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Plasma Membrane-associated Sialidase as a Crucial Regulator of Transmembrane Signalling

Taeko Miyagi*, Tadashi Wada, Kazunori Yamaguchi, Keiko Hata and Kazuhiro Shiozaki

Division of Biochemistry, Miyagi Cancer Center Research Institute, Natori, 981-1293, and CREST, JST, Japan

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Mammalian sialidases, glycosidases responsible for the removal of sialic acids from glycoproteins and glycolipids, has been implicated to participate in many biological processes as well as in lysosomal catabolism. Among those forms identified to date, plasma membrane-associated sialidase, Neu3, is a key enzyme in degradation of gangliosides, for which it exhibits a special substrate preference. This sialidase has been shown to control transmembrane signalling for many cellular processes, including cell differentiation, cell growth and apoptosis, and human orthologue NEU3 is markedly up-regulated in various cancers. It is known to suppress apoptosis in cancer cells. Furthermore, its overexpression causes impaired glucose tolerance and hyper-insulinaemia together with overproduction of insulin in enlarged islets in the transgenic mice. The present review primarily summarizes our recent results, focusing on Neu3 as a regulator of transmembrane signalling.

Key words: cancer, diabetes, gangliosides, sialidase, transmembrane signalling.

Abbreviations: EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; FAK, focal adhesion kinase; IR, insulin receptor; IRS-1, insulin receptor substrate I; LacCer, lactosylceramide.

Sialic acids are acidic monosaccharides generally found in the terminal positions on a variety of glycoproteins and glycolipids. They are widely distributed in nature and have been extensively characterized as biologically important molecules with structural diversity (*1–3*). They actually play crucial roles in various biological processes by influencing chemical and biological features of glycoconjugates, probably due to their negative charge. Sialic acids show striking differences in quantity as well as structure during cell differentiation, proliferation and carcinogenesis, and may contribute as virulence factors in bacterial and viral infection.

Sialidases catalyse the removal of α-glycosidically linked sialic acid residues from glycoproteins and glycolipids, which is the initial step in the degradation of these glycoconjugates. These enzymes exist in common in metazoan animals, from echinoderms to mammals, and are also found in various viruses and other microorganisms including fungi, protozoa and bacteria, and even in forms mostly lacking sialic acids. Sialidases of mammalian origin have been implicated not only in lysosomal catabolism but also in modulation of functional molecules involved in many biological processes (*3–5*), whereas in microorganisms the same enzymes appear to play roles limited to nutrition and pathogenesis (*6*). Four types of mammalian sialidases have been cloned to date and designated as Neu1, Neu2, Neu3 and Neu4. They differ in their subcellular localization and enzymatic properties, as well as in the chromosomal localization, and are expressed in a tissue-specific manner. To understand their physiological functions, we cloned sialidase genes, and were able to obtain a rat Neu2 gene as the first example of mammalian sialidase (*7*). Subsequently, Neu3 genes were cloned from libraries for bovine (*8*) and human (*9*) brains. Among the sialidases, the plasma membrane-associated sialidase Neu3 appears to play particular roles in controlling transmembrane signalling by modulation of gangliosides, and its aberrant expression is closely related to the pathogenesis of cancer and the diabetic phenotype. It is of special interest that human orthologue NEU3 acts in two ways, not only through catalytic hydrolysis of gangliosides but also through protein interactions with other signalling molecules.

CHARACTERIZATION OF SIALIDASE Neu3

The characteristic features of the primary structures of the four human sialidases are compared in Fig. 1. Three of them are localized predominantly in the lysosomes (*10–12*), cytosol (*13*) and plasma membranes (*9*, *14*) and the fourth sialidase, NEU4, has been suggested to exist in lysosomes (*15*), mitochondria (*16*) and certain intramembranous components (*16*, *17*), 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 (*18*), despite the lack of any particular similarity in the overall primary structures and in functions. 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.

*To whom correspondence should be addressed. Tel: +81-22-384-3151, Fax: +81-22-381-1195, E-mail: miyagi-ta173@pref.miyagi.jp

