeed, low throughput methods (e.g., Edman degradation and site-directed mutagenesis) played pivotal roles for O-GlcNAc identification on proteins of interest in the early days. With the development of enrichment and identification techniques in recent years, mass spectrometry-based proteomics began to be exploited as a sensitive and high throughput tool for large-scale identification of O-GlcNAc proteins (Wang et al. 2008; Ma et al. 2014; Thompson et al. 2018). It becomes possible to identify tens of hundreds of O-GlcNAc sites in one single experiment by using proteomics (Wang et al. 2010; Zhao et al. 2011; Trinidad et al.

2012; Alfaro et al. 2012; Ma et al. 2015; Wang et al. 2017; Xu et al., 2017; Woo et al. 2018; Qin et al. 2018; Li et al. 2019).

Although there are a number of databases developed for other PTMs, including dbPTM (Huang et al., 2019), O-GlycBase (Gupta et al. 1999), UniCarbKB (Campbell et al. 2014), GlyGen (York et al. 2020), Glycosciences.DB (Bohm et al. 2019), GlyTouCan (Tiemeyer et al. 2017), and PhosphoSite Plus (Hornbeck et al. 2019). Until now a few databases have been created to specifically accommodate the rapid accumulation of O-GlcNAc information on proteins. The database of O-GlcNAcylated proteins and sites (dbOGAP) which was constructed in 2011 contains ~400 O-GlcNAcylation sites and has not been updated (Wang et al. 2011). Undoubtedly, there is an urgent need to create a comprehensive and curated O-GlcNAc-specific database. Herein, we describe O-GlcNAcAtlas, a manually curated database of experimentally identified O-GlcNAc sites and proteins in the past decades. By enabling users to search and retrieve data easily, O-GlcNAcAtlas is proposed to facilitate site-specific functional analysis of O-GlcNAc proteins.

2. Methods

The system flow of the construction of the O-GlcNAcAtlas is presented in Figure 1. Specifically, O-GlcNAcAtlas was compiled through a manual curation of the literature accessed from PubMed between 1984 and Dec. 30, 2019. The following search items: 'O-linked β -N-acetylglucosamine', 'O-GlcNAc', or 'O-GlcNAcylation' were used. O-GlcNAc sites information in each publication was retrieved and evaluated by at least one of the curators. Besides O-GlcNAc sites, related information (including species, sample type, peptide sequence, protein name and site-mapping methods used) was also extracted. To determine the positions of O-GlcNAcylated Ser/Thr residues, the experimentally identified peptides were then mapped to UniProtKB protein entries based on database identifier or sequence similarity. The O-GlcNAcylated peptides/sites that could not align exactly to a protein sequence were annotated with curators' comments.

Figure 1. Assembly of experimentally identified O-GlcNAc sites and proteins for a comprehensive database O-GlcNAcAtlas.

Finally, each mapped O-GlcNAc site was attributed to the corresponding literature (PubMed ID). Of special note, to avoid and minimize misleading and confusion, rigorous selection criteria was applied to the O-GlcNAc sites and proteins selected. For large-scale proteomics studies, proteins without O-GlcNAc peptides/sites identified were not included. Each entry from low-throughput studies were also carefully curated.

A user-friendly web-based graphical user interface was created with HTML, CSS, and Bootstrap. The backend server is running on a collection of services developed using Python programming language (version 3.8.1) and coupled with the MySQL database. All entries, given a unique O-GlcNAcAtlas identification number, were organized in the MySQL database.

3. Current state of O-GlcNAcAtlas

Literature mining from PubMed yielded a total of 2236 O-GlcNAc-relevant articles (Figure 2A). Among them, 225 articles contain O-GlcNAc sites on proteins (Figure 2A). Each publication was retrieved and evaluated by at least one of the curators, with O-GlcNAc sites and related

Figure 2. (A) The accumulated number of O-GlcNAcylation-related publications and publications identifying O-GlcNAc sites from 1984 through Dec. 2019. Accumulation of unambiguous O-GlcNAc sites (B) and ambiguous O-GlcNAc sites (C) as well as their corresponding peptides and proteins identified from 1984 through Dec. 2019.

recorded and compiled. Clearly there has been increased interest in O-GlcNAc studies, especially site-specific functional studies in the past decade.

