Glycoconjugate Journal (2022) 39:677–683
https://doi.org/10.1007/s10719-022-10067-7

MINI REVIEW

Sialidase NEU3 and its pathological significance

Taeko Miyagi[1] · **Koji Yamamoto**[2]

Received: 25 January 2022 / Revised: 7 May 2022 / Accepted: 25 May 2022 / Published online: 8 June 2022

Abstract
Sialidases (EC 3.2.1.18, also called neuraminidases) catalyze the removal of α-glycosidically linked sialic acid residues from glycoproteins and glycolipids; this is the initial step in the degradation of these glycoconjugates. Sialidases of mammalian origin have been implicated in not only lysosomal catabolism but also the modulation of functional molecules involved in many biological processes. To date, four types of mammalian sialidases have been cloned and designated as Neu1, Neu2, Neu3 and Neu4. These sialidases differ in their subcellular localization and enzymatic properties, as well as their chromosomal localization, and they are expressed in a tissue-specific manner. Among the sialidases, the plasma membrane-associated sialidase Neu3 appears to play particular roles in controlling transmembrane signaling through the modulation of gangliosides, and its aberrant expression is closely related to various pathogeneses, including that of cancer. Interestingly, the human orthologue NEU3 acts in two ways, catalytic hydrolysis of gangliosides and protein interactions with other signaling molecules. Aberrant NEU3 expression can induce various pathological conditions. This review briefly summarizes recent studies, focusing on the involvement of NEU3 in various pathological phenomena.

Keywords Sialidase · Gangliosides · cancer · Hepatic steatosis · Pulmonary fibrosis · Transmembrane signaling

Characterization of sialidase NEU3

Human sialidases NEU1, NEU2, and NEU3 are localized predominantly in the lysosomes [1–4], cytosol [5] and plasma membranes [6–8], respectively, and the fourth sialidase, NEU4, has been suggested to exist in lysosomes [9], mitochondria [10] and certain intramembranous components [11, 12], from gene transfection studies. All contain several Asp boxes (-Ser-X-Asp-X-Gly-X-Thr-Trp-) and the Arg-Ileu-Pro sequence, which is conserved sequences also found in sialidases from microorganisms [13], despite the lack of any particular similarity in the overall primary structures.

The amino acid identity of NEU1 to the other sialidases is relatively low (19–24%), while NEU2, NEU3 and NEU4 show 34–40% homology to each other. NEU1 generally shows the strongest expression, 10–20 times greater than those of NEU3 and NEU4, whereas NEU2 expression is extremely low at the most at only 4000 to.

10,000th of the NEU1 value in a range of tissues [14], as assessed by a quantitative real-time RT–PCR using a standard curve for each cDNA. Human sialidase NEU1, a target gene for sialidosis, possesses narrow substrate specificity, with oligosaccharides and glycopeptides serving as good substrates. NEU2 and NEU4, in contrast, are able to hydrolyze glycoproteins and gangliosides at near neutral pH and at pH 4.6 *in vitro* respectively. On the other hand, the human plasma membrane-associated sialidase, NEU3, preferentially hydrolyses gangliosides but scarcely acts on other substrates, such as oligosaccharides and glycoproteins *in vitro* [6]. However, recent study demonstrated that NEU3 enhances EGFR activation without affecting EGFR expression and acts on its sialylation levels using colorectal cancer cells [15]. The general information of human sialidases NEU1-4 is available in The Human Protein Atlas (https://www.proteinatlas.org).

The plasma membrane-associated sialidase was first cloned from a bovine brain library [6], based on peptide sequence information from the purified enzyme protein [16], and later from a human skeletal muscle cDNA library

✉ Taeko Miyagi
miyagi-ta@nifty.com

[1] Division of Cancer Chemotherapy, Miyagi Cancer Center Research Institute, Natori, Japan

[2] Faculty of Health and Medical Care, Saitama Medical University, Moroyama, Saitama, Japan

[7], and from the human genome data base [8]. In COS-7 cells transiently expressing the sialidase, the major subcellular localization was found to be plasma membranes by Percoll density gradient centrifugation of cell homogenates and by immune-fluorescence staining of the cells [17], although the enzyme does not contain any α-herical transmembrane segment. The primary sequences covering the entire coding region of the corresponding human, mouse and rat genes display an 83, 79 and 78% overall identity with the bovine gene, respectively. The bovine and human enzymes specifically hydrolyze gangliosides in the presence of Triton X-100 *in vitro*, whereas the murine enzymes act on oligosaccharides, 4MU-NeuAc (a synthetic substrate), and glycoproteins as well to a certain extent [18]. Gangliosides GD3, GM3, GD1a and even GD1b are good substrates for these enzymes, except for GM1 and GM2. However, desialylation of GM1 and GM2 by the murine enzymes is evident in the presence of GM2 activator protein [19], supporting the existence of an asialo-derivative GA2 pathway for catabolism of GM2 in the mouse. Unlike the bovine and murine enzymes with only one activity peak at pH nearly 4.6, the human enzyme, NEU3, shows two peaks in its pH curve, at pH 4.5–4.8 and at pH 6.0–6.5 [7].

