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REVIEW

Where catabolism meets signalling: neuraminidase 1 as a modulator of cell receptors

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Abstract Terminal sialic acid residues are found in abundance in glycan chains of glycoproteins and glycolipids on the surface of all live cells forming an outer layer of the cell originally known as glycocalyx. Their presence affects the molecular properties and structure of glycoconjugates, modifying their function and interactions with other molecules. Consequently, the sialylation state of glycoproteins and glycolipids has been recognized as a critical factor modulating molecular recognitions inside the cell, between the cells, between the cells and the extracellular matrix, and between the cells and certain exogenous pathogens. Sialyltransferases that attach sialic acid residues to the glycan chains in the process of their initial synthesis were thought to be mainly responsible for the creation and maintenance of a temporal and spatial diversity of sialylated moieties. However, the growing evidence also suggests that in mammalian cells, at least equally important roles belong to sialidases/neuraminidases, which are located on the cell surface and in intracellular compartments, and may either initiate the catabolism of sialoglycoconjugates or just cleave their sialic acid residues, and thereby contribute to temporal changes in their structure and functions. The current review summarizes emerging data demonstrating that neuraminidase 1 (NEU1), well known for its lysosomal catabolic function, can be also targeted to the cell surface and assume the previously unrecognized role as a structural and functional modulator of cellular receptors.

Keywords Neuraminidase · Sialidase · Lysosome · Plasma membrane · Catabolism · Exocytosis · Secretion · Immune response · Phagocytosis · Cytokines · Elastin · Cell growth factors · Muscle differentiation

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Abbreviations

4MU-NeuAc	(2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid
CathA	cathepsin A/protective protein
ddNeuAc	2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid
EBP	elastin-binding protein
FcγR	Fc receptors for immunoglobulin G
GAL	β-galactosidase
GS	galactosialidosis
IGF-II	insulin-like growth factor II
IL-4	interleukin 4
IFN	interferon
IR	insulin receptor
LAMP-1	lysosome associated membrane protein 1
LPS	lipopolysaccharide
LSD	lysosomal storage disease
NEU1	neuraminidase 1

NEU2	neuraminidase 2
NEU3	neuraminidase 3
NEU4	neuraminidase 4
PDGF	platelet-derived growth factor
SGC	sialoglycoconjugate
Sia	sialic acid
SMC	smooth muscle cells
Syk	serine-tyrosine kinase
TLR	Toll-like receptor

Synthesis and processing of sialylated glycoconjugates

Sialic acids (Sia) are a family of nine-carbon α-keto acids found predominantly at the non-reducing end of oligosaccharide chains of glycoproteins and glycolipids (gangliosides), generally linked to either the 3- or 6-hydroxyl group of galactose (Gal), the 6-hydroxyl group of *N*-acetylglucosamine (GlcNAc), or *N*-acetylgalactosamine (GalNAc). Sia can also exist as a (2-8)-linked homopolymer in polysialylated gangliosides and glycoproteins.

Sialoglycoconjugates (SGC) are found in abundance on cell surfaces of animals and bacteria, forming a dense array of glycans often referred to as the sialome [1]. The sialome has been recently compared to the "canopy of a forest" that covers the cell membrane with a diverse array of complex sialylated structures [1]. Similarly sialylated is the inner surface of the lysosomal and endosomal membranes [2]. SGC are not evenly distributed on the membranes but rather form dynamic microdomains, so called "clustered saccharide patches" [3–6]. A well-recognized family of such sialylated clusters is termed "glycosynapses," and comprised of glycolipids, *O*-linked mucin-type glycoproteins, or a complex of gangliosides and sialylated membrane proteins [7]. As well, the majority of soluble secreted and lysosomal proteins contain Sia as parts of their glycan chains.

In mammals, the content of SGC strongly depends on the cell and tissue type, and significantly changes during development. These changes have been well documented at the level of total Sia released from the sample either by enzymatic treatment or by acid hydrolysis, or at the level of underlying glycan chains by labeling with antibodies or lectins specific for individual glycans [8]. Less is known about the changes in the levels of individual proteins or lipids carrying sialylations.

Sia are involved in a huge variety of biological processes, stemming from their diverse physical and chemical properties [9]. The most important role of Sia is the direct participation in diverse masking and recognition events, mediating a variety of biological phenomena, such as cell differentiation, interaction, migration, and metastasis (reviewed in [9–11]). Also, being among the most common ligands (or receptors) for pathogenic and non-pathogenic viruses, bacteria and protozoa, Sia modulate their ability to infect and to propagate within the mammalian organisms (reviewed in [9–11]). Described above glycosynapses are known to mediate cell signaling and are involved in cell adhesion, motility and growth [7].