O-GlcNAcAtlas consists of two datasets, depending on the ambiguity of O-GlcNAc sites mapped. Dataset-I contains unambiguously assigned O-GlcNAc sites, while Dataset-II is for O-GlcNAc sites ambiguously identified (mainly due to the low localization scores by software tools especially for peptides with clustered Ser/Thr residues). Despite the ambiguity of specific modification sites, the corresponding peptides can be positively identified, so do the O-GlcNAc proteins. Considering Dataset-2 provides useful information, it has been kept into the database. Overall, 9348 O-GlcNAc sites were unambiguously identified, corresponding to 8151 peptides and 3918 proteins (Figure

2B). In addition, 3028 peptides on 1507 proteins were found to be O-GlcNAcylated, corresponding to ~6520 ambiguous sites (Figure 2C).

Among the 9348 unambiguous O-GlcNAc sites, >98% were identified during 2010-2019 (Figure 3A). Moreover, >98% of all sites were unambiguously assigned by mass spectrometry (Figure 3B). While ~15% of all sites were identified by two or more publications, the majority (85%) were found only once (Figure 3C). And it turns out that the distribution of Ser residues and Thr residues is 62%:38% (slightly less than a ratio of 2:1) (Figure 3D).

Figure 3. Distribution of unambiguously identified O-GlcNAc sites. (A) Classification of O-GlcNAc sites according to their year of publication. (B) O-GlcNAc sites by different identification methods (including MS, NMR, Edman degradation and site-directed mutagenesis). (C) The identification frequencies of the O-GlcNAc sites by mass spectrometry. (D) Distribution of Ser/Thr residues modified by O-GlcNAc. (Note: MS, mass spectrometry; NMR, nuclear magnetic resonance spectroscopy)

Besides 3918 proteins with unambiguous O-GlcNAc sites, 1507 proteins were matched with ambiguous O-GlcNAc sites. Providing 854 proteins were overlapped between the two sets, in

total 4571 O-GlcNAc proteins were identified (Figure 4A). Among the O-GlcNAc proteins, ~77% (3535 out of 4571 proteins) were identified by one study (Figure 4B). However, 27 proteins were

Figure 4. Distribution of 4571 O-GlcNAcylated proteins. (A) Proteins matched with unambiguous sites and ambiguous sites. (B) A representation of the number of times a specific protein is identified. The majority of proteins (77%) are only identified by one publication. (C) Distribution of proteins in different species. (D) Number of human proteins identified from human cultured cells and other sources studied.

identified at least 10 times (Table 1). Although ~62% of proteins are derived from human, 38% (1728 out of 4571 proteins) are from other organisms (mainly common model systems, such as mouse, rat, C. elegans, Drosophila, Arabidopsis, and wheat) (Figure 4C). The details of O-GlcNAc proteins/sites information from different species are shown in Table 2. Regarding human proteins, most O-GlcNAc proteins were identified from model cell lines (e.g., HeLa cells and HEK293 cells) (Figure 4D). Moreover, hundreds of O-GlcNAc proteins were also identified from tissues/cells of special research interest (e.g., primary T cells and brain).

Table 1. A list of 27 proteins identified independently in at least 10 publications.

Entry name (UniProt)	Protein name	Gene symbol	Number of times identified
HCFC1_HUMAN	Host cell factor 1	HCFC1	16
NU153_HUMAN	Nuclear pore complex protein Nup153 (153 kDa nucleoporin) (Nucleoporin Nup153)	NUP153	14
UBP2L_HUMAN	Ubiquitin-associated protein 2-like	UBAP2L	14
NU214_HUMAN	Nuclear pore complex protein Nup214 (214 kDa nucleoporin) (Nucleoporin Nup214)	NUP214	14
UBAP2_HUMAN	Ubiquitin-associated protein 2 (UBAP-2)	UBAP2	12
RPRD2_HUMAN	Regulation of nuclear pre-mRNA domain-containing protein 2	RPRD2	12
PRC2C_HUMAN	Protein PRRC2C (BAT2 domain-containing protein 1)	PRRC2C	12
LMNA_HUMAN	Prelamin-A/C	LMNA	12
QSER1_HUMAN	Glutamine and serine-rich protein 1	QSER1	11
RBP2_HUMAN	E3 SUMO-protein ligase RanBP2	RANBP2	11
MINT_HUMAN	Msx2-interacting protein	SPEN	11
EMSY_HUMAN	BRCA2-interacting transcriptional repressor EMSY	EMSY	11
ZFR_HUMAN	Zinc finger RNA-binding protein	ZFR	11
MAP4_HUMAN	Microtubule-associated protein 4 (MAP-4)	MAP4	11
AHNAK_HUMAN	Neuroblast differentiation-associated protein AHNAK (Desmoyokin)	AHNAK PM227	11
WNK1_HUMAN	Serine/threonine-protein kinase WNK1	WNK1	11
POGZ_HUMAN	Pogo transposable element with ZNF domain	POGZ	11
PRC2B_HUMAN	Protein PRRC2B	PRRC2B	10
SON_HUMAN	Protein SON	SON	10
YTHD1_HUMAN	YTH domain-containing family protein 1	YTHDF1	10
CDK12_HUMAN	Cyclin-dependent kinase 12	CDK12	10
LIN54_HUMAN	Protein lin-54 homolog	LIN54	10
RBM14_HUMAN	RNA-binding protein 14	RBM14	10
BPTF_HUMAN	Nucleosome-remodeling factor subunit BPTF	BPTF	10
YTHD3_HUMAN	YTH domain-containing family protein 3	YTHDF3	10
IF4G1_HUMAN	Eukaryotic translation initiation factor 4 gamma 1 (eIF-4-gamma 1)	EIF4G1	10
MAFK_HUMAN	Transcription factor MafK (Erythroid transcription factor NF-E2 p18 subunit)	MAFK	10