NEU3 plays a crucial role in the regulation of transmembrane signaling through the modulation of ganglioside degradation and by direct interaction with signaling molecules. Subsequent analysis of membrane topology has suggested that the sialidase might be localized partially on the cell surface as a peripheral membrane protein and also in endosomes [20], where it interacts with various signaling molecules such as EGFR, caveolin-1, and Integrin β4 [21]. In response to growth stimuli such as EGF treatment, NEU3 mobilizes to membrane ruffles together with Rac-1, a small G protein participating in actin reorganization and cell motility, and enhances cell movement [22]. Furthermore, there is evidence that NEU3 interacts with phosphatidic acid in the inner-leaflet of membranes, leading to activation and translocation to the cell surface [23], and also modulates clathrin-mediated endocytosis, probably by affecting the structural organization and subcellular distribution of the clathrin adaptor AP-2 complex [24]. In this context, the portion of NEU3 having the catalytic region would be facing the extracellular environment, thus allowing its interaction with ganglioside substrates inserted in the outer leaflet of the plasma membrane. This would be also consistent with the fact that NEU3 is able to remove sialic acid residues from gangliosides present on adjacent cells [23, 25]. Interestingly, recent study revealed from the new aspects that NEU3 is S-acylated by its post -translational modification restricted to the cytosolic side of membranes [26], indicating that NEU3 may contain a cytosolic exposed domain. Thus, NEU3 plays a crucial role in the regulation of transmembrane signaling through the modulation of ganglioside catabolism and by direct interaction with signaling molecules.

Aberrant expression of sialidase NEU3 in cancer

Carbohydrate portions of glycoproteins and glycolipids undergo neoplasia associated alterations, including increase in branching of asparagine-linked glycans, increase in the number and/or length of polylactosaminoglycan chains, and increase in sialylation [27]. Altered sialylation of glycolipids is also observed as a ubiquitous phenotype, leading to the appearance of tumor-associated antigens, aberrant adhesion, and blocking of transmembrane signaling [28]. In earlier days, alterations of sialidase activity against gangliosides were described to be associated with malignant transformation in BHK-transformed cells [29]. An increase of a ganglioside sialidase activity had also been linked with induction of anchorage-independent growth in mouse epidermal JB6 cells on exposure to phorbol esters [30]. Those observations suggested that ganglioside hydrolyzing sialidase might be involved in carcinogenesis. In fact, NEU3 was found marked up-regulation in various cancers, whereas NEU1 and NEU4 showed a tendency of down-regulation [31].

After gene cloning, up-regulation of NEU3 was observed in various neoplasms including colon, renal, ovarian and prostate cancers, the two obvious exceptions being down-regulation in acute lymphoblastic leukemia [32] and in glioblastoma [33] in relation to disease progression. NEU3 mRNA levels were first found in human colon cancers to be increased 3- to 100-fold as carcinogenesis, compared to adjacent non-tumor mucosa [34]. In situ hybridization analysis with an antisense probe demonstrated positive signals to be localized to carcinoma cells, rather than surrounding stromal cells. During sodium butyrate induced apoptosis, human colon cancer cells showed down-regulation of NEU3 expression and in contrast, upregulation of NEU1. Transfection of a NEU3 gene into cancer cells was found to inhibit this sodium butyrate induced apoptosis, accompanied by increase in Bcl-2 protein and decreased caspase expression. Human colon cancer specimens were found to exhibit marked accumulation of lactosylceramide, a possible NEU3 product, compared with adjacent non-cancerous mucosa. In line with these results, addition of the glycolipid to cultures reduced apoptotic cells during sodium butyrate treatment. In colon cancer cells, NEU3 may differentially regulate cell proliferation through integrin-mediated signaling depending on the extracellular matrix [35], causing increased adhesion to laminins and consequent cell-division, but rather decrease in cell adhesion to fibronectin, collagen I and IV.

Triggered by laminins, NEU3 markedly enhanced tyrosine phosphorylation of intergrin β4, with recruitment of Shc and Grb-2, stimulating phosphorylation of focal adhesion kinase and ERK1/2.

Significant increase of NEU3 mRNA levels has been noted in renal cell carcinomas [36], correlating with elevation of interleukin (IL)-6, a pleiotropic cytokine. NEU3 is activated by IL-6 and directs IL-6- mediated signaling via the PI3K/Akt cascade in a positive feedback manner, and thus contributes to a malignant phenotype, including suppression of apoptosis and promotion of cell motility in renal cell carcinoma ACHN cells.

Upregulation of NEU3 has also been detected in prostate cancer, with a significant correlation to malignancy as assessed by the Gleason score [37]. In androgen-sensitive LNCaP cells, forced overexpression of NEU3 significantly induced expression of the progression-related transcription factor EGR-1, the androgen receptor and PSA both with and without androgen, the cells becoming sensitized to androgen. NEU3-mediated induction was abrogated by inhibitors of PI-3 kinase and MAPK, in line with increased phosphorylation of AKT and ERK1/2 in NEU3-overexpressing cells. NEU3 siRNA introduction caused reduction of cell growth of androgen-independent PC-3 cells in culture and of transplanted tumors in nude mice. There is further evidence of involvement in expression of malignant properties, in that NEU3 knockdown contributed to decreased cell motility, invasion and in vivo bone metastasis with decreased expression of the matrix metalloproteinases, MMP-2 and MMP-9, in PC-3 cells. The data suggest that NEU3 regulates tumor progression of prostate cancer through androgen receptor signaling, and is likely to be involved in bone metastasis.

