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MUCINS IN PROTOZOAN PARASITES

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1. ABSTRACT

Studies on host-pathogen interactions have led to the discovery of various cell surface associated and secretory molecules. Mucins and mucin-like molecules have recently been described in several protozoan parasites, at different stages of the life cycle. These share many structural and compositional features with mammalian mucins, but vary in several other aspects. It is now becoming evident that mucins in parasite are involved in cell-cell interaction and cell surface protection, thus helping the parasite to establish infection. A large number of mucin like genes from the parasite genome have been reported, and their expression differ during the developmental stages of the parasite. In this review, we describe the structure and functions of mucin and mucin-like molecules in parasitic protozoa.

2. INTRODUCTION

Mucins are a family of cell-surface associated or secreted glycoproteins that play role in cell-cell as well as cell–molecule interactions. In mammals, these are high molecular weight glycoproteins characterized by extensive O-glycosylation at serine and/or threonine residues. Most of these mucins have a variable central domain which contains repetitive Ser/Thr and Pro rich sequences and is flanked by non-repetitive N- and C- termini (1).

In addition to higher organisms, mucins or mucin like molecules have recently been described in some protozoan parasites. These include kinetoplastid (*Trypanosoma*, *Leishmania*), apicomplaxan (*Cryptosporadium*) and amoebic (*Entamoeba*) parasites (2-6). Like mammalian mucins, these molecules are heavily glycosylated and are rich in Thr/Ser and Pro. In number of parasites, different groups of mucin molecules exist in different life cycle stages. These mucins seem to perform essential functions, such as cell-cell/cell-molecular interaction and protection of the parasite, in order to help the parasite in establishing infection (4,7). The primary focus of this review is to present a consolidated and a comparative profile of mucins and mucin-like molecules in various parasitic protozoa. Emphasis has also been given to highlight their multiple roles, particularly towards the establishment of infection.

3. MAMMALIAN MUCINS---AN OVERVIEW

Mucins are highly glycosylated proteins occurring either as secretory or membrane bound forms. The polypeptide backbone (apomucin) is rich in hydroxy amino acids, serine and threonine, which together with glycine, alanine and proline comprise nearly 50% of total amino acid residues of the protein, and are present as tandemly repeated sequences. The threonine and serine residues are the targets of O-glycosylation machinery and the extent of glycosylation is such that carbohydrates account for 50-85% of the dry weight of mucins. Secretory mucins are the major constituents of mucus secretions, lining the epithelial cells of digestive, respiratory and reproductive tracts (8,9). They are capable of forming gels at very low concentration by forming long thread like polymers resulting from the formation of disulphide linkages between monomers and intramolecular interactions of sugar side chains. Membrane bound mucins are present on the surface of various cell types and unlike secretory mucins, do not form oligomers and are hence,

Table 1. Amino acid sequence of Ser/Thr rich regions of parasite mucins

Organism	Mucin/mucin-like molecule	Ser/Thr rich sequences	Repetitive(R)/Non-repetitive (NR)	Reference
L. major	fPPG, mPPG,& PPG1	APSASSSSAPSSSSS[S]	R	28
L. mexicana	SAP1	[T][T](S/T)(S/T)(S/T)SSEG	R	34
L. mexicana	SAP2	[T][T](S/T)(S/T)(S/T)SSEG & [T](A/T)(S/T)(S/T)(S/T)SSD(A/V)	R	34
T. cruzi	60-200 kD	TTTTTTTTKPP & T_{4-25}AP	R	7
T. cruzi	35-50 kD (L group)	KNTTTTTTTSTTTS(S/K)AP	R	7
T. cruzi	35-50 kD (S group)	DQT_{17-20}NAPAKDT_{5-7}NAPAK	NR	7
C. parvum	gp45	SSSSSSSSSSSSSSSSSSTST	NR	21

smaller in size than their secretory counterparts (8,9). The membrane bound mucins also have O-glycosylated serine and threonine rich regions, but they lack tandem repeat sequences. The primary function of secretory mucins is to provide protection to the apical epithelial cell layers in digestive, respiratory and urinogenital tracts, from environmental factors like acidic pH, hydrolytic enzymes and pathogens. The cell surface mucins, in addition to their protective role, have shielding effect on various surface receptors, thereby helping in the regulation of their activity (1). So far, twelve human mucin genes have been identified designated as *MUC1-4, MUC5AC, MUC5B, MUC6-9, and MUC11-12* (8-14).

