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Biochimica et Biophysica Acta 1780 (2008) 532–537
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbagen

Review

Roles of plasma membrane-associated sialidase NEU3 in human cancers ☆

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Received 10 August 2007; received in revised form 21 September 2007; accepted 24 September 2007
Available online 2 October 2007

Abstract

Altered sialylation of glycosphingolipids is observed in cancer as a ubiquitous phenotype, leading to the appearance of tumor-associated antigens, aberrant adhesion and disturbance of transmembrane signaling. To understand the pathological significance of aberrant alterations of gangliosides in cancer, our studies have been focused on sialidase, which is responsible for the removal of sialic acids from glycoproteins and glycolipids. Among human sialidases so far identified, sialidase NEU3 is a key enzyme for ganglioside degradation because of its uniqueness both in its localization in the plasma membrane and in specifically hydrolyzing gangliosides. NEU3 is markedly up-regulated in many types of cancers including colon and renal carcinomas and suppresses apoptosis of cancer cells. The present paper briefly summarizes our recent results on the sialidase alterations and their significance in cancer. NEU3 is indeed closely related to malignancy and thus may be a potential target for cancer diagnosis and therapy.

Keywords: Sialidase; Gangliosides; Cancer; Plasma membrane; Apoptosis; Transmembrane signaling

1. Introduction

Aberrant sialylation in cancer cells is a characteristic feature associated with malignant behavior, such as invasiveness and metastasis. A general increase in sialylation is often found in cell surface glycoproteins of malignant cells, and altered sialylation of glycolipids is also observed as a ubiquitous phenotype [1–4]. Despite the number of reports describing involvement of sialic acids in cancer, the molecular mechanism and significance are not fully understood. Sialylation is mainly regulated by sialidase and sialyltransferase which cleaves sialic acid residues from and transfers them to glycoconjugates, respectively. In this paper, we have focused on a human ganglioside-specific sialidase to further the understanding of ganglioside neoplastic alterations.

Ganglioside sialidases have so far been suggested to play important roles in various cellular functions. Although sufficient information as to what types of ganglioside sialidase were involved was not available, the activity levels were found to fluctuate consistently with cell differentiation, cell growth, and malignant transformation. Alterations of the levels of ganglioside sialidase expression associated with malignant transformation have been described in 3T3-transformed cells [5] and in BHK-transformed cells [6]. We previously reported an increase of plasma membrane sialidase activity associated with induction of anchorage-independent growth in mouse epidermal JB6 cells exposed to phorbol esters [7]. However, little was known about the molecular mechanisms underlying such sialidase alterations until we succeeded in cloning ganglioside sialidase. Human sialidases have been cloned and identified (designated as NEU1, NEU2, NEU3, and NEU4) differing in subcellular localization and enzymatic properties (Table 1). We found that four types of sialidase behave in different manners during carcinogenesis [8]. Among the sialidases, NEU3 is a key enzyme for ganglioside degradation because of its strict substrate preference to gangliosides [9–11], which co-localize with this sialidase in the surface membranes. Unlike lysosomes, the membranes do not contain a set of glycosidases for degradation, implying that Neu3 probably plays crucial roles in regulation of cell surface functions other than catabolism. NEU1 [12–14] hydrolyzes preferentially oligosaccharides and glycopeptides but poorly gangliosides. On the other hand, NEU2 [15] and NEU4 [16–18] can act on gangliosides as well as oligosaccharides and glycoproteins at nearly neutral pH and acidic pH, respectively.

☆ This paper is dedicated to Prof. S. Hakomori on the occasion of "Glycobiology and Shingobiology 2007" Hakomori Commemorative Forum in Tokushima, Japan.