Recently, in adenocarcinoma of Non-Small Cell Lung Cancer (NSCLC), NEU3 overexpression was also found to stimulate the ERK pathway and this activation was completely abolished by gefitinib treatment [38]. Patients carrying EGFR mutations therefore may benefit from EGFR targeted therapies. These findings indicate that NEU3 can act directly on the ERK pathway through EGFR and both directly and indirectly with respect to EGFR on the Akt pathway. It has also been reported that NEU3 overexpression in glioblastoma U87MG cells activates PI3K/Akt signaling pathway, resulting in an increased radioresistance capacity and in an improved efficiency of double strand DNA-repair mechanisms after irradiation [39].

Furthermore, it was found that a significant increase of sialidase activity in the serum of patients with prostate cancer compared with that in healthy subjects having low activity by sensitive activity assay with GM3 substrates and by a sandwich ELISA method using two anti-NEU3 antibodies. Interestingly, sera additionally contained inhibitory activity against the sialidase and the sialidase inhibitor activities could be separated by exosome isolation, providing a potential utility for novel diagnosis of human cancer [40]. Another evidence of exosomes secreted by HeLa cells i*n vitro* shuttle NEU3 on their external surface as demonstrated by enzyme activity measurements, Western blot analysis, and dot blot analysis [41].

NEU3 as a signaling molecule

To define further the molecular mechanisms of NEU3 influence and its possible targets, the encoding gene has been silenced by siRNA or overexpressed in various human cancer cells [42]. Silencing caused apoptosis without specific stimuli, accompanied by decreased Bcl-xL and increased mda7 and GM3 synthase mRNA levels in HeLa cells, whereas overexpression resulted in the opposite effects. Human colon and breast carcinoma cell lines, HT-29 and MCF-7 cells, appeared to be similarly affected by treatment with the NEU3 siRNA, but interestingly noncancerous human fibroblasts and keratinocytes showed no significant changes. NEU3 siRNA was found to inhibit Ras activation and NEU3 overexpression to stimulate it with consequent influence on ERK and Akt. Ras activation by NEU3 was largely abrogated by PP2 (a src inhibitor) or AG1478 (an EGFR inhibitor). In fact, the siRNA introduction reduced phosphorylation of EGFR while overexpression promoted its phosphorylation in response to EGF. NEU3 co-immunoprecipitated with EGFR, and EGF-stimulation yielded a higher amount of immunoprecipitable NEU3. NEU3 was then found to activate Src kinase, and the clonogenicity was completely suppressed by a Src inhibitor, PP2. The activity-null mutants failed to activate Src and EGFR, indicating that ganglioside modulation by NEU3 may be necessary for the activation. NEU3 and Src were co-immunoprecipitated with EGFR in NEU3- and EGFR- transfected cells. These findings identify NEU3 as an essential participant in tumorigenesis through the EGFR/Src signaling pathway and a potential target for inhibiting EGFR-mediated tumor progression. These results indicate that NEU3 suppresses apoptosis of cancer cells by promoting EGFR phosphorylation, probably through its association with EGFR and consequent activation of Ras cascades, especially via the Ras/ERK pathway [43]. Up-regulation of NEU3 has also been established to have importance for the promotion stage of colorectal carcinogenesis in vivo, from experiments using NEU3 transgenic mice [44]. Thus, NEU3 was found to increase azoxymethane-induced aberrant crypt foci formation in colon mucosa by suppression of apoptosis, possibly via activation of EGF signaling.

Furthermore, transcription factors Sp1 and Sp3 bind to the assigned Sp1-binding motif of the NEU3 gene and

seem also to play a role in selection of the two clusters of transcriptional start site. NEU3 expression was found to be diversely regulated by Sp1/Sp3 transcription factors binding to alternative promoters [45]. Such transcriptional control might also account for the up-regulation of NEU3 in cancer, because Sp1 and Sp3 have been documented to play critical roles in regulating the transcription of genes involved in cell growth control and tumorigenesis [46]. In fact, the expression of NEU3 exhibited good correlations with those of Sp1 or Sp3 in cancer, implying a promoting role in NEU3 gene.

To summarize, the sialidase activates the molecules including FAK, ILK, Shc, integrinβ4 and EGFR, often upregulated in carcinogenesis, which may cause acceleration of progression to malignant phenotype in cancer cells. This sialidase could be a useful target for cancer diagnosis and therapy.

Involvement of NEU3 in non-cancerous pathological conditions

NEU3 promotes adipose tissue formation and hepatic steatosis

NEU3 up-regulation induced by intestine hypoxia-inducible factor (HIF-2α) was observed in human intestine biopsy specimens from individuals with or without obesity. HIF-2α signaling was positively correlated with body-mas index and hepatic toxicity. The regulation of ceramide metabolism by HIF2α increased NEU3 expression in hepatic steatosis. The causality of this correlation was verified in mice with an intestine-specific disruption of Hif-2a; these mice exhibited lower levels of high-fat-diet-induced hepatic steatosis and obesity than those observed in control mice. The results suggest that intestinal HIF-2α could be a viable target for hepatic steatosis therapy [47]. Other observations also confirmed that mice lacking Neu3 showed lower levels of high-fat diet -induced adipose tissue and hepatic steatosis compared with control mice, and injections of a sialidase inhibitor DANA (N-acetyl-2,3dehydro- 2-deoxyneuraminic acid) reduced weight and steatosis [48].