4. MUCIN/MUCIN-LIKE MOLECULES IN PROTOZOAN PARASITES

4.1. Occurrence

Many protozoan parasites with different stages exhibit a digenetic life cycle between insect vector and mammalian host. For instance, *Trypanosoma cruzi*, responsible for Chagas' disease, has four stages. Two of the three extracellular forms, epimastigotes and metacyclic trypomastigotes, are present in the vector while the parasite exists as blood stream trypomastigotes and intracellular amastigotes in vertebrate hosts (15). Likewise, the life cycle of *Leishmania*, the causative agent for a wide range of Leishmaniases diseases, involves motile, flagellated promastigotes in the vector midgut, while a non-motile, intracellular amastigotes exists in mammalian hosts (16). *Cryptosporidium parvum*, an emerging zoonotic protozoan parasite, causes self-limiting gastrointestinal disease in immunocompetent hosts, but results in a more severe life threatening diarrhoea in immunocompromised individuals (17).

Among all the parasitic organisms, mucins from *T. cruzi* have been studied most extensively. They are linked to the surface membrane through a glycosylphosphatidylinositol (GPI) anchor and are present in all the developmental stages of the parasite. *T. cruzi* expresses two distinct groups of mucins; epimastigote and metacyclic trypomastigote express a group of mucins ranging from 35-50 kDa (2,18), while the cell derived blood stream trypomastigotes produce mucins with a large smear from 60-200 kDa in immunoblots (3,19). Proteophosphoglycans (PPGs), expressed by both promastigote and amastigote stages in many species of *Leishmania*, are the structural equivalents of mammalian mucins. *Leishmania* produces several forms of PPGs. Promastigotes express secreted acid phosphatases (SAPs), filamentous PPG (fPPG) and membrane bound PPG (mPPG), whereas the amastigotes are known to secrete amastigote PPG (aPPG) (reviewed in 4). Recently, PPGs, similar to those present in *Leishmania*, have also been detected in *Entamoeba histolytica*, the causative agent of amoebic dysentery (20). Mucin-like molecules, such as a high molecular weight surface glycoprotein, GP900 (5), and some other smaller surface glycoproteins gp15 and gp45 (6,21), have also been described in *C. parvum*. Additionally, intracellular form of this parasite also express HC10 gene, which codes for a mucin-like molecule with 984 residues (22).

4.2. Composition and structure

Mammalian mucins primarily have central domain containing repetitive Ser/Thr and Pro rich sequences, which are heavily O-glycosylated (1). Protozoan parasites express mucins/mucin-like molecules that have striking structural and compositional similarities to their mammalian counterparts. The heavy glycosylation results in the protease resistance and extended conformation of the polypeptide chain, as observed in PPGs of *Leishmania*. The oligosaccharide chains project out from the protein backbone. As a result, the *Leishmania* SAPs and PPGs have a thread like appearance in electron microscope similar to that observed with mammalian secretory mucins (23,24).

Though parasitic mucins do not share much homology amongst themselves, they are rich in Ser and/or Thr residues and present as repetitive/non-repetitive sequences (Table 1). These constitute the primary sites for glycosylation (2,25,26). The PPGs of *Leishmania* vary in carbohydrate and amino acid content. In *L. major* promastigotes the dry weight of PPG predominantly comprise of carbohydrates (76%) and phosphates (20%) and only 4% is contributed by amino acids. In contrast, the