In mammals, the synthesis of SGC is performed by a family of sialyltransferases that catalyze the transfer of Sia from CMP-Sia to an acceptor carbohydrate [12]. Twenty mammalian sialyltransferases identified to date show a high variation in terms of specificity, tissue and cellular distribution, and induction profile, thus reflecting remarkable functional diversity of their substrates [12]. Hydrolytic cleavage of Sia linked to mono- or oligosaccharide chains of glycoconjugates is catalyzed by the family of exoglycosidases called neuraminidases (*N*-acylneuraminosyl glycohydrolases; EC 3.2.1.18) or sialidases [9].

Pioneering studies on the biogenesis of membrane sialoglycoproteins established that the half-life of the Sia residues in the glycan chains of glycoproteins is several times shorter than the half-life of other sugar residues and of the proteins themselves [13, 14], suggesting that, besides catabolism, neuraminidases are also involved in "trimming" Sia residues from glycoconjugates. Further work demonstrated selective desialylation and resialylation of plasma membrane gangliosides and glycoproteins [15, 16], establishing that the net status of sialoglycoconjugates depends on the interplay of neuraminidases and sialyltransferases triggered by numerous external or internal factors.

Mammalian neuraminidase 1 (NEU1) and its catabolic role

Neuraminidases have been identified in numerous viral, bacterial, fungal, protozoan, avian and mammalian species [17, 18]. To date 4 members of the neuraminidase family (neuraminidase-1 (NEU1) [19–21]; neuraminidase-2 (NEU2) [22, 23]; neuraminidase-3 (NEU3, also known as ganglioside sialidase) [24–26], and neuraminidase-4 (NEU4) [27, 28]) with different, although partially overlapping tissue expression, intracellular localization and substrate specificity, have been identified in mammals. NEU1 was initially localized to the lysosomes [19, 21], NEU2 to the cytosol [23, 30, 31] and NEU3 to caveolae microdomains of plasma membranes [32] as well as the endosomal and lysosomal membranes [33]. Both the human and mouse *NEU4* genes are spliced in 2 different forms resulting in the appearance of two NEU4 isoforms, differing in the first 12N-terminal amino acid residues [28, 34]. The short isoform is found predominantly on the ER [28, 35], whereas the long is targeted both to the mitochondria [34, 35] and/or lysosomes [29]. In contrast to

NEU2, NEU3 and NEU4, which show tissue-specific expression patterns [reviewed in 36], NEU1 is ubiquitously expressed in all tissues with the highest expression in the kidney, pancreas, skeletal muscle, liver, lungs, placenta and brain [19]. In these tissues NEU1 generally shows 10–20 times higher expression than NEU3 and NEU4, and ~10^3-10^2 higher expression than NEU2 [34, 37].

The human *NEU1* gene is located on chromosome 6 in the locus of the major histocompatibility complex (6p21.3) [19–21, 38]. The single splice product results in a synthesis of the 46–48 kDa protein [19–21]. In many tissues NEU1 was reported to be membrane-bound and requiring high concentrations of detergents for its solubilisation (reviewed in [39]), although there is still no complete understanding of the mechanism of its interaction with cellular membranes. Lukong *et al.* suggested that NEU1 is an integral membrane protein carrying the C-terminal transmembrane domain followed by a Tyr-containing internalization signal for targeting the lysosomal membrane [40]. They also proposed that in the lysosome, the transmembrane domain of NEU1 can be further cleaved, resulting in a soluble form of the enzyme that has been purified from several mammalian tissues [reviewed in 39]. In contrast, homology models of NEU1 tertiary structure [reviewed in 36] do not predict the existence of trans-membrane segments, suggesting that NEU1 rather associates with the membrane by other mechanisms such as lipid anchors, or through interactions with other membrane protein(s).

The analysis of human fibroblasts overexpressing NEU1 by immunoelectron microscopy has shown that the enzyme is also present at the plasma membrane, as well as in the exocytic and/or endocytic vesicles [41]. This localization could be considered as transient and attributed to the fusion of lysosomal and plasma membranes during the lysosomal exocytosis, but numerous studies have shown that the cell surface pool of NEU1 is detected under specific conditions and in particular cell types including activated lymphocytes, neutrophils and monocytes [40, 42, 43].