NEU3 activates the cardiac cell response to chronic hypoxia by inducing HIF1α

Hypoxia is a common feature of many congenital heart defects (CHD), contributing significantly to these pathophysiologies. Increased levels of HIF-1α, NEU3, EGFR and their downstream targets were observed in right atrial appendage biopsies from cyanotic patients, compared with acyanotic controls. NEU3 was found to play a central role in activating the cell response to chronic hypoxia by inducing the up-regulation of HIF-1α; this could be a novel approach to treating several CHD pathologies [49].

NEU3 reduces cardiac fibrosis by inhibiting the TGF-β signaling pathway

Cardiac fibrosis is a key physiological response to cardiac tissue injury, protecting the heart from wall rupture. No efficient antifibrotic therapies are presently available. The involvement of the transforming growth factor beta (TGF-β) signaling pathway has been recognized in myofibroblast activation and fibrosis progression, and the direct involvement of GM3 in TGF-β receptor1(TGF-R1) activation has been shown. Based on this observation, the induction of NEU3 significantly reduced cardiac fibrosis in primary cultures of human cardiac fibroblasts by inhibiting the TGF-β signaling pathway, decreasing collagen I deposition. These results suggest that modulating of the GM3 cell content might represent a novel approach to treating cardiac fibrosis [50].

NEU3 promotes pulmonary fibrosis by inducing TGF-β1accumulation

The extensive desialylation of glycoconjugates and the upregulation of sialidases have been observed in fibrotic lesions in human and mouse lungs. The fibrosis-associated cytokine TGF-β1 upregulates sialidases in human airway epithelium cells, lung fibroblasts, and immune system cells. Conversely, the addition of sialidases to human peripheral blood mononuclear cells induces the accumulation of extracellular TGF-β1, forming a sialidase - TGF-β1 – sialidase-positive- feedback loop. Monocyte-derived fibrocytes also activated fibroblasts, and sialidases might potentiate fibrocyte differentiation. A sialylated glycoprotein, serum amyloid P (SAP), inhibits fibrocyte differentiation, and sialidases attenuate SAP function. Interestingly, injections of the sialidase inhibitors DANA and oseltamivir (Tamiflu) attenuated pulmonary fibrosis in a bleomycin-treated mouse pulmonary fibrosis model, by breaking the feedback loop, causing the downregulation of sialidase and TGF-β1 accumulation [51]. Furthermore, the same group investigated only Neu3 among the four mammalian sialidases, was detected in the bronchoalveolar lavage fluid from mice with bleomycin-induced pulmonary fibrosis. NEU3 upregulated the extracellular accumulation of the profibrotic cytokines IL-6 and IL-1β, and IL-6 induced NEU3 in human peripheral blood mononuclear cells, suggesting that NEU3 might be involved in a positive feedback loop that potentiates fibrosis. In a bleomycin-induced pulmonary model, male and female *Neu3*$^{-/-}$mice had significantly less inflammation, a lower profibrotic cytokine active TGF-β1 level,

and less pulmonary fibrosis than male and female C57BL/6 mice. These results suggested that NEU3 participates in fibrosis and that NEU3 could be a target for the development of treatments for pulmonary fibrosis [52].

Mouse Neu3 induction triggers intestinal inflammation and colitis in a model of Salmonella enterica infection, a major source of human foodborne illness

Intestinal inflammation is considered to be the central pathological feature of colitis and the inflammatory bowel diseases. Gastric infections of Gram-negative *Salmonella enterica* Typhimurium (ST), is a major source of human food poisoning, caused inflammation of murine intestinal tissue. ST progressively disabled a host mechanism of protection by inducing endogenous sialidase activity, which accelerated clearance of intestinal alkaline phosphatase (IAP), and linked to a Toll-like receptor 4 (TLR4)-dependent mechanism of IAP desialylation with accumulation of the IAP substrate and TLR4 ligand. The administration of IAP or the antiviral sialidase inhibitor zanamivir was therapeutic by maintaining IAP abundance and function [53]. Based on these observations, using *Neu3*-null mice, Neu3 was identified for the development of colitis in a mouse model of recurrent human food-poisoning involving repeated transient *ST* infections during the adult lifespan. This pathway may serve as an effective target for future human inflammatory bowel diseases [54].