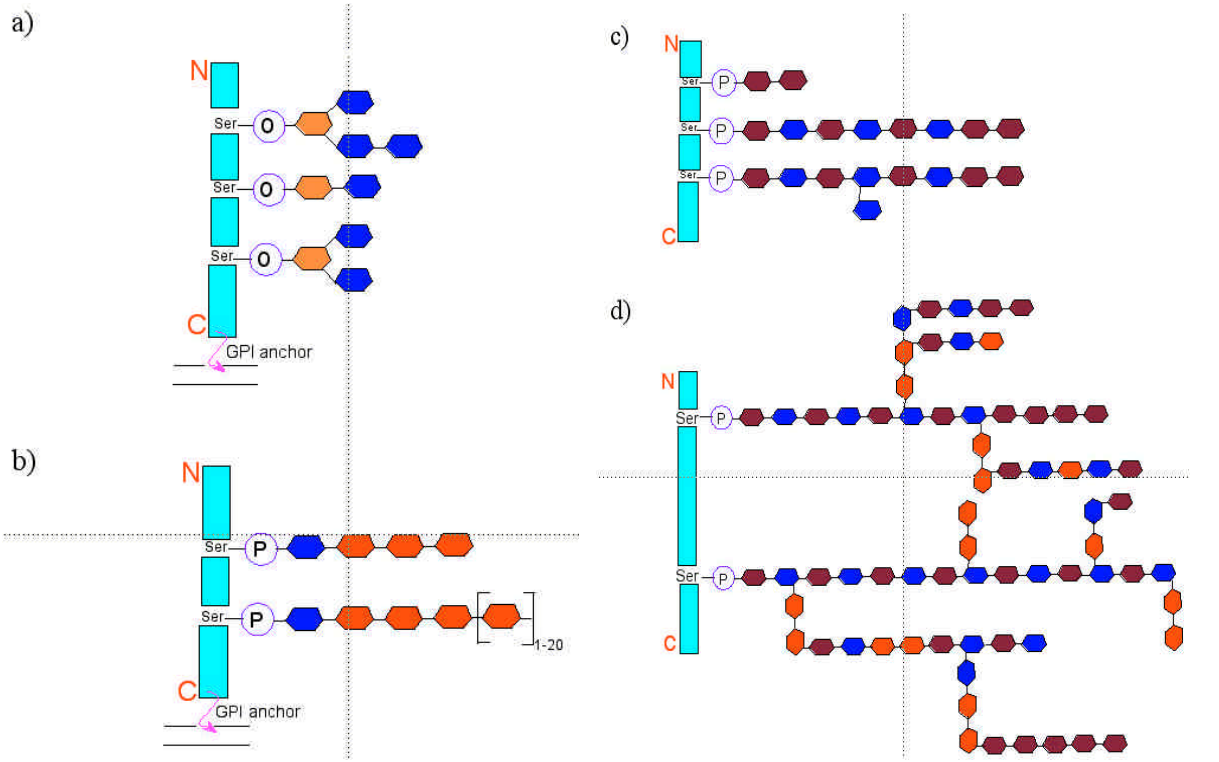

Figure 1. A schematic representation highlightening the important features in structure and glycosylation pattern of mucins/mucin-like molecules in parasitic protozoa. (a) *T. cruzi* 35-50 kD mucin, (b) *E. histolytica* PPG, (c) *L. major* pPPG ,(d) *L. mexicana* aPPG. Circled O and P represent O-linked glycosylation and phosphoglycosylation respectively. The glycosyl moieties are represented as colored hexagons (orange = N-acetyl glucosamine, red = glucose, blue = galactose and brown = mannose). The figure does not indicate the nature of glycosidic bonds between different monosaccharides. In *Leishmania* PPGs, the sugar residues are phosphorylated and are connected by both glysosidic and phosphoglycosidic bonds.

aPPG has higher amino acid content (70% carbohydrate, 14% phosphate and 16% amino acids) (24)(27). In fPPG, serine is the main amino acid present in the backbone, and together with alanine and proline accounts for 87% of the total amino acid content. Interestingly, more than 80% of the serine residues are glycosylated in fPPG (24). In mPPG of promastigotes, the N-terminal region has leucine-rich repeats and the central domain contains nearly 100 copies of serine-rich repeat (28) (Table 1). GP900 of *C. parvum*, like mPPG of *Leishmania*, is a surface bound mucin-like molecule and shares homology with human MUC2 and MUC5 mucins. This 1832 residue protein consists of cysteine-rich domains separated by polythreonine domains (5). Another 60 kDa, 330 amino acid long mucin like molecule has been identified in *C. parvum*. It is cleaved proteolytically into 45 kDa and 15 kDa surface protein. The gene responsible for this exhibits high degree of polymorphism amongst different isolates (6). Similarly, HC10 gene product of intracellular form of *C. parvum* is also rich in threonine and serine (22).

