The Ocular Surface xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Review Article

Glycoprotein 340 in mucosal immunity and ocular surface

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ARTICLE INFO

Keywords:
Bacteria
Contact lens
Dry eye
Gp340
Infection
Inflammation
Ocular surface
Tears

ABSTRACT

Glycoprotein 340 (Gp340) is an innate immune receptor with well-defined roles in mucosal tissues. It is a normal component of mucosal fluids such as tears, breast milk, and saliva, and it is expressed in tissues such as the vagina, gastrointestinal tract, oral cavity, lung alveoli, and pancreas. In the eye, it is expressed in the lacrimal gland, cornea, conjunctiva, and retina. Investigations of the protein in wet-surfaced epithelia of the body show that the effects of Gp340 can be beneficial or harmful depending on the conformation in which it exists. In a fluid phase, Gp340 appears to be protective against mucosal infection, while in a surface-associated form it appears to promote infection. On the ocular surface, it is dysregulated in dry eye disease and inhibits twitching motility of *P. aeruginosa* in tears. This review discusses what is known about Gp340 in wet-surfaced mucosal epithelia and highlights the potential roles of the protein in ocular surface immunity, inflammation, and infections.

1. Introduction

Glycoprotein 340 (Gp340) is a 340-kDa extracellular protein which belongs to the scavenger receptor cysteine-rich (SRCR) superfamily of proteins. It is encoded by the "*deleted in malignant brain tumor-1*" (*DMBT1*) gene, located on human chromosome 10q26.13 [1]. Gp340 is also referred to as DMBT1 and salivary agglutinin because all three have identical protein core and are encoded by the same gene [2–6]. Being a glycoprotein, Gp340 is heavily glycosylated by post-translational modifications which accounts for 25–40% of the total molecular weight of the protein [2,7].

Gp340 is a normal component of mucosal fluids such as tears, saliva, and breast milk [8–10]. It is expressed on the ocular surface and other mucosal epithelial tissues such as gastrointestinal tract, oral cavity, lung alveoli, and pancreas, as part of the innate immune system [1,11–13]. Gp340 is also expressed in lower quantities in the brain, uterus, testis, and mammary glands [13]. As an innate immune receptor, Gp340 confers protection against pathogens on the ocular surface and other moist-surfaced epithelial surfaces where they are expressed. It is also involved in epithelial cell differentiation [14]. The effects of Gp340 may either be beneficial or harmful depending on the conformation in which the protein exists. In the fluid phase, Gp340 inhibits cariogenesis, HIV-1, and influenza A infections. However, in the surface-associated form, it promotes cariogenesis and HIV-1 infections. Gp340 also has both stimulatory and inhibitory effects on the complement system [15].

The purpose of this review is to describe the current state of our understanding of Gp340 on the ocular surface in relation to other mucosal tissues/surfaces, with emphasis on its roles and effects in normal physiology and altered states of the ocular surface.

2. Overview of Gp340

Human Gp340 exists in two variants, namely the secretory and cell-associated types [16,17]. The secretory variant is expressed in tissues in the eye, lung, oral cavity, and breast whereas the cell-associated form is expressed on the vaginal and cervical epithelia [18,19]. The secretory Gp340 exists in two conformations; the soluble (fluid-phase) and the immobilized forms [20]. The soluble form is found in tears, respiratory mucosal secretions, saliva, and break milk while the immobilized is found on the hydroxyapatite surface in the oral cavity [16,20]. In the context of this review, the cell-associated variant and the immobilized form are classified as surface-associated conformation.

Gp340 contains four major well-defined domains: 1) the scavenger receptor cysteine-rich (SRCR) domain, 2) the SRCR interspersed domain (SID), 3) the C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein-1 (CUB) domain, and 4) the zona pellucida (ZP) domains (see Fig. 1). The SRCR domain is found in a variety of secreted and cell-surface proteins and contains 100–110 amino acids. Two groups of SRCR domains have been characterized [21]. Group A SRCR domains contain six cysteine residues and are encoded by two exons, while group B SRCR domains consist of eight cysteine residues and are

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https://doi.org/10.1016/j.jtos.2018.04.006
Received 8 February 2018; Received in revised form 18 April 2018; Accepted 24 April 2018

Abbreviations

ADDE	aqueous-deficient dry eye
CLDE	contact lens-related dry eye
CUB	C1r/C1s, urchin embryonic growth factor and bone morphogenetic protein-1
DED	dry eye disease
DMBT1	deleted in malignant brain tumor-1
EDE	evaporative dry eye
Gp340	Glycoprotein 340
IAV	influenza A virus
IL-17	interleukin 17
IL-22	interleukin 22
MBL	mannose-binding lectin
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
SID	SRCR interspersed domain
SRCR	scavenger receptor cysteine-rich
SS	Sjögren's syndrome
TFF	trefoil factor
TFF2	trefoil factor 2
TFF3	trefoil factor 3
TLR	toll-like receptor
TLR4	toll-like receptor 4
ZP	zona pellucida