In contrast to other mammalian, bacterial or viral neuraminidases, the enzymatic activity of NEU1 is triggered by its association with the lysosomal protective protein/cathepsin A (CathA). NEU1, CathA, and β-galactosidase (GAL) form a lysosomal multienzyme complex, with a mass of ~1200 kDa (reviewed in [36, 39, 44]). It is thought that through association with CathA, NEU1 and GAL acquire their active and stable conformation in the lysosome [36, 39, 44]. However, since the tertiary structure of NEU1 and its complex with CathA and Gal is still unknown, the mechanism of NEU1 enzymatic activation in the complex remains mainly unclear. Recently, Bonten *et al.* studied the interaction between the soluble recombinant NEU1 and CathA expressed in insect cells and showed that in the absence of CathA, NEU1 self-associates into inactive chain-like oligomers [45]. CathA presumably competes with NEU1 for the same binding site and can reverse the self-association of NEU1 by causing the disassembly of NEU1 oligomers and the formation of 102 kDa CathA-NEU1 heterodimers [45]. The authors, however, did not observe further association of these heterodimers into high-molecular weight complexes, suggesting that the interaction between the recombinant forms of CathA and NEU1 can be different from that occurring in the cell.

In the lysosome, NEU1 is involved in the catabolism of sialylated glycoconjugates, primarily oligosaccharides and glycoproteins [46–50], so its genetic deficiency results in a severe metabolic disease sialidosis (OMIM #256550), caused by the lysosomal storage of these macromolecules (reviewed in [44]). In addition, the genetic deficiency of CathA results in the secondary deficiencies of NEU1 and GAL, and causes the lysosomal storage disorder galactosialidosis (GS OMIM #256540) (reviewed in [51]). Both disorders clinically manifest with skeletal and gait abnormalities, progressive impaired vision, bilateral macular cherry-red spots, ataxia, seizures and myoclonus syndrome. Severe early-onset forms are also associated with a dysmorphic phenotype, dysostosis multiplex, mental retardation and hepatosplenomegaly [44, 51]. Knockout mouse models of sialidosis (*NEU1* KO) and galactosialidosis (*CathA* KO) develop a severe systemic disease closely resembling human conditions [52, 53]. In addition, a spontaneous mouse model of NEU1 deficiency, an SM/J mouse originally characterized by altered sialylation of several lysosomal glycoproteins [54], shows reduced levels of Neu1 activity (20-30% of normal) because of a point mutation in the gene promoter and/or L209I amino acid substitution in the protein [55–57]. SM/J mice as well as B10.SM mice that have NEU1 SM/J mutations transferred to a B.10 genetic background causing a similar reduction of NEU1 activity do not show any gross abnormalities but display altered immune reactions [87, 91, 92] described in details in the sections below.

Role of NEU1 in regulation of receptors and signalling pathways

In contrast to the above described catabolic role of NEU1, which has been known for decades, its participation in diverse cellular regulatory mechanisms has been discovered only recently, mainly through studies of animal models of neuraminidase deficiency. Below we summarize data pointing to the essential roles of NEU1 in the regulation of exocytosis, carcinogenesis, the immune response, generation of extracellular matrix, cell proliferation and differentiation, through desialylation of specific protein targets (See Fig. 1).