Carbohydrate content analysis of parasite mucin revealed certain interesting features (Figure 1). In *T. cruzi*, unlike most mammalian mucins, where the first residue attached to the hydroxyl group of amino acid is N-acetyl galactosamine, the O-linked glycoside is N-acetylglucosamine (GlcNAc). Further, this GlcNAc residue might remain as such or may be modified by attachment of mono-, di-, tri-, tetra- and pentasaccharide (18). There is evidence of inter strain variation in the glycosylation in *T. cruzi* mucins (25). In PPG molecules of *Leishmania*, the attachment of oligosaccharides represent a novel type of protein-glycan linkage, termed phosphoglycosylation, in which the phosphosugars are attached to the hydroxyl group of Ser/Thr residue by a phosphodiester bond. The glycans are attached as disaccharide repeat units PO_4-6Galβ1-4Man, which accounts for more than 40% of the total carbohydrate content. In addition, other phosphorylated glycans like trisaccharide PO_4-6[Galβ1-3]Galβ1-4Man and unphosphorylated oligosaccharides like Galβ1-4Man and Man α1-2Man are also present (24). In *L. mexicana* promastigotes and amastigotes, stage-specific glycosylation of PPGs has been observed. Glycan chains in aPPG appear to be more diverse, branched, and multiphosphorylated (29,30) (Figure 1d). Similarly, GPI-anchored PPGs of *E. histolytica* are modified with oligosaccharide having a general structure $[Glc\alpha1\text{-}6]_nGlc\beta1\text{-}6Gal$, where n = 2-23 (20) (Figure 1b).

The membrane bound mucin of *T. cruzi*, *T. carassii*, *C. parvum* and mPPG of *Leishmania* promastigotes are attached to the plasma membrane by GPI

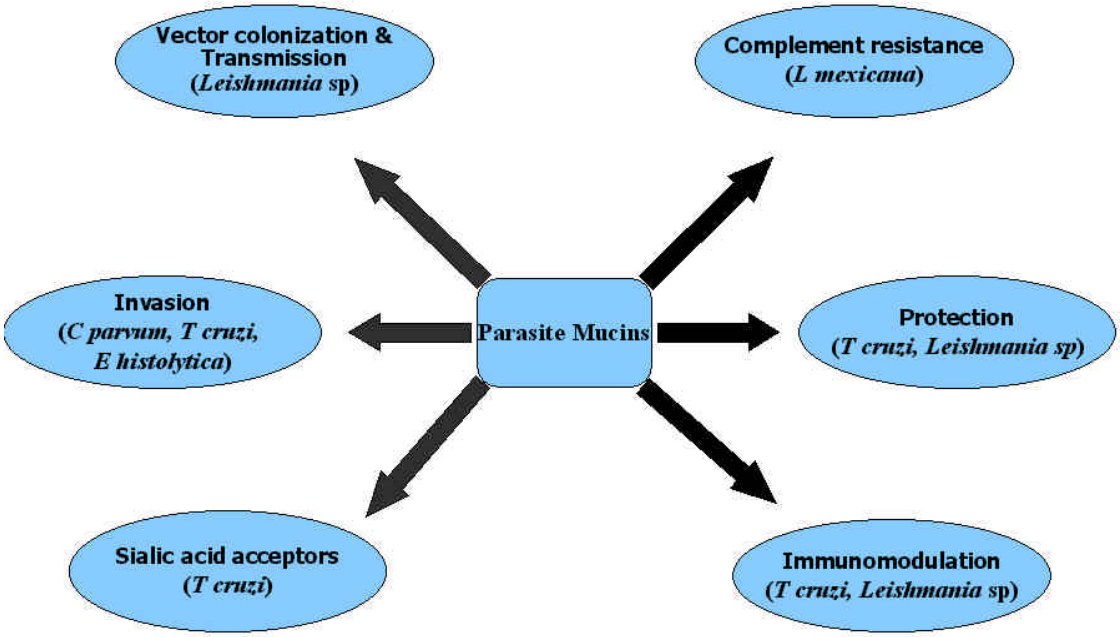

Figure 2. A schematic diagram showing various functions of mucin/mucin-like molecules in parasitic protozoa.

anchors. In *T. cruzi* the lipid components of GPI anchors have been demonstrated to undergo modification during the differentiation of non-infective epimastigotes to infective metacyclic trypomastigotes in insect vector. Ceramide phospatidylinositol content is higher in metacyclic trypomastigotes (26).