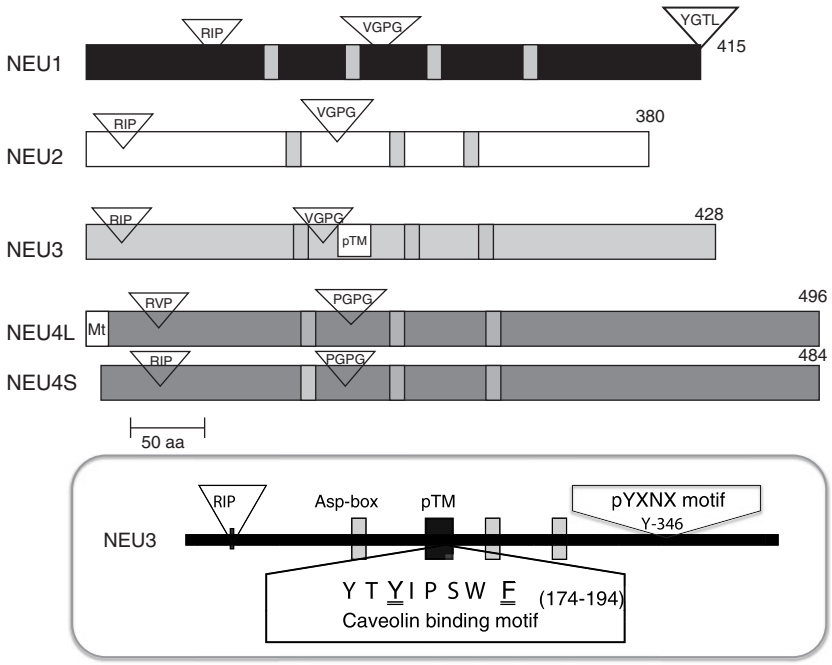

Fig. 1. **Schematic characteristics of primary structure of four human sialidases and binding motifs of NEU3.** All contain several Asp boxes (shaded light grey box, -Ser-X-Asp-X- Gly-X-Thr-Trp-) and the Arg-Ileu-Pro sequence, conserved sequences found in microbial sialidases. NEU1 features a possible lysosomal C-terminal targeting motif (YGTL), NEU3 possesses long hydrophobic stretch as a putative transmembrane domain (pTM), and NEU4 consists of two isoforms differing in the presence and absence of 12 N-terminal amino acid residues (Mt), which may act in mitochondrial targeting. The lower panel indicates caveolin-binding domain and pYXNX motif via its SH2 domain found in NEU3. A mutation with a single amino acid change (double underlined) in the caveolin-binding motif leads to inhibition of recruitment of NEU3 to the caveolae.

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 hydrolyse glycoproteins and gangliosides at near neutral pH and at pH 4.6, respectively. The human plasma membrane-associated sialidase, NEU3, almost specifically hydrolyses gangliosides but scarcely acts on other substrates, including oligosaccharides and glycoproteins. The properties of the murine sialidases are similar to those of the corresponding human sialidases, except for the variation in substrate specificity described below.

The plasma membrane-associated sialidase was first cloned from a bovine brain library (*8*), based on peptide sequence information from the purified enzyme protein (*19*), and later from a human brain library (*9*). In COS-7 cells transiently expressing the bovine sialidase, the major subcellular localization was found to be plasma membranes by Percoll density gradient centrifugation of cell homogenates and by immunofluorescence staining of cells. 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 hydrolyse gangliosides in the presence of Triton X-100, and the murine enzymes act on oligosaccharides, a synthetic substrate, 4MU-NeuAc, and glycoproteins as well to a certain extent. 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 (*20*), 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 (*9*).

On administration of the radiolabelled ganglioside GD1a to murine Neu3-transfected cells, Neu3 was shown to hydrolyse ganglioside substrates in intact living cells at a neutral pH, with probable involvement in cell–cell interactions through hydrolysis of gangliosides at the surfaces of neighbouring cells (*21*). Unlike the bovine and mouse Neu3 sialidases, the human orthologue NEU3 is not always detected on the cell surface and may exist in other intramembranous components. 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*). Recent 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 endosomal structures (*23*).

PARTICIPATION OF SIALIDASE Neu3 IN NEURITE FORMATION

Unlike lysosomes, plasma membranes do not contain a set of glycosidases for degradation, implying that Neu3 in this site probably plays crucial roles in regulation of cell surface functions rather than catabolism.

Kopitz *et al*. (*24*) reported that 2,3-dehydro- 2-deoxy-*N*-acetylneuraminic acid, a sialidase inhibitor, reduces differentiation markers in human neuroblastoma cells and that a cell surface sialidase may take part in growth inhibition and neural differentiation by providing the reaction product GM1 as a ligand for galectin 1, without affecting cell apoptosis. After cloning, we documented Neu3 to participate in neurite formation in mice and in human neuroblastoma cells, as well as in regulation and regeneration in rat hippocampus neurons. During 5-bromodeoxyuridine-induced Neuro2a cell differentiation, endogenous sialidase was increased in the activity and mRNA levels, and transfection of the Neu3 gene accelerated neurite arborization (*25*). In human neuroblastoma NB-1 cells, NEU3 expression also increased when neurite outgrowth was induced by dibutyryl cAMP. While treatment with dibutyryl cAMP alone enhanced the outgrowth of dendrite-like processes, NEU3 transfection gave rise to a more prominent outgrowth of neurites with axon-like characteristics, even in the absence of dibutyryl cAMP (*26*).

In rat hippocampus neurons, Neu3 has been shown to induce axon specification by enhancing TrkA activity locally, which triggers phosphatidylinositol-3-kinase- and Rac1-dependent inhibition of RhoA signalling and modifies local actin stability to define axonal fate (*27*, *28*).