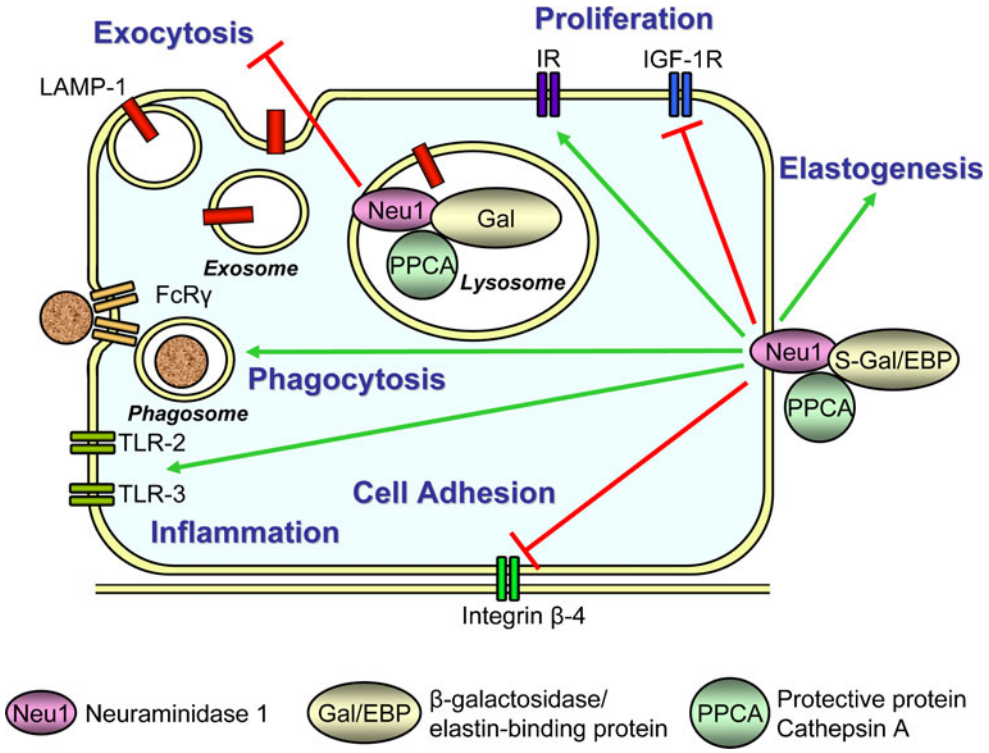

Fig. 1 Proposed roles of neuraminidase 1 in regulation of cell signalling. Components of the multienzyme lysosomal complex, neuraminidase 1 (NEU1), protective protein/cathepsin A (PPCA) and β-galactosidase (Gal), are sorted from the trans-Golgi network to the endosomal pathway where they participate in the lysosomal catabolism of glycoconjugates. NEU1-PPCA-Gal complex is also sorted to the vesicles destined to the plasma membrane. The 67-kDa splice variant of β-galactosidase S-Gal/EBP, NEU1 and CathA form the elastin receptor targeted to the plasma membrane and involved in extracellular assembly of elastic fibers. NEU1 activated by PPCA within NEU1-PPCA-Gal and NEU1-PPCA- S-Gal/EBP complexes desialylates and activates receptors for phagocytosis (FcRγ), inflammation (TLR-1 and TLR-2) and cell proliferation/glucose uptake (IR). It also desialylates and inhibits receptors for cell adhesion (Integrin β-4), exocytosis (LAMP-1) and cell proliferation (IGF-1R)

Exocytosis Regulated lysosomal secretion or lysosomal exocytosis is presently recognized as an important part of the diverse regulatory mechanisms in the immune system and a number of other cell types including membrane repair in fibroblasts, entry of trypanosomes into cells, and the secretion of viruses and morphogens [reviewed in 58, 59]. A number of cellular proteins involved in either the binding of lysosomes to and their movement along the microtubules or the fusion of lysosomal and plasma membranes (adaptor protein-3, Lyst protein, Rab proteins, Munc 13 proteins, Rab27a effector proteins and SNARE proteins) have already been identified as regulators of lysosomal exocytosis [58, 59]. Genetic deficiencies of many of these proteins in humans and mice result in impaired or partially impaired lysosomal secretion [58, 59]. In contrast, bone marrow stromal cells and neutrophils from *NEU1* KO mice showed enhanced secretion of lysosomal proteases and glycosidases and an increased presence of heavily sialylated lysosome associated membrane protein 1 (LAMP-1) on the cell surface both indicative of enhanced lysosomal exocytosis [60]. The authors suggest that NEU1 acts as a negative regulator of lysosomal exocytosis through its participation in the processing of LAMP-1, which is implicated in the process of lysosomal exocytosis and the fusion of lysosomes with exosomes according to the earlier work of Kima *et al.* [61], who studied its role in the mechanism of entry of trypanosomes into non-phagocytic Chinese hamster ovary (CHO) cells. To gain entry, *Trypanosoma cruzi* trypomastigotes enhance the recruitment of lysosomes to the host cell surface, so the lysosome fusion at the site of parasite entry leads to the formation of a parasitophorous vacuole with lysosomal properties [61]. The increased expression of LAMP-1 at the cell surface makes CHO cells more susceptible to trypomastigote invasion in a microtubule-dependent fashion and enhances the exocytosis of lysosomal β-hexosaminidase, whereas mutation of critical residues in the lysosome-targeting motif of LAMP-1 abolish the enhancement of *T. cruzi* invasion [61]. In the cultured cells and tissues from NEU1-deficient mice LAMP-1 shows increased glycosylation (sialylation) and a prolonged half-life resulting in the overall increase of its intracellular level and induced lysosomal exocytosis, whereas the siRNA-mediated inhibition of LAMP-1 expression reverses the phenotype [60].

The enhanced secretion of serine proteases from hematopoietic cells in the bone marrow niche of *NEU1* KO mice leads to the inactivation of extracellular serpins, premature degradation of VCAM-1, and loss of bone marrow retention, providing a link between the NEU1 deficiency and impaired long-term bone marrow engraftment [60]. Moreover, a recent paper from the same group shows that the increased lysosomal exocytosis from marginal cells of the stria vascularis into the endolymph was linked to reduced endolymphatic potential, dysfunction of transduction in sensory hair cells and hearing loss reported in *NEU1* KO mice, also observed in sialidosis patients [62].