Together with NEU1, NEU3 triggers atherosclerosis by desialylating low-density lipoproteins and increasing their uptake by macrophages

The hypothesis that sialidases contribute to the development of atherosclerosis by removing sialic acid residues from glycan chains of the LDL glycoprotein and glycolipids has been examined. Atherosclerosis progression was investigated in apolipoprotein E and LDL receptor knockout mice with a genetic deficiency of Neu1, 3, and 4. Desialylation of the LDL glycoprotein, and apolipoprotein B 100, by human NEU1 and NEU3, increased the uptake of human LDL in human cultured macrophages and by macrophages in aortic root lesions in *Apoe*$^{-/-}$ mice via asialoglycoprotein receptor 1. The genetic inactivation or pharmacological inhibition of Neu1 and Neu3 significantly delayed the formation of fatty streaks in the aortic root without affecting the plasma cholesterol and LDL levels in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$mouse models of atherosclerosis. These results suggest that NEU1 and NEU3 trigger the initial phase of atherosclerosis and the formation of aortic fatty streaks by desialylating LDL and increasing uptake by resident macrophages [55].

Attempts to obtain selective inhibitors for NEU3

Oseltamivir (Tamiflu) and zanamivir (Relenza), used clinically effective anti-influenza drugs, are inhibitors of viral sialidase which differ in primary structures and enzyme properties but possess tertiary structures similar to those of human sialidases. Using human recombinant NEU3 enzyme with GM3 as substrate, oseltamivir carboxylate scarcely affected the activities of any of the sialidases, even at 1 mM, while zanamivir was found to inhibit significantly the activity in the micromolar range (Ki, 3.7 ± 0.48), providing a contrast to the low nanomolar concentrations for the viral sialidases [56]. To obtain small molecule inhibitors as research tools, a library of DANA analogues with modifications at C4 and C9 positions has been designed, and selective inhibitors targeting NEU3 were obtained. The inhibitor has a K_i of 320 ± 40 nM and a 15-fold selectivity over other human sialidases, shown by blocking glycolipid processing in vitro [57]. They were observed to cause significant retardation of in vitro cell migration assay on fibronectin coated surfaces in breast cancer and prostate cancer cell lines [58]. Based on these observations, further attempts may lead to the development of therapeutics for pathological conditions caused by NEU3.

Conclusions

The investigation of mammalian sialidases has uncovered a great deal of information regarding the molecular basis of aberrant sialylation on various pathological conditions, including cancer. Sialidase alterations could create potential applications in the diagnosis and cure of cancer. Interestingly, recent studies have demonstrated the involvement of NEU3 in various pathological conditions including hepatic steatosis, pulmonary and cardiac fibrosis, and possible inflammatory bowel diseases through the desialylation of signaling molecules. Overall, these results show that NEU3 plays important roles in many physiological phenomena and that changes in its expression can lead to pathological conditions. Further understanding of the role of NEU3 in these pathologies, and the discovery of its specific inhibitors, antibodies and siRNAs for controling the expression could lead to new tools for treatment.

Acknowledgements We would like to express our sincere gratitude to the late professor Hakomori, S.I. for his many pioneering studies and his leadership in the field of glycolipids in cancer.

Declarations

Conflict of interest None declared conflict of interests.

