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Review

Sweet escape: Sialic acids in tumor immune evasion

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ABSTRACT

Sialic acids represent a family of sugar molecules derived from neuraminic acid that frequently terminate glycan chains and contribute to many biological processes. Already five decades ago, aberrantly high expression of sialic acids has been proposed to protect cancer cells from recognition and eradication by the immune system. Today, increased understanding at the molecular level demonstrates the broad immunomodulatory capacity of tumor-derived sialic acids that is, at least in part, mediated through interactions with immunoinhibitory Siglec receptors. Here we will review current studies from a sialic acid sugar perspective showing that tumor-derived sialic acids disable major killing mechanisms of effector immune cells, trigger production of immune suppressive cytokines and dampen activation of antigen-presenting cells and subsequent induction of anti-tumor immune responses. Furthermore, strategies to modulate sialic acid expression in cancer cells to improve cancer immunotherapy will be discussed.

Contents

1. Introduction

Every living cell is surrounded by a dense layer of glycans that are attached to cell surface glycoproteins and glycolipids. Glycans are composed of various monosaccharides and show an enormous, cell-specific structural diversity illustrating their importance in many biological processes at the molecular level [1]. Despite their abundance on the cell membrane, many physiological functions and effects of glycans are not yet understood. Nevertheless, it is known for decades that glycosylation changes reflect, and are causative for several pathological conditions. Upon malignant transformation, tumor cells present a significantly different glycosylation pattern relative to their normal counterparts, and several cancer-specific glycans have been identified

Abbreviations: $SLe^{A/X}$, sialyl Lewis antigen A and X; STn, sialyl Tn antigen; PSA, polysialic acid; SAMPs, self-associated molecular patterns; Siglecs, sialic acid-binding immunoglobulin-like lectins; ITIMs, immunoreceptor tyrosine-based inhibitory motifs; NK cell, natural killer cell; NKT cell, natural killer T cell; CTLs, cytotoxic T cells; DISC, death-inducing signaling complex; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell; DC, dendritic cell; Neu5Gc, N-glycolylneuraminic acid; TACA, tumor-associated carbohydrate antigen; GBM, glioblastoma multifore

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that promote tumor growth and progression. Among the glycosylation changes reported in cancer, aberrantly high expression of sialic acid sugar-carrying glycans (sialoglycans) is commonly found [2,3].

Sialic acids represent a family of about fifty derivates of neuraminic acids that share a common nine-carbon (C1-9) backbone. In general, sialic acids terminate glycan chains of all vertebrate and many invertebrate cells and contribute to protein stability and trafficking as well as cell–cell and cell–extracellular matrix interactions. Most cells possess a specific machinery to synthesize the different sialic acids from precursor carbohydrates in the cytoplasm. Following transport, Golgi-resident sialyltransferases incorporate these sialic acids into the glycans of glycoproteins and glycolipids. To date, more than 20 different sialyltransferases have been identified, each attaching sialic acids via different glycosidic linkages (α2,3; α2,6 or α2,8) to underlying sugars [4]. In cancer cells, overexpression of sialyltransferases leads to increased synthesis of sialoglycoconjugates that are deposited on the cell surface. Possibly, overexpression of sialyltransferases also leads to the neoformation of cancer sialoglycans, however this hypothesis remains to be proven [5,6]. Prominent cancer-associated sialoglycan structures include the sialogangliosides fucosyl-GM1, GD1a, GM2, GD2, GM3 and GD3, the sialyl Lewis antigens A and X ($SLe^{A/X}$), the sialyl Tn (STn) antigen, polysialic acid (PSA) and mucins [3,7–9]. Due to hypersialylation, cancer cells acquire distinct characteristics including resistance to apoptosis and enhanced migratory properties which correlate with tumor aggressiveness and a poor prognosis for patients [6,10,11]. An increasing amount of evidence advocates tumor sialoglycans as potent immune modulators acting at the tumor/immune interface. It becomes more and more apparent that hypersialylation provides a selective advantage for tumor cells to escape from anti-tumor immunity and is even involved in manipulating immune cell function to benefit tumor growth.

In this review, we examine recent findings emphasizing that tumor cells escape from host anti-tumor immunity through aberrant expression of sialic acids. In particular, the effect of tumor-derived sialoglycans on the function of different immune cell subsets involved in anti-tumor immunity will be discussed. An overview of the immunomodulatory events at the tumor sialoglycan/immune interface is provided in Fig. 1.