In our opinion, the increased exocytosis of lysosomal proteases from NEU1-deficient cells provides an important insight into pathophysiology of sialidosis. However, direct evidence for the LAMP-1-dependent mechanism has yet to be provided since *LAMP-1* KO mice show normal secretion of lysosomal cathepsin D and do not display any signs suggesting decreased exocytosis of lysosomes or lysosome-related organelles [63]. Moreover, a recent study by Caspar *et al.* did not show any difference between the invasion of *T. cruzi* extracellular amastigotes in embryonic fibroblasts from *LAMP-1* KO mouse and wild type cells [64]. It is possible, therefore, that the increased amount and/sialylation of LAMP-1 (or other glycoproteins) on the lysosomal membrane simply modifies its properties favoring the process of its merging with the plasma membrane during exocytosis.

Migration, invasion and adhesion of cancer cells Cancer cells are associated with a significant over-representation of Sia on the surface glycoproteins as compared with normal cells [*e.g.* 65–68]. Lipid- and protein-bound Sia are also elevated in the plasma from cancer patients as compared with healthy individuals [66, 69–72] and are linked to acute phase condition and chronic disease [*e.g.* 73, 74], indicating that Sia-containing glycoproteins/peptides can potentially serve as biomarkers for cancer. Similarly important, alterations in the levels of all 4 neuraminidases have been detected in cancer cells and correlated with their malignancy [reviewed in 75, 76]. In particular, the level of NEU1 activity and expression in different clones of transformed rat fibroblast 3Y1 cells and mouse adenocarcinoma colon 26 cells inversely correlated with their metastatic potential [77, 78]. Further study from the same group demonstrated that the overexpression of NEU1 in mouse B16 melanoma cells reversed their metastatic capacity as detected by the suppression of the experimental pulmonary metastasis as well as their invasiveness in collagen gels and motility on colloidal gold-coated glass plates [79]. Although these experiments did not identify the molecular mechanism of the observed changes in metastatic capacity, they potentially implicated NEU1 as a negative regulator of malignant properties of cancer cells.

Further insight into the NEU1 role in cancer malignancy came from the recent work by Uemura *et al.* [80] who showed that *NEU*1 overexpression in colon cancer HT-29 cells significantly reduced their liver metastasis potential after transplantation in mice as well as migration, invasion and adhesion properties *in vitro*, whereas *NEU1* silencing caused the opposite effect. The authors demonstrated that NEU1 overexpression in HT-29 cells resulted in the desialylation of the laminin receptor, integrin β4, essential to carcinoma migration and invasion. Desialylation of integrin β4 caused its decreased phosphorylation, attenuation of downstream kinases and suppression of cell adhesion to laminin [80]. Besides, NEU1 overexpression caused downregulation of matrix metalloproteinase-7, also associated with cancer metastasis [80]. The proposed hypothesis that integrin β4 is one of the NEU1 target molecules controlling the malignant properties in cancer cells was indirectly supported by the fact that chemical inhibition of *O*-linked integrin β4 glycosylation caused effects similar to that of NEU1 overexpression. However, since NEU1 desialylated multiple proteins on the cell surface, it is not clear whether the downregulation of integrin β4-mediated signaling was the only mechanism involved, and whether similar events occur in the other types of cancer cells.

Modulation of immune response and inflammation The important role of Sia in the function of immune cells has been well documented. In particular, members of the Siglec (sialic acid binding immunoglobulin-like lectins) superfamily contribute in the scavenging function of macrophages, pathogen uptake and antigen presentation [reviewed in 3]. Similarly, the sialylation level of the cell surface substantially affects the capacity of resting B cells to stimulate the proliferation of allogeneic and antigen specific syngeneic T cells [81–85], and increases the phagocytosis capacity of influenza virus-infected HeLa cells [86]. Moreover, a neuraminidase with an acidic pH optimum was detected on the surface of activated mouse T cells where it was shown to be essential for the early production of interleukin 4 (IL-4) and the interaction of T cells with the antigen presenting cells [87–89]. It has also been shown that neuraminidase present on the surface of T cells converted the group specific component (Gc) protein into a factor necessary for the inflammation-primed activation of macrophages [90, 91]. T-cells derived from the introduced above SM/J or B10.SM strains of mice with reduced NEU1 activity failed to convert Gc and synthesize IL-4, while B cells of these mice produced less IgG1 and IgE [87, 91, 92]. Induction of NEU1 on the surface of activated T lymphocytes was further directly shown to contribute to general cell surface desialylation and to the production of interferon (IFN)-gamma [93].