References

1. Bonten, E., van der Spoel, A., Fornerod, M., Grosveld, G., d'Azzo, A.: Characterization of human lysosomal neuraminidase defines the molecular basis of the metabolic storage disorder sialidosis. Genes Dev. **10**, 3156–3169 (1996)
2. Milner, C.M., Smith, S.V., Carrillo, M.B., Taylor, G.L., Hollinshead, M., Campbell, R.D.: Identification of a sialidase encoded in the human major histocompatibility complex. J. Biol. Chem. **272**, 4549–4558 (1997)
3. Pshezhetsky, A.V., Richard, C., Michaud, L., Igdoura, S., Wang, S., Elsliger, M.A., Qu, J., Leclerc, D., Gravel, R., Dallaire, L., Potier, M.: Cloning, expression and chromosomal mapping of human lysosomal sialidase and characterization of mutations in sialidosis. Nat. Genet. **15**, 316–320 (1997)
4. Carrillo, M.B., Milner, C.M., Ball, S.T., Snoek, M., Campbell, R.D.: Cloning and characterization of a sialidase from the murine histocompatibility-2 complex: low levels of mRNA and a single amino acid mutation are responsible for reduced sialidase activity in mice carrying the Neu1a allele. Glycobiology. **7**, 975–986 (1997)
5. Miyagi, T., Konno, K., Emori, Y., Kawasaki, H., Suzuki, K., Yasui, A., Tsuik, S.: Molecular cloning and expression of cDNA encoding rat skeletal muscle cytosolic sialidase. J. Biol. Chem. **268**, 26435–26440 (1993)
6. Miyagi, T., Wada, T., Iwamatsu, A., Hata, K., Yoshikawa, Y., Tokuyama, S., Sawada, M.: Molecular cloning and characterization of a plasma membrane-associated sialidase specific for gangliosides. J. Biol. Chem. **274**, 5004–5011 (1999)
7. Wada, T., Yoshikawa, Y., Tokuyama, S., Kuwabara, M., Akita, H., Miyagi, T.: Cloning, expression, and chromosomal mapping of a human ganglioside sialidase. Biochem. Biophys. Res. Commun. **261**, 21–27 (1999)
8. Monti, E., Bassi, M.T., Papini, N., Riboni, M., Manzoni, M., Venerando, B., Croci, G., Preti, A., Ballabio, A., Tettamanti, G., Borsani, G.: Identification and expression of NEU3, a novel human sialidase associated to the plasma membrane. Biochem. J. **349**, 343–351 (2000)
9. Seyrantepe, V., Landry, K., Trudel, S., Hassan, J.A., Morales, C.R., Pshezhetsky, A.V.: Neu4, a novel human lysosomal lumen sialidase, confers normal phenotype to sialidosis and galactosialidosis cells. J. Biol. Chem. **279**, 37021–37029 (2004)
10. Yamaguchi, K., Hata, K., Koseki, K., Shiozaki, K., Akita, H., Wada, T., Moriya, S., Miyagi, T.: Evidence for mitochondrial localization of a novel human sialidase (NEU4) Biochem. J. **390**, 85–93 (2005)
11. Monti, E., Bassi, M.T., Bresciani, R., Civini, S., Croci, G.L., Papini, N., Riboni, M., Zanchetti, G., Ballabio, A., Preti, A., Tettamanti, G., Venerando, B., Borsani, G.: Molecular cloning and characterization of NEU4, the fourth member of the human sialidase gene family. Genomics. **83**, 445–453 (2004)
12. Bigi, A., Morosi, L., Pozzi, C., Forcella, M., Tettamanti, G., Venerando, B., Monti, E., Fusi, P.: Human sialidase NEU4 long and short are extrinsic proteins bound to outer mitochondrial membrane and the endoplasmic reticulum, respectively. Glycobiology. **20**, 148–157 (2010)
13. Roggentin, P., Schauer, R., Hoyer, L.L., Vimr: E R. The sialidase superfamily and its spread by horizontal gene transfer: Mol. Microbiol. **9**, 915–921 (1993)
14. Koseki, K., Wada, T., Hosono, M., Hata, K., Yamaguchi, K., Nitta, K., Miyagi, T.: Human: cytosolic sialidase NEU2-low general tissue expression but involvement in PC-3 prostate cancer cell survival.Biochem Biophys Res Commun. 428,142–149 (2012)