4.3. Mucin genes

Organization of various mucin and mucin like genes in parasitic protozoa has been studied. Mucins in *T. cruzi* are encoded by a large family of mucin genes, TcMUC, that occupies approximately 1% of the parasite genome. Approximately, 484 mucin genes are present in the haploid genome (31). This number might further increase based on the recent discovery of another subfamily TcSMUC (32). The coding region of these genes is made up of sequences encoding conserved signal peptide and GPI-anchoring signals, and central either repetitive or non-repetitive regions containing Thr/Pro rich sequences that are the targets of O-glycosylation (31,32). Together, these gene families are divided into subfamilies based on the sequence similarities in their coding and untranslated regions and their expression is regulated in a stage dependent manner. The TcMUC subfamily A which contains a hypervariable region, several tandem repeats, a threonine rich region and sequences for GPI anchorage, is thought to be expressed in cell derived trypomastigotes in mammals and codes for the core protein of high molecular weight (60-200 kDa) mucins. While, the insect derived epimastigotes and metacyclic trypomastigotes are thought to express the members of TcSMUC subfamily encoding the core protein of 35/50 kDa mucins (7,31,32). Some of these genes appear to be genetically linked to the trans-sialidase genes, which again represent a large gene family. This organization might be important for the coordinated expression of mucins with trans-sialidase, an enzyme which transfers sialic acid from host proteins to GPI-anchored surface mucins of the parasite (33). In *L. mexicana*, two separate genes, *lmsap1* and *lmsap2* code for SAP1 and SAP2. The gene products are identical except for the difference in the nature of Ser/Thr repeats (34). Species-specific differences have also been observed in the nature of Ser rich repeat sequences of *ppg1* genes of *L. major* and *L. mexicana*, which code for mPPG in these species (28) (Table 1).

4.4. Functions

Parasite mucins have been implicated to be involved in multiple functions (summarized in Figure 2).

4.4.1. Role in cellular interactions and parasite protection

Mucins, being present in a high copy number and covering the entire cell surface, play crucial role towards cellular interactions and form a protective coat around the parasite in the hostile environment of vector and host. This is attributed to the presence of sialic acid that results in the stabilization of the mucin coat (35). *T. cruzi* lacks the capability to synthesize sialic acid and mucins have been demonstrated to be acceptors of sialic acid from host in a reaction catalyzed by parasitic surface bound trans-sialidases (36). Although it has been reported that the removal of sialic acid from 35/50 kDa mucins of insect derived metacyclic trypomastigotes, and not from tissue culture derived trypomastigotes (expressing 70-200 kDa mucins), enhanced their infectivity (37). Negative charge conferred by sialic acid also contributes to the protective property of the mucin coat. This was supported by the recent observation where sialylated mucins provide resistance against the lytic anti-α-Gal antibodies present in

chronic Chagas' disease patients (35). Also, sialylated mucin coat protects the parasite from the damage induced by enzymes and oxidants used in host defence (38). Moreover, there is a possibility that sialylation of β-Gal residues might prevent the binding of opsonins and other antibodies, hence preventing parasite clearance (35). GP900 in *C. parvum* is believed to play an important role in the invasion of host cells by the sporozoites and is subsequently shed. Several monoclonal antibodies, which recognize mucin-like molecules in *Cryptosporidium* were shown to have a neutralizing effect on the infection by the parasite (6,39). PPG of *E. histolytica*, due to high copy number and heavy glycosylation, is thought to play a protective role for the trophozoits. It may also play an important function in host parasite interactions, and towards virulence of the parasite (20).

In *T. cruzi*, the N-terminal hypervariable region of mature TcMUC apomucins is hydrophilic and glycosylated. It is probably exposed to the host and is thought to allow wide range of interaction with a variety of host receptors, thereby, allowing the parasite to infect wide variety of cells in the hosts. This might also explain the wide host range exhibited by the parasite. This region is also suggested to be of help in preventing maturation of immune response against some important sequences by providing antigenic variation (31).