During the differentiation of circulating blood monocytes and monocytic cell lines into macrophages, *NEU1* expression is induced 12 fold, and the newly produced pool of the enzyme is targeted mostly to the cell surface [43, 94]. In contrast to other cellular neuraminidases, NEU2, NEU3 and NEU4, whose expression either remains unchanged or reduced, NEU1 mRNA protein and activity are specifically increased during the differentiation, coinciding with a significant induction of the transcriptional activity of the *NEU1* gene promoter [43]. NEU1 and its activator CathA are first targeted to the lysosome and then are sorted to the LAMP-2-negative, MHC II-positive vesicles, which later merge with the plasma membrane [43]. The biological importance of this phenomenon was clarified in a gene-targeted mouse model with ~10% of the residual NEU1 activity in tissues [95]. Macrophages and immature dendritic cells from NEU1-deficient mice showed increased sialylation of the cell surface and compromised ability to engulf gram-positive and gram-negative bacteria, as well as IgG-opsonized and non-opsonized particles and IgG-coated red blood cells, suggesting that all types of phagocytosis are affected [96]. The observed effect was relevant to the deficiency of Neu1 activity since the treatment of the cells with the exogenous mouse NEU1, which reduced the sialylation of the cell surface to the normal levels, completely restored the phagocytosis [96]. The authors also showed that the absence of NEU1 in particular affected transduction of signals from the Fc receptors for immunoglobulin G (FcγR). The macrophages from NEU1-deficient mice showed increased sialylation and impaired phosphorylation of FcγR, as well as markedly reduced phosphorylation of serine-tyrosine kinase (Syk) in response to treatment with IgG-opsonized beads. Therefore it is conceivable that cell surface NEU1 activates phagocytosis in macrophages and dendritic cells through desialylation of surface receptors [96], although it would be interesting to investigate whether other alterations in cell signaling (primarily in Siglec-mediated response) can also contribute to the observed impaired phagocytosis in NEU1 deficient mice.

FcγR is probably not the only immune receptor regulated by NEU1. The recently published studies by Amith *et al.* suggested that NEU1 could also be involved in activation of cell surface Toll-like receptors (TLR) that play key roles in activating immune responses during infection [97]. The authors showed that ligand binding to TLR-2, -3 and −4 rapidly induces NEU1 activity in bone marrow-derived macrophages, as well as in macrophage and dendritic cell lines, and that the interaction of activated NEU1 with TLRs promotes intracellular signaling [97]. In macrophage and dendritic cell lines, lipopolysaccharide (LPS)-induced interaction of TLR-4 with the signal transducer protein, MyD88 and subsequent activation of NFkappaB signaling pathway were dependent on the presence of NEU1 because TLR-4-derived signalling was impaired in the cells from NEU1-deficient mice, allowing the authors to speculate that NEU1 changes activity of the receptor by removing Sia from its glycan chains [98].

At the same time, the above work reported that the LPS-induced NEU1 activity and TLR-derived signalling were effectively (IC_{50} 1.2 μmol) blocked by oseltamivir phosphate (Tamiflu) [98], which contradicted previously published data that Tamiflu scarcely inhibited any neuraminidase including NEU1 even at 1 mM concentration [99]. In the follow-up report from the same group, Tamiflu was also found to be highly potent (IC_{50} 3.9 μM) in inhibiting neuraminidase (presumably NEU1) activity induced on the surface of live TrkA receptor-positive rat adrenal pheochromocytoma (PC12) cells by treatment with nerve growth factor [100]. The authors speculate that the reasons for such inhibitory potency of Tamiflu are unknown but may be due to "a unique orientation of NEU1 with the molecular multi-enzymatic complex". In our opinion, further studies remain necessary to determine whether the effect of Tamiflu detected *in cellulo* is caused by its action on NEU1 or by its indirect action on other cellular proteins.

Dependence of LPS-induced cytokine production on neuraminidase activity in dendritic cells was also reported by a recent study of Stamatos *et al.* [101]. The authors showed that monocyte-derived dendritic cells treated with broad neuraminidase inhibitor 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (ddNeuAc) show decreased expression of IL-6, IL-12p40, and TNF-α in response to LPS treatment and increased cell surface sialylation. However, in contrast to the study by Amith *et al.*, the authors of this work speculate that the neuraminidase implicated in TLR signalling is NEU3 and not NEU1. They show that 1 mM zanamivir, a pharmacologic inhibitor of Neu3 [99] also reduced cytokine expression from LPS treated dendritic cells [101]. Also, levels of IL6 or TNF-α produced by the LPS-treated dendritic cells derived from NEU1-knockout mice were similar to those measured for wild type cells [101]. Unfortunately, similarly to the study of Amith *et al.* the work of Stamatos *et al.* lacks essential controls necessary to confirm the roles of NEU1 and NEU3 in regulation of cytokine production. In particular, the 1 mM concentration of zamanivir used in this study to treat the cells is close to its *Ki* (1,121±250 mM) for NEU1 [99]. There is no evidence that zamanivir at a 1 mM concentration in the culture medium can not at least partially inhibit NEU1 exposed at the cell surface. Also, the authors neither comment on the apparent difference in the levels of cytokines produced by the ddNeuAc- and zamanivir-treated cells nor do they provide a direct evidence for NEU3 involvement in cytokine production,

such as experiments in cells with the knocked out or knocked down *NEU3* gene.