LOCALIZATION OF SIALIDASE NEU3 IN CAVEOLAE

Human NEU3 has been found located in rafts of neuroblastoma cells (*29*) and in caveolae of HeLa cells, closely associated with caveolin-1 (*30*). Deduced caveolin-binding motifs ($\phi X\phi XXXX\phi$ and $\phi XXXX\phi XX\phi$, where ϕ is an aromatic residue W, F or Y) (*31*) are present in most caveolae-associated proteins, including Src kinase, mitogen-activated protein kinase, and the EGF receptor. In the human NEU3, we identified a possible analogous region (YTYYIPSW, 179–186 residues) within the hydrophobic stretch of the putative transmembrane domain sequence. To determine whether the binding site in NEU3 is functional, the following strategies were employed. In transfectants expressing a polyhistidine-tagged form of NEU3, caveolin-1 was co-eluted with NEU3 on affinity column chromatography. A mutation with a single amino acid change in the caveolin-binding motif led to inhibition of recruitment of the sialidase to the microdomain, accompanied by reduction of the enzyme activity. NEU3 also failed to associate with caveolin-enriched microdomains by cholesterol depletion with β-cyclodextrin, with concomitant decrease of the sialidase activity, whereas NEU3 was activated by increased caveolin-1 expression. A tight association of NEU3 with caveolin-1 was supported further by co-immunoprecipitation of NEU3 by an anti-caveolin-1 antibody. These results strongly suggest that NEU3 functions as a caveolin-related signalling molecule within caveolin-rich microdomains.

ABERRANT EXPRESSION OF SIALIDASE NEU3 IN CANCER

Observations on sialidase activity in cancer cells have suggested that endogenous sialidase might be related to transformation and tumour invasiveness. In fact, four types of mammalian sialidases have been found to behave in different manners during carcinogenesis (*5*). Three of the sialidases, Neu1, Neu2 and Neu4 showed a tendency of down-regulation (*32*), while Neu3 showed marked up-regulation. Alterations of sialidase activity against gangliosides are associated with malignant transformation in 3T3-transformed cells (*33*), and in BHK-transformed cells (*34*). An increase of membrane ganglioside sialidase activity has been linked with induction of anchorage-independent growth in mouse epidermal JB6 cells on exposure to phorbol esters (*35*). Since these data suggest that a plasma membrane-associated ganglioside sialidase might be involved in carcinogenesis, after gene cloning, we investigated NEU3 expression in surgical specimens of human colon cancer (*36*). The mRNA levels were found to be increased 3- to 100-fold as compared to adjacent non-tumour mucosa, associated with significant elevation of sialidase activity. *In situ* hybridization confirmed high sialidase expression in epithelial elements of the adenocarcinomas. During sodium butyrate-induced apoptosis, human colon cancer cells show down-regulation of NEU3 expression and in contrast, up-regulation of the lysosomal sialidase NEU1. Transfection of a NEU3 gene into cancer cells inhibits this apoptosis accompanied by increased Bcl-2 and decreased caspase 3 expression. Colon cancer cells and tissues have been found to exhibit a marked accumulation of lactosylceramide (LacCer), a possible NEU3 product, and addition of this glycolipid to cell cultures reduced apoptotic cells during sodium butyrate treatment. The results indicate that high expression of NEU3 in cancer cells leads to protection against programmed cell death. In colon cancer cells, NEU3 differentially regulates cell proliferation through integrin-mediated signalling depending on the extracellular matrix (*37*). It causes increased adhesion to laminins and consequent cell proliferation, but rather decrease in cell adhesion to fibronectin, collagen I and IV. Triggered by laminins, NEU3 can clearly stimulate phosphorylation of focal adhesion kinase (FAK) and extracellular signal-related kinase (ERK), without any activation of fibronectin. Furthermore, NEU3 markedly enhances tyrosine phosphorylation of integrin-β4 only on laminin-5, with recruitment of Shc and Grb-2, and the sialidase is co-immunoprecipitated by anti-integrin-β4 antibody, suggesting that the association with integrin-β4 might facilitate promotion of integrin-derived signalling on laminin 5.

NEU3 mRNA levels are also significantly increased in renal cell carcinomas (RCCs) (*38*), correlating with elevation of interleukin (IL)-6, a pleiotropic cytokine that has been implicated in immune responses and the pathogenesis of several cancers, including RCCs. In ACHN human renal carcinoma cells, IL-6 treatment has been shown to enhance NEU3 promotor luciferase activity 2.5-fold and endogenous sialidase activity significantly. NEU3 transfection and IL-6 treatment may both result in suppression of apoptosis and promotion of cell motility, causing synergistic effects in combination. NEU3 hardly affects MAPK or IL-6-induced STAT3 activation but may promote the PI3K/Akt cascade in both IL-6-dependent and -independent ways.

Furthermore, IL-6 was found to promote Rho activation and this was potentiated by NEU3, leading to increased cell motility that was affected by LY294002, a PI3K inhibitor. In contrast, NEU3 silencing by siRNA resulted in decreased Akt phosphorylation and inhibition of Rho activation. As described in colon tumours, glycolipid analysis showed decrease in ganglioside GM3 and increase in LacCer after NEU3 transfection, these lipids apparently affecting cell apoptosis and motility. Thus, NEU3 activated by IL-6 enhances IL-6-mediated signalling largely via the PI3K/Akt cascade in a positive feedback manner and contributes to expression of a malignant phenotype in RCCs. To summarize these observations on NEU3 in cancer, the sialidase activates molecules including FAK, ILK, Shc, integrin-β4 and also Met, which are often up-regulated in carcinogenesis, and may accelerate development of a malignant phenotype.