15. Mozzi, A., Forcella, M., Riva, A., Difrancesco, C., Molinari, F., Martin, V., Papini, N. Bernasconi, B., Nonnis, S., Tedeschi, G., Mazzucchelli, L., Monti, E., Fusi, P. Frattini M.: NEU3 activity: enhances EGFR activation without affecting EGFR expression and acts on its sialylation levels. Glycobiology. **25**, 855–868 (2015)
16. Hata, K., Wada, T., Hasegawa A,Kiso M,Miyagi, T.: Purification and characterization of a membrane-associated ganglioside sialidase from bovine brain. J. Biochem. (Tokyo). **123**, 899–905 (1998)
17. Akita, H., Miyagi, T., Hata, K., Kagayama, M.: Immunohistochemical evidence for the existence of rat cytosolic sialidase in rat skeletal muscles. Histochem. Cell. Biol. **107**, 495–503 (1997)
18. Hasegawa, T., Yamaguchi, K., Wada, T., Takeda, A., Itoyama, Y., Miyagi, T.: Molecular cloning of mouse ganglioside sialidase and its increased expression in Neuro2a cell differentiation. J. Biol. Chem. **275**, 8007–8015 (2000)
19. Li, S.C., Li, Y.T., Moriya, S., Miyagi, T.: Degradation of G(M1) and G(M2) by mammalian sialidases. Biochem. J. **360**, 233–237 (2001)
20. Cirillo, F., Ghiroldi, A., Fania, C., Piccoli, M., Torretta, E., Tettamanti, G., Gelfi, C.: Anastasia L.NEU3 sialidase protein interactors in the plasma membrane and in the endosomes. J. Biol. Chem. **291**, 10615–10624 (2016)
21. Miyagi, T., Wada, T., Yamaguchi, K., Hata, K., Shiozaki, K.: Plasma membrane- associated sialidase as a crucial regulator of transmembrane signalling. J. Biochem. **144**, 279–285 (2008)
22. Yamaguchi, K., Hata, K., Wada, T., Moriya, S., Miyagi, T.: Epidermal growth factor-induced mobilization of a ganglioside-specific sialidase (NEU3) to membrane ruffles. Biochem Biophys Res Commun. 346, 484–490 (2006). (2006)
23. Shiozaki, K., Takahashi, K., Hosono, M., Yamaguchi, K., Hata, K., Shiozaki, M., Bassi, R., Prinetti, A., Sonnino, S., Nitta, K., Miyagi, T.: Phosphatidic acid-mediated activation and translocation to the cell surface of sialidase NEU3, promoting signaling for cell migration.FASEB J,29,2099–2111(2015)
24. Rodriguez-Walker, M., Vilcaes, A.A., Garbarino-Pico, E., Daniotti, J.L.: Role of plasma-membrane-bound sialidase NEU3 in clathrin-mediated endocytosis. Biochem. J. **470**, 131–144 (2015)
25. Valaperta, R., Chigorno, V., Basso, L., Prinetti, A., Bresciani, R., Preti, A., Miyagi, T., Sonnino, S.: Plasma membrane production of ceramide from ganglioside GM3 in human fibroblasts. FASEB J. **20**, 1227–1229 (2006)
26. Rodriguez-Walker, M., Daniotti, J.L.: Human sialidase Neu3 is S-acylated and behaves like an integral membrane protein. Sci. Rep. **7**, 4167 (2017)
27. Lau, K.S., Dennis, J.W.: N-Glycans in cancer progression. Glycobiology. **18**, 750–760 (2008)
28. Hakomori, S.I.:Glycosynaptic, microdomains controlling tumor cell phenotype through alteration of: cell growth, adhesion, and motility. FEBS Lett. **584**, 1901–1906 (2010)
29. Schengrund, C.-L., Lausch, R.N., Rosenberg, A.: Localization of sialidase in the plasma membrane of rat liver cells. J. Biol. Chem. **248**, 4424–4428 (1973)
30. Miyagi, T., Sagawa, J., Kuroki, T., Matsuya, Y., Tsuiki, S.: Tumor-promoting phorbol ester induces alterations of sialidase and sialyltransferase activities of JB6 cells. Jpn J. Cancer Res. **81**, 1286–1292 (1990)
31. Miyagi, T., Wada, T., Yamaguchi, K., Shiozaki, K., Sato, I., Kakugawa, Y., Yamanami, H., Fujiya, T.: Human sialidase as a cancer marker. Proteomics. **8**, 3303–3311 (2008)
32. Mandal, C., Tringali, C., Mondal, S., Anastasia, L., Chandra, S., Venerando, B.: Down regulation of membrane-bound Neu3 constitutes a new potential marker for childhood acute lymphoblastic leukemia and induces apoptosis suppression of neoplastic cells. Int. J. Cancer. **126**, 337–349 (2010)