The last immune receptor reported to be functionally regulated through NEU1-mediated desialylation is the hyaluronic acid (HA) receptor, CD44 implicated in multiple cell-cell and cell-matrix interactions. Earlier work showed that Sia in the surface glycan chains of CD44 and the homologous LYVE-1 receptor can mask their binding to hyaluronic acid [102, 103]. Recently, indirect evidence that NEU1 desialylates and activates CD44 was obtained from studying splenic CD4+ T cells treated with neuraminidase inhibitors or derived from SM/J mice with reduced NEU1 activity [104]. Using an experimental mouse asthma model, the authors show that the Th2 cytokine concentration and absolute number of Th2 cells were reduced in the bronchoalveolar lavage fluid from the NEU1-deficient SM/J mice as compared to wild type mice [104], although it is not clear whether this phenotype was solely due to the increased CD44 sialylation.

Altogether, the summarized data implicate NEU1 as a regulator of multiple pathways in the immune cells, suggesting that it may become a potential target molecule for treatment of disorders related to immunity and inflammation.

Modulation of elastic fiber assembly Constitutive cell surface targeting of NEU1 and its activating partner CathA was first reported when both proteins were identified as components of the elastin receptor, which also contains the elastin-binding protein (EBP), a 67 kDa enzymatically inactive, alternatively spliced variant of lysosomal β-galactosidase, S-gal [105–108]. Anti-NEU1 and anti-CathA antibodies showed an identical pattern of immunolocalization on the surface of elastin-producing cells, as did an antibody recognizing the S-Gal/EBP [109]. The complex containing S-Gal/EBP, NEU1 and CathA could be purified from a cell membrane fraction of human fibroblasts using affinity chromatography on elastin columns.

All three components of the elastin receptor were shown to actively participate in the formation of elastic fibers [110]. While the EBP initially plays the role of the intracellular molecular chaperone for hydrophobic and non-glycosylated tropoelastin and assures its proper secretion, the cell surface-residing NEU1 catalyzes the removal of the terminal sialic acids from carbohydrate chains of microfibrillar proteins, forming the structural scaffold of new elastic fibers, unmasking their penultimate galactosugars. The exposed galactosugars, in turn, interact with the galactolectin domain of the EBP, thereby inducing the release of transported tropoelastin molecules and facilitating their subsequent assembly into elastic fibers.

The crucial role of EBP in the formation of elastic fibers is potentially supported by connective-tissue, skeletal and cardiovascular defects observed in G_{M1} gangliosidosis (OMIM #230500) and Morquio B (OMIM #253010) patients with mutations in the *GAL* gene that lack both β-Gal and EBP [111, 112]. These patients also show cardiac valve deformations, aortic stenosis, and intimal thickening in the coronary arteries and in the pulmonary artery [113, 114]. Clinical symptoms related to the defects in the elastic fiber formation (most often cardiomyopathies) have been also documented in GS and sialidosis patients [115]. Similarly, the NEU1-deficient fibroblasts from sialidosis patients have impaired elastogenesis, which could be reversed after the transfection of cells with vectors containing NEU1 and CathA cDNA [110]. Impaired elastogenesis connected to the defective development of aorta, skin and lungs has also been detected in *NEU1* KO mice [116]. In particular, the elastic lamellae in the aorta of the *NEU1* KO mice were thinner and separated by hypertrophic smooth muscle cells that were surrounded by an excess of the sialic acid-containing moieties. The concentration of elastin in the aorta was significantly reduced but the production of tropoelastin was normal, suggesting the elastic fiber defects result from impaired extracellular assembly [116]. Altogether, the reviewed data implicate NEU1 and the important functional component of the elastin receptor contributing to the normal development of elastic fibers, a crucial component of the cardiovascular and respiratory systems.

Modulation of cell proliferation Finally, two recent publications demonstrated that NEU1 is involved in the fine tuning of a group of homologous receptors regulating the cellular mitogenic response to growth factors. The first study [117] demonstrated that the NEU1 residing on the cell surface of arterial smooth muscle cells (SMC) contributes to downregulation of cellular proliferation by desialylation of cell surface receptors interacting with the platelet-derived growth factor BB (PDGF-BB receptor) and insulin-like growth factor (IGF-II) receptor, potent stimulators of cell proliferation also implicated in the progression of atherosclerosis [118–122]. The inhibition of endogenous NEU1 with ddNeuAc or anti-NEU1 antibody in cultures of arterial SMC and normal dermal fibroblasts coincided with the increase of their proliferation rates. In addition, the treatment of the cells with exogenous neuraminidase from *Clostridium perfringens* (sharing substrate specificity with mammalian NEU1) resulted in the reduction of cellular proliferation and eliminated the PDGF-BB- and IGF-II-induced increase in the phosphorylation of β-subunits of their respective receptors. Fibroblasts derived from sialidosis patients had a significantly stronger mitogenic response to the same doses of PDGF-BB and IGF-II than fibroblasts of the normal skin, suggesting that NEU1 deficiency resulted in a greater number of cell surface receptors remaining sialylated and thus more responsive to their respective growth factors [117].