2. Sialic acids in immune regulation

The dense layer of glycans protruding from the cell surface is one of the first structures recognized by immune cells that constantly screen host cell surfaces for (malignant) aberrations or presence of pathogens. Indeed, immune cells express multiple distinct carbohydrate-binding receptor families that modulate their function and that have recently been reviewed elsewhere [12]. Among the various carbohydrates present in cell surface glycans, the diverse family of sialic acids is of particular interest [13]. Being vertebrate specific, expression of sialic acids allows discrimination of pathogens lacking sialic acid expression from sialylated host cells. Interestingly, pathogens have been identified that have evolved strategies to express host sialic acids as molecular mimicry to evade the host immune system [14,15].

A growing body of evidence suggests that sialic acids control immune homeostasis and dampen inappropriate immune activation in order to avoid or limit damage of sialylated host cells. The importance of sialic acids herein became apparent in a cohort of patients lacking the expression of 9-O acetylated sialic acids due to a defect in the sialic acid acetylesterase (SIAE) gene. These patients developed a broad spectrum of autoimmune diseases ranging from rheumatoid arthritis to type I diabetes [16]. In line with these findings, expression of sialoglycans on colonic epithelia cells or neurons has been indicated to prevent immune activation and to protect the gut mucosa and nervous system, respectively [17,18]. Together, the importance of sialic acids in the discrimination of *self* and *non-self* and as immune inhibitory signals preventing inappropriate immune activation has brought forth the idea that sialic acids act as *self-associated molecular patterns* (SAMPs, Ajit Varki 2011) [19]. This implies that there are sialic acid-recognizing receptors that transmit inhibitory signals to control immune activation. So far, three sialic acid-binding lectins have been identified including Selectins, factor H and the family of Sialic acid-binding immunoglobulin-like lectins (Siglecs). Selectins (P-, L-and E-Selectin) belong to the family of C-type lectins and are well-known for their binding to SLe^x and involvement in leukocyte trafficking, but also cancer metastasis. Factor H is a central regulatory protein in the alternative complement pathway discussed in more detail in Section 3.1 [4]. Siglecs comprise a family of more than 14 I-type lectins expressed by virtually all immune cells that specifically recognize diverse sialoglycans (Box 1). Siglecs are type I transmembrane proteins with an N-terminal sialic acid-binding site and most of them possess one or more immunoreceptor tyrosine-based inhibitory motifs (ITIMs) at their C-terminus [4,20]. In general, binding of sialic acid ligands to immune inhibitory Siglecs results in inhibition of immune cell activation and function. Therefore, the sialic acid/Siglec pathway has been proposed to signal self-recognition limiting immune activation and destruction of host cells [19].

3. Sialic acids in tumor immune evasion

Already fifty years ago, Barbara H. Sanford and others proposed that aberrantly high expression of sialic acids on tumor cells allows immune escape (Box 2). They suggested that the dense layer of sialic acids found on tumor cells masks surface antigens preventing recognition by the immune system (antigen masking). This concept was supported by the observation that removal of sialic acids from tumor cells using bacterial sialidases strongly increased their immunogenicity and hindered growth in immunocompetent mice [28,29]. Subsequently, sialidase-treated tumor cells were exploited as preventive and therapeutic vaccine in several animal tumor models. These vaccines showed impressive results in animal tumor models and culminated in clinical trials in men. However, efficacy of sialidase-treated tumor vaccines could not be demonstrated unequivocally in cancer patient cohorts between 1970 and 1990 [30,31]. Nevertheless, these early important studies indicated a vital role for sialic acids in tumor immunology and defined them as potential target for tumor immunotherapy.

Today, advances in glycobiology and immunology renewed the interest into tumor sialoglycan research and revealed their strong immunomodulatory potential beyond the concept of antigen masking. A growing body of evidence indicates that tumor sialoglycans affect numerous immune relevant processes and make a major contribution to immune evasion. Below, we review immunomodulatory processes at the tumor sialoglycan-immune cell interface in the context of tumor immune escape.

3.1. Sialic acids in complement system evasion

In vertebrates, sialoglycans were suggested to characterize host cells as *self* and have been reported to prevent activation of the complement system by recruitment of the complement control protein factor H to the cell surface [13,19]. Factor H has several polyanionic binding sites that bind to sialoglycans, glycosaminoglycans and other negatively charged molecules on the surface of host cells. Surface-bound factor H hinders deposition and amplification of the complement-activating protein C3b on the cell surface and downstream activation of the alternative complement pathway. This regulatory mechanism has been suggested to prevent inappropriate complement activation and killing of sialylated host cells [47]. Pathogens that lack sialic acid expression do not recruit factor H and activate the complement cascade [48]. Some pathogens like as *Neisseria meningitides*, have been found to utilize host sialic acids as molecular mimicry to recruit factor H to their surface and avoid lysis by the complement system [49,50]. Although the role of the complement system in tumorigenesis is not yet understood, it has been suggested that tumor cells escape from complement activation by covering their membrane with sialoglycans [51,52]. Removal of sialic