The fPPGs are secreted by the promastigotes of *Leishmania* in the growth medium and form a gel like network in which the promastigotes tend to aggregate. fPPG also forms a gel-like matrix in the cardia and stomodeal valve of the sandfly where metacyclic promastigotes are embedded. This gel like plug is thought to improve the efficiency of transmission during the bloodmeal, by blocking the lumen of the stomodeal valve which makes it difficult for the sandfly to engorge the bloodmeal (40). It is also suggested that fPPG help in the survival of the parasite in the hostile environment of insect midgut and successful colonization in the vector (41,42). For mPPG, it is speculated that the sugar residues in phosphoglycan might serve as ligands for macrophage and sandfly midgut binding and acceptors of complement (28). aPPG represent the only identified secretory product of *Leishmania* amastigotes. It is secreted in large amounts in the parastiophorous vacuole where the parasite resides and can reach to a concentration of mg/ml. Due to its polyanioic nature it has been suggested to contribute to the expansion of parasitophorous vacuole (43).

Additionally, mucins have also been thought to confer resistance to parasite from complement-mediated lysis. In *T. cruzi* this is achieved by the inhibition of the cleavage of convertase (44). aPPG in *Leishmania* is potent in activating the complement by mannose-binding lectin pathway. At a very low concentration (10 μg/ml), it was demonstrated to deplete the complement, suggesting that aPPG released from the infected cells in lesion might lead to the local depletion of the complement *in vivo* (45). This view supported by the observation that *L. mexicana* amastigotes isolated from mouse lesions lack C3 fragments from their surface while aPPG deficient *L. major* amastigotes are opsonised by C3b/C3bi (45). In addition to protecting the parasite from complement mediated lysis, aPPG seems to harvest the complement activation to the benefit of the parasite. The cleavage products of complement C3, C4, and C5 (C3a, C4a, C5a) are anaphylactic and are capable of attracting the circulating monocytes, the host cells of *Leishmania* parasite (40).

4.4.2. Immunomodulatory effects

Cytokines are important in regulating the parasite replication as well as the immune response in infected animals (46). Parasite mucins have been shown to modulate the cytokine production for parasite survival. AgC10, a mucin like antigen of *T. cruzi*, can bind to the surface of macrophages, probably by interacting with CD62L, and results in increased intracellular $Ca^{2+}$and induction of IL-1β, but impairs TNF α and IL-2 by monocytes in response to LPS stimulation (47). Similar effects have been observed in *T. cruzi* infected cells. This was further substantiated by the observation that AgC10 binding mAb C10 substantially decreased the effect of parasite infection on cytokine production. Based on these observations it was proposed that *T. cruzi* utilizes AgC10 for imbalancing the immune response in its favor (47). IL-12 is known to mediate host resistance to *T. cruzi* (and other intracellular pathogens) while TNF-α along with IFN-γ, mediates the killing of parasite by macrophages. In another study, it was demonstrated that trypomastigote-specific GPI-anchors are the important elements that signal for cytokine production which results in macrophage activation (48).

PPGs of *Leishmania* promastigotes also showed immunomodulatory effects similar to those observed with *T. cruzi* mucins. On one hand, they inhibit the LPS induced TNF α production by the macrophages, which is beneficial to the parasite while on the other side, in combination with IFN-γ, they induce NO production, which has parasiticidal effects (49).

5. PERSPECTIVE

In summary, this review has outlined the important structural and functional aspects of mucins and mucin-like molecules in different protozoan parasites. Clearly the studies of such molecules are at the upcoming stage in the field of parasitology to understand several basic questions. Mucin genes, constituting a heterogeneous family, are expressed in different stages as well as species of parasites. How this microheterogenity is generated and how exactly these diverse structural features contribute towards the myriad functions of protozoan parasite mucins, particularly in establishing the infection, is an important question. Furthermore, whether these molecules could be utilized as potential target(s) for chemotherapeutic interventions of parasitic infections needs to be investigated. Answers to these and related questions should eventually lead to the development of novel strategies to control protozoan parasitic infections.

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Abbreviations: GPI, glycosylphosphatidylinositol; PPGs, proteophosphoglycans; SAPs, secreted acid phosphatases; fPPG, filamentous proteophosphoglycan; mPPG, membrane bound proteophosphoglycan; aPPG, amastigote proteophosphoglycan; GlcNAc, N-acetylglucosamine; mAb, monoclonal antibody

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