The follow-up study [123] demonstrated that in addition to the IGF-II receptor, the homologous IGF-1R receptor and insulin receptor (IR) can be also affected by NEU1 and that desialylation of these two receptors affects their activity in opposite directions. The physiological (0.5–1 nM) and therapeutic (10 nM) doses of insulin stimulated the proliferation of cultured skeletal muscle progenitors L6WT through IR, and the effect could be further enhanced following the desialylation of this receptor by exogenous mammalian NEU1, or by the addition of *Clostridium perfringens* neuraminidase to the cell culture. In contrast, the inhibition of endogenous NEU1 with ddNeuAc or with the anti-NEU1 antibody abolished the proliferative response in L6 cells treated with 1 or 10 nM doses of insulin. A similar inhibitory effect was observed when the expression of endogenous NEU1 has been inhibited by the siRNA. On the other hand, the supra-physiological (100 nM) concentration of insulin induced a more potent proliferative response in L6 myoblasts, which was transmitted through IGF-1R. This response was inhibited following treatment of the cells with exogenous neuraminidases, and enhanced in cultures treated with ddNeuAc, the anti-NEU1 antibody or NEU1 siRNA [123]. These data endorsed the notion that the enzymatic activity of cell surface NEU1 could structurally alter and functionally inactivate IGF-1R, thereby decreasing the proliferative responsiveness of skeletal myoblasts to extremely high doses of insulin. They also prompted the hypothesis that desialylation of IGF-1R could serve as a negative modulator that prevents the overzealous response of myoblasts to extremely high dose of insulin. It is likely that the desialylation of the receptors directly affects their ability to undergo the autophosphorylation prerequisite for the subsequent downstream signals in such a way that either increases (IR) or decreases (IGF-1R) their insulin-induced activation. Indeed, Tyr-phosphorylation of IR as well as activation of the downstream Akt kinase in L6 cells co-treated with 1 or 10 nM of insulin and exogenous neuraminidases were markedly higher than in cells treated with insulin alone [123]. In contrast, pretreatment of L6 myoblasts with NEU1 or *Clostridium perfringens* neuraminidase abolished the phosphorylation of IGF-1R induced by 100 nM insulin [123].

The above mentioned results suggesting a crucial role of NEU1 in skeletal muscle growth correlate well with the observation that the expression of NEU1 is up-regulated by the muscle regulatory factor, myogenic determination gene (MyoD) on early stages of myogenesis and that the experimental overexpression of NEU1 in myoblastic cells coincides with their heightened proliferation and inhibition of the differentiation cascade [124]. Besides, early-onset sialidosis type II patients and mice with the KO *NEU1* gene present with severe progressive muscular atrophy [52, 125]. It is necessary to mention, however, that muscular atrophy in sialidosis patients and NEU1 KO mice can also be explained by the expansion of perimysial and endomysial connective tissue, coupled to the increased proliferation of fibroblast-like cells, abnormal deposition of collagen fibers, and enhanced proteolytic activity in the extracellular matrix linked to the deficiency of NEU1 [98].

Conclusion

In conclusion, multiple data endorse the novel role for mammalian neuraminidase NEU1 as an important factor modulating the activity of cell surface receptors. Future studies should reveal both the mechanism by which sialylation alters receptor activity and the exact biological role played by this new type of regulation. Nevertheless, the net sialylation of receptors likely constitutes another, not fully appreciated and understood level in a complicated process controlling initiation of cellular signaling in such diverse processes as phagocytosis and pro-inflammatory response in macrophages, migration, invasion and adhesion of cancer cells, proliferation of skeletal smooth muscle progenitors and assembly of elastic fibers by arterial smooth muscle cells (Fig. 1). Moreover, considering the diversity of involved pathways, it is tempting to speculate that NEU1 may even coordinate activities of independent signalling pathways all aimed at achieving the same biological goal. For example, during the immune response, the induction of NEU1 activates both phagocytosis and interleukin production in macrophages and dendritic cells, induces antibody production by B cells and increases adhesion of neutrophils and lymphocytes.

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