To define the molecular mechanisms underlying the NEU3 effects, its encoding gene was silenced by siRNA or overexpressed in human cancer cells (*39*). NEU3 silencing caused apoptosis with no specific stimuli, accompanied by decreased Bcl-xL and increased differentiation markers including mda7 and GM3 synthase in HeLa cells, whereas overexpression resulted in the opposite. 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 non-cancerous human fibroblast WI-38 and NHDF cells and keratinocyte NHEK cells, despite significant reduction (66, 92 and 64%, respectively) in NEU3 mRNA, showed no significant changes. NEU3 siRNA was found to inhibit and NEU3 overexpression to stimulate Ras activation with consequent influence on ERK and Akt. Since Ras activation by NEU3 was largely abrogated by PP2 (a src inhibitor) or AG1478 (an EGFR inhibitor), we tested whether NEU3 stimulates EGF receptor phosphorylation in HeLa cells. Consistent with previous observations by others (*40*, *41*), the siRNA introduction reduced phosphorylation of epidermal growth factor receptor (EGFR) and in contrast, the overexpression promoted the phosphorylation and the receptor dimerization in response to EGF. With anti-phospho-EGFR antibodies to specific tyrosine residues, the level of EGFR phosphorylation at Tyr-845 could be shown to be the highest among those at Tyr-992, 1045, 1068, 1148 and 1173 in NEU3 transfected HeLa cells, suggesting that NEU3 may primarily stimulate EGFR phosphorylation in which Src kinase is involved (*42*). Opposite to the case with NEU3 transfection, phosphorylation at Tyr-845 was markedly decreased by NEU3 silencing. NEU3 was co-immunoprecipitated with the EGFR, and EGF stimulation yielded a higher amount of immunoprecipitable NEU3. These results indicate that NEU3 suppresses apoptosis of cancer cells by promoting EGFR phosphorylation probably through its association with EGFR and consequently activation of Ras cascades, especially via the Ras/ERK pathway as illustrated in Fig. 2. Up-regulation of NEU3 is now considered to be essential for the survival of various cancer cells.

With regard to ganglioside alterations related to these cellular events, NEU3-silenced HeLa cells showed increase in globotriaosylceramide (Gb3) accompanied by elevation of Gb3 synthase (α1-4 galactosyltransferase)

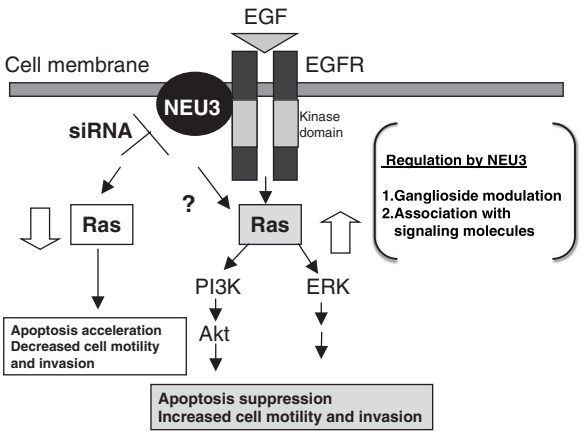

Fig. 2. **Roles of NEU3 in apoptosis of cancer cells via EGFR.** NEU3 siRNA inhibits and NEU3 overexpression stimulates Ras activation, with consequent influence on ERK and Akt through modulation of tyrosine phosphorylation of EGFR.

mRNA, and overexpression increased LacCer as assessed by thin layer chromatography. Furthermore, LacCer rescued from cell death induced by NEU3-siRNA to a considerable extent when it was added exogenously into the cell culture, whereas Gb3 showed a tendency to decrease cell growth in the cells. Thus, these glycolipids might be possible components involved in cell apoptosis of cancer cells. When HeLa cells were treated with LacCer, significant promotion of EGFR phosphorylation was observed in response to EGF, indicating that accumulation of the glycolipids as a result of the sialidase reaction may lead to apoptosis suppression as a consequence of activation of EGF signalling. Thus, NEU3 may protect against cell death both by promoting EGFR phosphorylation probably through its association with EGFR as well as through accumulation of glycolipid products such as LacCer, leading to consequent activation of the Ras/ERK pathway.