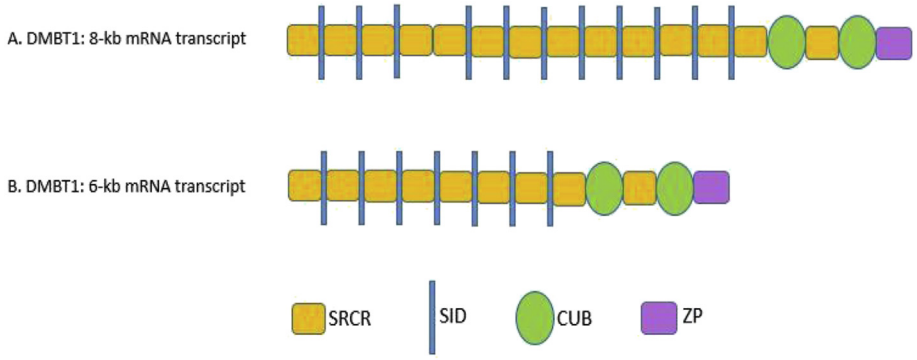

Fig. 1. Schematic overview of the structural organization of Gp340. Gp340 contains up to 14 Group B SRCR domains. Interspersed between successive SRCRs are SIDs which are the sites for posttranslational glycosylation. There are 2 CUB domains which mediate Gp340's interactions with extracellular proteins. Located at the C-terminus is a single ZP domain which is primarily involved in Gp340 oligomerization. Alternative splicing results in mRNA transcripts which code for Gp340 with different number of SRCRs. The 8-kb transcript (A) codes for Gp340 with 14 SRCR domains, while the 6-kb transcript (B) codes for protein with nine SRCR domains.

encoded by a single exon [22]. The SRCR domains of Gp340 are of the group B type [23]. There is variability in the number of SRCR domains in Gp340 due to alternative splicing occurring in both SRCR and SID regions at mRNA level which results in mRNA transcripts of different sizes. The most extended transcript with 8 kb size consists of 7656 nucleotides and codes for Gp340 with 14 SRCR domains with the first 13 in tandem. The shortest mRNA transcript is of 6-kb size, consists of 5802 nucleotides and codes for Gp340 with only nine SRCR domains [19]. Fourteen N-linked glycosylation sites and several O-linked glycosylation sites exist in Gp340. These glycosylation sites are present in the SIDs, located between SRCR domains in tandem (except between the fourth and fifth SRCR domains). Following the SRCR domains and SIDs are two CUB domains separated by the 14th SRCR domain. The CUB domain is a 110-residue protein motif which consists of four conserved cysteine residues and exhibits a β-sandwich fold [24]. The CUB domains mediate the interaction of Gp340 with extracellular proteins [24]. At the C-terminus of the polypeptide is a single ZP domain which consists of 260 amino acids with 8 conserved cysteine residues. The ZP domain is primarily involved in oligomerization of Gp340 [25].

3. Gp340 and innate immunity

The innate immunity is an evolutionarily conserved system which functions as a first-line of defense against invading microbial pathogens and other potential untoward effects on the host [26]. It consists of nonspecific defense mechanisms that come into play immediately or within hours of the host's exposure to antigens. Gp340 is known to be an innate immune molecule with broad-spectrum antimicrobial functions. It is considered as a pattern recognition receptor (PRR), which identifies pathogens by engaging pathogen-associated molecular patterns (PAMPs) [27]. PAMPs are the conserved structures in microbial cells which are essential for the existence of microbes. PAMPs include lipopolysaccharides on gram-negative bacteria, lipoteichoic acid on gram-positive bacteria, peptidoglycans, mannan, glucan, porins, flagellin, and bacterial RNA and DNA [28]. Recognition of PAMPs by PRRs, in turn, stimulates the innate immune system. Thus, as a PRR, Gp340 stimulates innate immunity.

Due to its ability to bind and agglutinate broad spectrum of microbes, Gp340 also plays a vital role in the innate protection against oral microbial infections [29,30]. For instance, fluid-phase Gp340 has been implicated in the clearance of microorganisms from the oral cavity [31]. It achieves this by aggregating microbial species which in turn get washed down the acidic gut where they face extinction.

4. Gp340 and complement activation

The complement system is an essential component of the innate immune response. It augments the opsonization of bacteria by antibodies and promotes the bactericidal functions of antibodies [26]. It thus complements the roles of antibodies and serves as a link between innate and adaptive immunity. The complement system is activated via three pathways: (1) the classical pathway which is activated by antibody or by direct binding of complement component C1q to the pathogen surface, (2) the lectin pathway which is triggered by mannose-binding lectin (MBL), a normal serum constituent that binds a mannose-containing carbohydrate on the surface of some class of bacteria, and (3) the alternative pathway which is triggered directly on pathogen surfaces [26]. All three pathways generate a critical enzymatic activity that, in turn, generates the effector molecules of the complement system. The main effects of complement activation are opsonization of pathogens, recruitment of inflammatory mediators, and direct killing of pathogens [26].

A study examining the effect of Gp340 on the complement system found Gp340 to modulate complement activation [15]. The effect of Gp340 on complement activation was investigated by incubating fluid-phase and surface-associated Gp340, each with serum and measuring the deposition of downstream complement factors. While surface-associated Gp340 activated the classical and lectin pathways, fluid-phase Gp340 inhibited the lectin pathway. The study further found fluid-