Table 2. Summary of O-GlcNAc sites and proteins identified from different species.

Species	Dataset-I		Dataset-II		Total proteins
	Unambiguous sites	Proteins matched with unambiguous sites	Ambiguous Sites	Proteins matched with ambiguous sites	
Human	5654	2273	5074	1202	2843
Mouse	2315	1017	573	98	1045
Arabidopsis	334	167	559	138	200
Wheat	386	182			182
Rat	428	159	84	21	171
Caenorhabditis elegans	66	57	88	11	65
Drosophila	103	36	131	33	37
Others	62	27	11	4	28

4. O-GlcNAcAtlas web-server

To facilitate the use of the O-GlcNAcAtlas resource, a web interface has been developed for users to browse and search efficiently for their O-GlcNAcylated proteins of interest. O-GlcNAcAtlas can be searched using UniProt accession, protein name, or gene symbol as key words, and the results can be filtered further. The search output includes the basic annotations for all the matched entries (Figure 5A). The accession number of each entry is linked to the detailed annotation for the specific protein (Figure 5B). So far, O-GlcNAcAtlas supports several functions including data searching, browsing and retrieving. Moreover, search results can be directly downloaded and saved from the O-GlcNAcAtlas webpage.

5. Conclusion

To appreciate the tremendous efforts in O-GlcNAc research in the past 35 years, we aimed to create a comprehensive database of O-GlcNAc sites and proteins. O-GlcNAcAtlas not only includes data from case-by-case studies but also integrates high-throughput data from proteomics studies. For either low-throughput or high-throughput studies, we tried our best to carefully curate each entry, with curators' comments added. We fully respect the original authors' discoveries, but by adding curators' comments we hope viewers can pay attention to specific entries (e.g., the erroneously labeled modification residues or sites). With O-GlcNAcAtlas, we aim to provide a one-stop portal for the community to search O-GlcNAcylated proteins and the sites information. We

anticipate it will facilitate both basic and translational research to better understand protein O-GlcNAcylation at the molecular level.

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Abbreviation

O-GlcNAc, O-linked β -N-acetylglucosamine

MS, mass spectrometry

NMR, nuclear magnetic resonance spectroscopy

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Figure 3. Distribution of unambiguously identified O-GlcNAc sites. (A) Classification of O-GlcNAc sites according to their year of publication. (B) O-GlcNAc sites by different identification methods. (C) The identification frequencies of the O-GlcNAc sites by mass spectrometry. (D) Distribution of Ser/Thr residues modified by O-GlcNAc.

Figure 4. Distribution of 4571 O-GlcNAcylated proteins. (A) Proteins matched with unambiguous sites and ambiguous sites. (B) A representation of the number of times a specific protein is identified. The majority of proteins (77%) are only identified by one publication. However, 27 proteins were identified at least 10 times (listed in Table 1). (C) Distribution of proteins in different species. (D) Number of human proteins identified from human cultured cells and other sources studied.

Figure 5. A snapshot for searching O-GlcNAcAtlas, with ‘microtubule-associated protein tau’ as an example. (A) Tabular results for all the matched entries. (B) Main display page with detailed annotation and links to UniProtKB and PubMed.

Table 1. A list of 27 proteins identified independently in at least 10 publications.

Table 2. Summary of O-GlcNAc sites and proteins identified from different species.