IMPAIRED INSULIN SIGNALLING IN NEU3-TRANSGENIC MICE

Based on the results described above verifying up-regulation of NEU3 in human colon cancer, we attempted to generate colon cancers in NEU3-transgenic mice. Unexpectedly, the mice developed impaired insulin signalling and insulin-resistant diabetes mellitus by 18–22 weeks (*43*). Hyper-insulinaemia, islet hyperplasia and increase in the β-cell mass were observed together with extreme insulin resistance. As compared to the wild-type, insulin-stimulated phosphorylation of the insulin receptor (IR) and insulin receptor substrate I (IRS-1) was significantly reduced, and activities of phosphatidylinositol 3-kinase and glycogen synthase were also decreased. Overexpression of NEU3 in L6 myocytes and in 3T3-L1 adipocytes caused a significant decrease in insulin-stimulated phosphorylation of IR and IRS-1, and the transfection into 3T3-L1 cells resulted in delayed differentiation into adipocytes. Exogenous NEU3 in muscle was found to undergo tyrosine phosphorylation in response to insulin, leading to impaired insulin signalling probably

by accumulating GM1 and GM2, which are sialidase products causing inhibition of IR phosphorylation. These results indicated that insulin signalling to be inhibited in transgenic mice. When we measured NEU3 activity with substrate gangliosides, the increase due to insulin was 2.8- to 3.5-fold and 3.0- to 4.2-fold for the activity and phosphorylation, respectively; in transgenic muscle, the proportions clearly suggested that the activity is regulated by tyrosine phosphorylation in skeletal muscle responding to insulin. Since we postulated that the Grb2 adaptor protein, which contains SH2 and SH3 domains for interaction with signalling molecules, might bind to the pYXNX motif (where pY is a phosphotyrosine) (*44*) encompassing Y-346 of NEU3 via its SH2 domain, we examined this possibility using specific antibodies to Grb2 and NEU3. With muscle extracts, NEU3 protein was co-immunoprecipitated with Grb2 by anti-Grb2 antibody, and Grb2 protein was detected in immunoprecipitates with anti-NEU3 antibody, under conditions of almost equal amounts of endogenous Grb2. Larger amounts of NEU3 and Grb2 were recovered with anti-Grb2 and anti-NEU3 antibodies, respectively, after insulin stimulation, indicating that tyrosine-phosphorylated NEU3 associates more strongly with Grb2 in response to insulin. To confirm whether the phosphorylation site of NEU3 is responsible for the binding to Grb2, we prepared a NEU3 mutant, Y346A, featuring substitution of Ala for Tyr-346, transfected into L6 myocytes. In response to insulin, the wild-type NEU3 demonstrated a clearly enhanced association with Grb2, as observed in transgenic muscle, but the mutant showed marked decrease in sialidase activity (5–7% of the wild-type) and failed to co-immunoprecipitate with Grb2. In addition, IRS-1 phosphorylation scarcely changed in the mutant, while the NEU3 wild-type showed a significant reduction. Similarly, glycogen synthase activity was decreased in the wild-type, but the mutant exhibited the same level as the control. These data indicate that tyrosine phosphorylation and activation followed by association with Grb2 may be required for involvement of NEU3 in insulin signaling (Fig. 3). It remains unclear what type of protein kinase is involved in phosphorylation of NEU3 protein.

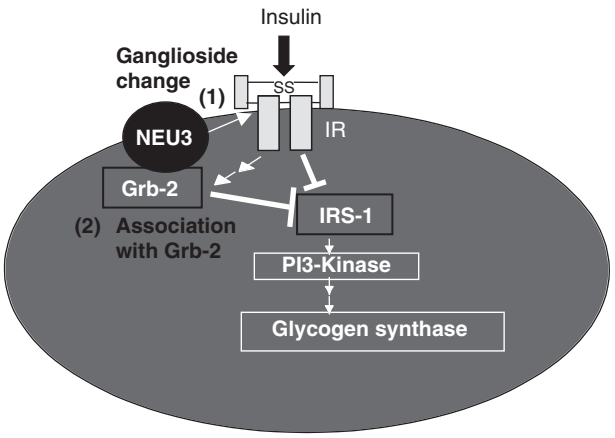

Fig. 3. **Schematic model of the hypothetical role of NEU3 in insulin signalling.** In the upstream pathway (1), NEU3 down-regulates IR phosphorylation through modulation of gangliosides like GM1 and GM2, and in the downstream pathway (2), NEU3 influences signalling negatively through interaction with Grb2 via SH2 domain.

DUAL REGULATION OF TRANSMEMBRANE SIGNALLING BY NEU3 SIALIDASE

As described earlier, NEU3 regulates transmembrane signalling by interacting with signalling molecules including caveolin-1, Rac-1, integrin-β4, Grb-2 and EGFR. Considering the mechanisms of NEU3 effects on the EGFR, enhancement of phosphorylation seems to be mediated in two ways. To determine whether association of NEU3 protein itself with the receptor influences the phosphorylation, we employed a NEU3 mutant without sialidase activity. The mutant NEU3, which expresses a similar level of protein to the wild-type, was co-immunoprecipitated with the EGFR in response to EGF to the same extent as the wild-type. The phosphorylation of EGFR was stimulated by 150% of that in vector-transfected cells, even though the mutant possessed almost no sialidase activity. But the enhancing effect was less than that with the wild-type. The results supported a stimulatory effect of NEU3 as a protein molecule interacting with the EGFR on receptor phosphorylation. When GFP–NEU3 fusion protein fragments covering the whole NEU3 region were examined for binding affinity, only fragments containing the putative transmembrane domain appeared to interact specifically with the EGFR (Shiozaki, M. *et al*. manuscript in preparation). On the other hand, LacCer, a NEU3 reaction product, actually stimulated the receptor phosphorylation when added to cell cultures, as described above. Therefore, in addition to modulation of gangliosides as the result of catalytic reaction, it is possible that NEU3 regulates the functions of molecules in upstream signalling pathways, such as growth factor receptors like the EGF receptor and the Met receptor, by forming molecular complexes with them. NEU3 association with EGFR is likely a more important trigger than glycolipid changes for the first-stage activation of the pathway, probably because a rapid response might be needed. This idea is consistent with our finding that NEU3 attenuates IR phosphorylation and consequent post-receptor signalling by changing ganglioside components and also by association with Grb-2. In response to insulin, NEU3 undergoes phosphorylation and associates with Grb-2 through the SH2 domain, giving rise to reduced IR tyrosine phosphorylation. Although further detailed investigations are needed to clarify these molecular mechanisms, it is of special interest that a glycosidase might exert an influence on signalling as a protein interacting with related molecules, in addition to its indirect impact through catalytic reaction.