33. Takahashi, K., Proshin, S., Yamaguchi, K., Yamashita, Y., Katakura, R., Yamamoto, K., Shima, S., Hosono, M., Miyagi, T.: Sialidase NEU3 defines invasive potential of human glioblastoma cells by regulating calpain-mediated proteolysis of focal adhesion proteins. BBA - General Subjects. **1861**, 2778–2788 (2017)
34. Kakugawa, Y., Wada, T., Yamaguchi, K., Yamanami, H., Ouchi, K., Sato, I., Miyagi, T.: Up-regulation of plasma membrane-associated ganglioside sialidase (Neu3) in human colon cancer and its involvement in apoptosis suppression. Proc. Natl. Acad. Sci. U S A. 99, 10718–10723 (2002)
35. Kato, K., Shiga, K., Yamaguchi, K., Hata, K., Kobayashi, T., Miyazaki, K., Saijo, S., Miyagi, T.: Plasma-membrane-associated sialidase (NEU3) differentially regulates integrin-mediated cell proliferation through laminin- and fibronectin-derived signalling. Biochem. J. **394**, 647–656 (2006)
36. Ueno, S., Saito, S., Wada, T., Yamaguchi, K., Satoh, M., Arai, Y., Miyagi, T.: Plasma membrane-associated sialidase is up-regulated in renal cell carcinoma and promotes interleukin-6-induced apoptosis suppression and cell motility. J. Biol. Chem. **281**, 7756–7764 (2006)
37. Kawamura, S., Sato, I., Wada, T., Yamaguchi, K., Li, Y., Li, D., Zhao, X., Ueno, S., Aoki, H., Tochigi, T., Kuwahara, M., Kitamura, T., Takahashi, K., Moriya, S., Miyagi, T.: Plasma membrane-associated sialidase (NEU3) regulates progression of prostate cancer to androgen-independent growth through modulation of androgen re- ceptor signaling. Cell. Death Differ. **19**, 170–179 (2012)
38. Forcella, M., Oldani, M., Epistolio, S., Freguia, S., Monti, E., Fusi, P., Frattini, M.: Non-small cell lung cancer (NSCLC), EGFR downstream pathway activation and TKI targeted therapies sensitivity: Effect of the plasma membrane-associated NEU3.PLoS One.12,e0187289.(2017)
39. Orizio, F., Triggiani, L., Colosini, A., Buglione, M., Pasinetti, N., Monti, E., Bresciani, R.: Overexpression of sialidase NEU3 increases the cellular radioresistance potential of U87MG glioblastoma cells. Biochem. Biophys. Res. Commun. **508**, 31–36 (2019)
40. Hata, K., Tochigi, T., Sato I.,3Kawamura S.,2, Shiozaki, K., Wada T.,1Takahashi T., Moriya, S. 1 Yamaguchi, K., Hosono, M., Miyagi, T. Increased sialidase activity in serum of cancer patients: Identification of sialidase and inhibitor activities in humanserum Cancer Sci,106,383–389(2015)
41. Paolini, L., Orizio, F., Busatto, S., Radeghieri, A., Bresciani, R., Bergese, P., Monti, E.: Exosomes secreted by HeLa Cell shuttle on their surface the plasma membrane-associated sialidase NEU3. Biochemistry. 56,6401–6408. (2017)
42. Wada, T., Hata, K., Yamaguchi, K., Shiozaki, K., Koseki, K., Moriya, S., Miyagi, T.: A crucial role of plasma membrane- associated sialidase in the survival of human cancer cells. Oncogene. **26**, 2483–2490 (2007)
43. Yamamoto, K., Takahashi, K., Shiozaki, K.,. Yamagushi, K., Moriya, S.,. Hosono, M., Shima, H., Miyagi, T. Potentiation of epidermal growth factor -mediated oncogenic transformation by sialidase NEU3 leading to Src activation.PLoS One, 10(3), e0120578, (2015)
44. Shiozaki, K., Yamaguchi, K., Sato, I., Miyagi, T.: Plasma membrane-associated sialidase (NEU3) promotes formation of colonic aberrant crypt foci in azoxymethane-treated transgenic mice. Cancer Sci. **100**, 588–594 (2009)
45. Yamaguchi, K., Koseki, K., Shiozaki, M., Shimada, Y., Wada, T., Miyagi, T.: Regulation of plasma-membrane-associated sialidase NEU3 gene by Sp1/Sp3 transcription factors. Biochem J. 430:107–117:. (2010)
46. Wierstra, I. Sp1: emerging roles– beyond constitutive activation of TATA-less housekeeping genes: Biochem. Biophys. Res. Commun. **372**, 1–13 (2008)
47. Xie, C., Yagai, T., Luo, Y., Liang, X., Chen, T., Wang, Q., Sun, D., Zhao, J., Ramakrishnan, S.K., Sun, L., Jiang, C., Xue, X., Tian, Y., Krausz, K.W., Patterson, A.D., Shah, Y.M., Wu, Y., Jiang, C., Gonzalez, F.J.: Activation of intestinal hypoxia-inducible factor 2α during obesity contributes to hepatic steatosis. Nat. Med. **23**, 1298–1308 (2017)
48. Pilling, D., Karhadkar, T.R., Gomer, R.H.: High-fat diet–induced adipose tissue and liver Inflammation and steatosis in Mice Are reduced by inhibiting sialidases. Am. J. Pathol. **191**, 131–143 (2021)
49. Piccoli, M., Conforti, E., Varrica, A., Ghiroldi, A., Cirillo, F., Resmini, G., Pluchinotta, F., Tettamanti, G., Giamberti, A., Frigiola, A., Anastasia, L.: NEU3 sialidase role in activating HIF-1alpha in response to chronic hypoxia in cyanotic congenital heart patients. Int. J. Cardiol. **230**, 6–13 (2017)
50. Ghiroldi, A., Piccoli, M., Creo, P., Cirillo, F., Rota, P., D'Imperio, S., Ciconte, G., Monasky, M.M., Micaglio, E., Garatti, A., Aureli, M., Carsana, E.V., Menicanti, L., Pappone, C., Anastasia, L.: Role of sialidase Neu3 and ganglioside GM3 in cardiac fibroblasts activation. Biochem. J. **477**, 3401–3415 (2020)
51. Karhadkar, T.R., Pilling, D., Cox, D., Gomer, N.: Sialidase inhibitors attenuate pulmonary fibrosis in a mouse model. Sci. Rep. **7**, 15069 (2017)
52. Karhadkar, T.R., Chen, W., Gomer, R.H.: Attenuated pulmonary fibrosis in sialidase-3 knockout (*Neu3*$^{-/-}$) mice. Am. J. Physiol. Lung Cell. Mol. Physiol. **318**, L165–L179 (2020)
53. Yang, W.H., Heithoff, D.M., Aziz, P.V., Sperandio, M., Nizet, V., Mahan, M.J., Marth, J.D. Recurrent infection progressively disables host protection against intestinal inflammation. Science. Dec 22;358(6370): eaao5610 (2017)
54. Yang, W.H., Westman, J.S., Heithoff, D.M., Sperandio, M., Cho, J.W., Mahan, M.J., Marth, J.D. Neu3 neuraminidase induction triggers intestinal inflammation and colitis in a model of recurrent human food-poisoning.Proc Natl Acad Sci U S A.118(29):e2100937118
55. Demina, E.P., Smutova, V., Pan, X., Fougerat, A., Guo, T., Zou, C., Chakraberty, R., Snarr Shiao, B.D., Roy, T.C., Orekhov, R., Miyagi, A.N., Laffargue, T., Sheppard, M., CairoCW, D.C., Pshezhetsky, A.V.: Neuraminidases 1 and 3 trigger atherosclerosis by desialylating low-density lipoproteins and increasing their uptake by macrophages. J. Am. Heart Assoc. **10**(4), e018756 (2021)
56. Hata, K., Koseki, K., Yamaguchi, K., Moriya, S., Suzuki,Y., Yingsakmongkon, Hirai, S., Sodeoka, G., von Itzstein, M., Miyagi, M., T.:Limited inhibitory effects of oseltamivir and zanamivir on human sialidases.Antimicrob. Agents Chemother. 52, 3484–3491 (2008)
57. Guo, T., Dätwyler, P., Demina, E., Richards, M.R., Ge, P., Zou C, Zheng R, Fougerat A. Pshezhetsky, A.V., Ernst, B., Cairo, C.W.: Selective Inhibitors of Human Neuraminidase 3. J. Med. Chem. **8**, 611990–612008 (2018)
58. Howlader, M.A., Guo, T., Chakraberty, R., Cairo, C.W. Isoenzyme-selective inhibitors migration: ACS Chem. Biol. **15**(6), 1328–1339 (2020)