CONCLUDING REMARKS

We can conclude that NEU3 is a key regulator of transmembrane signalling at the cell surface through both modulation of gangliosides as an enzyme and by interaction with other signal molecules as a protein. This was verified by experiments using a NEU3 mutant that

expresses the same amount of protein as the wild-type but almost no sialidase activity. NEU3 activates molecules like the EGFR, FAK, ILK, Shc and integrin β4, often up-regulated in carcinogenesis, through accumulation of the enzyme products such as LacCer and also forming complexes with these molecules. In fact, up-regulation of NEU3 occurs in various cancers and actually causes a diabetic phenotype in transgenic mice. Recent epidemiological reports (*45*, *46*) describing higher incidence of cancers in diabetic patients than in controls, have suggested that these diseases might be closely related to each other in pathogenesis. In this context, it is feasible that NEU3 possibly regulates common signalling pathways involved in pathogenesis of the both diseases. However, for full understanding of the functional roles of NEU3 and the regulation mechanisms, a number of problems still remain to be solved: for example, the significance and the mechanism of recruitment of some NEU3 primarily localized in intracellular membrane compartment to the cell surface, the metabolic relationship between NEU3 and the other human sialidases in ganglioside modulation, and the possible presence of physiological activators of NEU3. Further elucidation of the pathological roles of NEU3 should lead to potential applications in control of cancer and diabetes.

REFERENCES

1. Schauer, R. (2000) Achievements and challenges of sialic acid research. *Glycoconj. J.* **17**, 485–499
2. Vimr, E.R., Kalivoda, K.A., Deszo, E.L., and Steenbergen, S.M. (2004) Diversity of microbial sialic acid metabolism. *Microbiol. Mol. Biol. Rev.* **68**, 132–153
3. Miyagi, T. and Yamaguchi, K. (2007) Biochemistry of glycans: sialic acids in *Comprehensive Glycoscience* (Kamerling, J.P., Boons, G., Lee, Y.C., Suzuki, A., Taniguchi, N., and Voragen, A.G.J., eds.) pp. 297–322, Elsevier BV, Amsterdam
4. Monti, E., Preti, A., Venerando, B., and Borsani, G. (2002) Recent development in mammalian sialidase molecular biology. *Neurochem. Res.* **27**, 649–663
5. Miyagi, T., Wada, T., Yamaguchi, K., and Hata, K. (2004) Sialidase and malignancy: a minireview. *Glycoconj. J.* **20**, 189–198
6. Corfield, T. (1992) Bacterial sialidases—roles in pathogenicity and nutrition. *Glycobiology* **2**, 509–521
7. Miyagi, T., Konno, K., Emori, Y., Kawasaki, H., Suzuki, K., Yasui, A., and Tsuik, S. (1993) Molecular cloning and expression of cDNA encoding rat skeletal muscle cytosolic sialidase. *J. Biol. Chem.* **268**, 26435–26440
8. Miyagi, T., Wada, T., Iwamatsu, T., Hata, K, Yoshikawa, Y., Tokuyama, S., and Sawada, M. (1999) Molecular cloning and characterization of plasma membrane-associated sialidase specific for gangliosides. *J. Biol. Chem.* **274**, 5004–5011
9. Wada, T., Yoshikawa, Y., Tokuyama, S, Kuwahara, M., Akita, H., and Miyagi, T. (1999) Cloning, expression, and chromosomal mapping of a human ganglioside sialidase. *Biochem. Biophys. Res. Commun.* **261**, 21–27
10. Bonten, E., van der Spoel, A., Fornerod, M., Grosveld, G., and d'Azzo, A. (1996) Characterization of human lysosomal neuraminidase defines the molecular basis of the metabolic storage disorder sialidosis. *Genes Dev.* **10**, 3156–3169
11. Milner, C.M., Smith, S.V., Carrillo, M.B., Taylor, G.L., Hollinshead, M., and Campbell, R.D. (1997) Identification of a sialidase encoded in the human major histocompatibility complex. *J. Biol. Chem.* **272**, 4549–4558
12. Pshezhetsky, A.V., Richard, C., Michaud, L., Igdoura, S., Wang, S., Elsliger, M.A., Qu, J., Leclerc, D., Gravel, R., Dallaire, L., and Potier, M. (1997) Cloning, expression and chromosomal mapping of human lysosomal sialidase and characterization of mutations in sialidosis. *Nat. Genet.* **15**, 316–320
13. Monti, E., Preti, A., Rossi, E., Ballabio, A., and Borsani, G. (1999) Cloning and characterization of NEU2, a human gene homologous to rodent soluble sialidases. *Genomics* **57**, 137–143
14. Monti, E., Bassi, M.T., Papini, N., Riboni, M., Manzoni, M., Venerando, B., Croci, G., Preti, A., Ballabio, A., Tettamanti, G., and Borsani, G. (2000) Identification and expression of NEU 3, a novel human sialidase associated to the plasma membrane. *Biochem. J.* **349**, 343–351
15. Seyrantepe, V., Landry, S., Trudel, J.A., Hassan, J.A., Morales, C.R., and Pshezhetsky, A.V. (2004) Neu4, a novel human lysosomal lumen sialidase, confers normal phenotype to sialidosis and galactosialidosis cells. *J. Biol. Chem.* **279**, 37021–37029
16. Yamaguchi, K., Hata, K., Koseki, K., Shiozaki, Akita, H., Wada, T., Moriya, S., and Miyagi, T. (2005) Evidence for mitochondrial localization of a novel human sialidase (NEU4). *Biochem. J.* **390**, 85–93
17. 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., and Borsani, G. (2004) Molecular cloning and characterization of NEU4, the fourth member of the human sialidase gene family. *Genomics* **83**, 445–453
18. Roggentin, P., Rothe, B., Kaper, J.B., Galen, J., Lawrisuk, L., Vimr, E.R., and Schauer, R. (1989) Conserved sequences in bacterial and viral sialidases. *Glycoconj. J.* **6**, 349–353
19. Hata, K., Wada, T., Hasegawa, A., Kiso, M., and Miyagi, T. (1998) Purification and characterization of a membrane-associated ganglioside sialidase from bovine brain. *J. Biochem.* **123**, 899–905
20. Li, S.-C., Li, Y.-T., Moriya, S., and Miyagi, T. (2001) Degradation of GM1 and GM2 by mammalian sialidases. *Biochem. J.* **360**, 233–237
21. Papini, N., Anastasia, L., Tringali, C., Croci, G., Bresciani, R., Miyagi, T., Yamaguchi, K., Preti, A., Prinetti, A, Prioni, S., Sonnino, S., Tettamanti, G., Venerando, B., and Monti, E. (2004) The plasma membrane-associated sialidase Neu3 modifies the cell surface ganglioside pattern through cell-to-cell interactions. *J. Biol. Chem.* **279**, 16989–16995
22. Yamaguchi, K., Hata, K., Wada, T., Moriya, S., and Miyagi, T. (2006) Epidermal growth factor-induced mobilization of a ganglioside-specific sialidase (NEU3) to membrane ruffles. *Biochem. Biophys. Res. Commun.* **346**, 484–490
23. Zanchetti, G., Colombi, P., Manzoni, Anastasia, L., Caimi, L., Borsani, G., Venerando, B., Tettamanti, G., Preti, A., Monti, E., and Bresciani, R. (2007) Sialidase NEU3 is a peripheral membrane protein localized on the cell surface and in endosomal structures. *Biochem J.* **408**, 211–219
24. Kopitz, J., von Reitzenstein, C., André, S., Kaltner, H., Uhl, J., Ehemann, V., Cantz, M., and Gabius, H.J. (2001) Negative regulation of neuroblastoma cell growth by carbohydrate-dependent surface binding of galectin-1 and functional divergence from galectin-3. *J. Biol. Chem.* **276**, 35917–35923
25. Hasegawa, T., Yamaguchi, K., Wada, T., Takeda, A., Itoyama, Y., and Miyagi, T. (2000) Molecular cloning of mouse ganglioside sialidase and its increased expression in Neuro2a cell differentiation. *J. Biol. Chem.* **275**, 8007–8015

26. Proshin, S., Yamaguchi, K., Wada, T., and Miyagi, T. (2002) Modulation of neuritogenesis by ganglioside-specific sialidase (Neu3) in human neuroblastoma NB-1 cells. *Neurochem. Res.* **27**, 841–846
27. Rodriguez, J.A., Piddini, E., Hasegawa, T., Miyagi, T., and Dotti, C.G. (2001) Plasma membrane ganglioside sialidase regulates axonal growth and regeneration in hippocampal neurons in culture. *J. Neurosci.* **21**, 8387–8395
28. Da Silva, J.S., Hasegawa, T., Miyagi, T., Dotti, C.J., and Avad-Rodriguez, J.A. (2005) Symmetric membrane ganglioside sialidase activity specifies axonal fate. *Nat. Neurosci.* **8**, 606–615
29. Kalka, D., von Reitzenstein, C., Kopitz, J., and Cantz, M. (2001) The plasma membrane ganglioside sialidase cofractionates with markers of lipid rafts. *Biochem. Biophys. Res. Commun.* **283**, 989–993
30. Wang, Y., Yamaguchi, K., Wada, T., Hata, K., Zhao, X., Fujimoto, T., and Miyagi, T. (2002) Close association of ganglioside-specific sialidase, Neu3, with caveolin in membrane microdomains. *J. Biol. Chem.* **277**, 26252–26259
31. Okamoto, T., Schlegel, A., Scherer, P.E., and Lisanti, M.P. (1998) Caveolins, a family of scaffolding proteins for organizing "preassembled signaling complexes" at the plasma membrane. *J. Biol. Chem.* **273**, 5419–5422
32. Yamanami, T., Shiozaki, K., Wada, T., Yamaguchi, K., Uemura, T., Kakugawa, Y., Fujiya, T., and Miyagi, T. (2006) Down-regulation of sialidase NEU4 may contribute to invasive properties of human colon cancers. *Cancer Sci.* **98**, 299–307
33. Yogeeswaran, G. and Hakomori, S. (1975) Cell contact-dependent ganglioside changes in mouse 3T3 fibroblasts and a suppressed sialidase activity on cell contact. *Biochemistry* **14**, 2151–2156
34. Schengrund, C.-L., Lausch, R.N., and Rosenberg, A. (1973) Localization of sialidase in the plasma membrane of rat liver cells. *J. Biol. Chem.* **248**, 4424–4428
35. Miyagi, T., Sagawa, J., Kuroki, T., Matsuya, Y., and Tsuiki, S. (1990) Tumor-promoting phorbol ester induces alterations of sialidase and sialyltransferase activities of JB6 cells. *Jpn. J. Cancer Res.* **81**, 1286–1292
36. Kakugawa, Y., Wada, T., Yamaguchi, K., Yamanami, H., Ouchi, K., Sato, I., and Miyagi, T. (2002) Up-regulation ofplasma membrane-associated ganglioside sialidase (Neu 3) in human colon cancer and its involvement in apoptosis suppression. *Proc. Natl Acad. Sci. USA* **99**, 10718–10723
37. Kato, K., Shiga, K., Yamaguchi, K., Hata, K., Kobayashi, T., Miyazaki, K., Saijo, S., and Miyagi, T. (2006) Plasma membrane-associated sialidase (NEU3) differentially regulates integrin-mediated cell proliferation through laminin- and fibronectin- derived signaling. *Biochem. J.* **394**, 647–656
38. Ueno, S., Saito, S., Wada, T., Yamaguchi, K., Satoh, M., Arai, Y., and Miyagi, T. (2006) Plasma membrane-associated sialidase is up-regulated in renal cell carcinoma and promotes the interleukin-6 -induced apoptosis suppression and cell motility. *J. Biol. Chem.* **281**, 7756–7764
39. Wada, T., Hata, K., Yamaguchi, K., Shiozaki,K., Koseki, K., Moriya, S., and Miyagi, T. (2007) A crucial role of plasma membrane-associated sialidase (NEU3) in the survival of human cancer cells. *Oncogene* **26**, 2483–2490
40. Bremer, E.G., Schlessinger, J., and Hakomori, S. (1986) Ganglioside-mediated modulation of cell growth. Specific effects of GM3 on tyrosine phosphorylation of the epidermal growth factor receptor. *J. Biol. Chem.* **261**, 2434–2440
41. Wang, X.O., Sun, P., and Paller, A.S. (2003) Ganglioside GM3 blocks the activation of epidermal growth factor receptor induced by integrin at specific tyrosine sites. *J. Biol. Chem.* **278**, 48770–48778
42. Biscardi, J.S., Maa, M.C., Tice, D.A., Cox, M.E., Leu, T.H., and Parsons, S.J. (1999) c-Src-mediated phosphorylation of the epidermal growth factor receptor onTyr845 and Tyr1101 is associated with modulation of receptor function. *J. Biol. Chem.* **274**, 8335–8343
43. Sasaki, A., Hata, K., Suzuki, S., Sawada, M., Wada, T., Tateno, Obinata, M., Suzuki, Y., and Miyagi, T. (2003) Overexpression of plasma membrane-associated sialidase attenuates insulin signaling in transgenic mice. *J. Biol. Chem.* **278**, 27896–27902
44. Lowenstein, E.J., Daly, R.J., Batzer, A.G., Li, W., Margolis, B., Lammers, R., Ullrich, A., Skolnik, E.Y., Bar-Sagi, D., and Schlessinger, J. (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* **70**, 431–442
45. Nishii, T., Kono, S., Abe, H., Eguchi, H., Shimazaki, K., Hatano, B., and Hamada, H. (2001) Glucose intolerance, plasma insulin levels, and colon adenomas in Japanese men. *Jpn. J. Cancer Res.* **92**, 836–840
46. Yoshida, I., Suzuki, A., Vallee, M., Matano, Y., Masunaga, T., Zenda, T., Shinozaki, K., and Okada, T. (2006) Serum insulin levels and the prevalence of adenomatous and hyperplastic polyps in the proximal colon. *Clin. Gasteroenterol. Hepatol.* **4**, 1225–1231