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doi:10.1016/j.bbagen.2007.09.016

Table 1
Comparison of four types of human sialidases

	NEU1	NEU2	NEU3	NEU4
Major location	Lysosomes	Cytosol	Plasma membrane	Lysosomes[17] Mitochondria[18] Intracellular Membranes[16,18]
Major Substrate	Oligosaccharides 4MU-NeuAc	Oligosaccharides 4MU-NeuAc Glycoproteins Gangliosides	Gangliosides	Oligosaccharides 4MU-NeuAc Glycoproteins Gangliosides
Optimal pH	4.4-4.6	6.0-6.5	4.6-4.8	4.4-4.5
Total amino Acids	415	380	428	496 (484)
Chromosome Location	6p 21.3	2q 37	11q13.5	2q37.3
References	[12–14]	[15]	[10,11]	[16–18]

As NEU2 appears to be hardly expressed in human tissues, NEU4 might be also involved in ganglioside degradation, although they are not localized in the plasma membranes. Using the human NEU3 gene, we have studied ganglioside alterations and their significance in cancer in humans.

2. NEU3 as a ganglioside-specific human sialidase localized in the caveolae

A ganglioside-specific sialidase was first cloned from a bovine brain library [9], based on the peptide sequence information from the purified enzyme protein [19]. As PCR with oligonucleotide primers for the bovine enzyme produced the same size of cDNA fragment with an 87% amino acid identity using human cDNA as the template, we isolated a complete human NEU3 cDNA [10] with the fragment probe. The nucleotide sequence of the human cDNA consists of 2052 nucleotides and translation of this open reading frame generates a protein of 428 amino acids with a molecular mass of 48251Da. The sequence contains a Arg–Ile–Pro sequence, three Asp-boxes, and a putative trans-membrane domain, showing an overall 83% amino acid identity to the bovine sialidase, and 19%, 34% and 38% sequence identity with NEU1, NEU2 and NEU4 human sialidases, respectively. Hydrolysis by NEU3 was essentially specific to gangliosides other than GM1 and GM2, in the presence of Triton X-100 with considerable activity at a neutral pH as well as under acidic conditions. Fluorescent *in situ* hybridization with a cosmid clone as a probe allowed the human sialidase gene localized to chromosome 11 at q 13.5. The subcellular localization of the expressed sialidase was assessed by immunofluorescence staining of transfected cells. The major subcellular localization of the expressed bovine sialidase was assessed to be the plasma membrane by Percoll density gradient centrifugation of cell homogenates and by immunofluorescence staining of transfected COS-7 cells. Analysis of the membrane topology with a protease protection assay suggested that this sialidase has a type I membrane orientation with its amino-terminus facing the extracytoplasmic side and lacking a signal sequence. When the radioabeled ganglioside GD1a was administrated to murine Neu3-transfected cells, Neu3 was shown to hydrolyze the ganglioside in intact living cells at a neutral pH mainly through cell-to-cell interactions. This probably indicates that the sialidase is involved in cell–cell interaction through hydrolyzing gangliosides at the surface of neighboring cells [20]. Unlike the bovine and mouse Neu3 sialidases, the human NEU3 is not always detected on the cell surface but may exist in other cellular membranes and can be functionally moved to and concentrated at leading edges in response to growth stimuli. In response to epidermal growth factor, the sialidase redistributed rapidly to the ruffling cell membranes of squamous carcinoma-derived A431 cells and co-localized with Rac-1, leading to increased cell motility [21].

NEU3 also functions as a caveolin-interacting protein within caveolin-rich microdomains by a putative caveolin-binding motif within the molecule [22]. In the mice overexpressing NEU3 impaired glucose tolerance and hyper-insulinemia were observed together with overproduction of insulin in enlarged islets and subsequently to insulin-resistant diabetes mellitus [23]. NEU3 expression was shown to participate in neuronal differentiation [24] through ganglioside modulation, as the murine homolog gene plays essential roles in neurite formation [25] and regeneration [26]. These results suggest that NEU3 plays a role as a signal mediator by controlling gangliosides dynamically.

3. Up-regulation of NEU3 in colon cancer

We first examined the NEU3 mRNA level with quantitative RT-PCR using surgical specimens of human colon cancer [27]. The level was increased in all cases of colon cancer tissues tested ($n=32$) by 3- to 100-fold associated with significant elevation in sialidase activity as compared to adjacent non-tumor mucosa (Fig. 1a). In situ hybridization showed NEU3 expression in epithelial elements of adenocarcinomas. In cultured human colon cancer cells, the sialidase level was down-regulated in the process of differentiation and apoptosis induced by sodium butyrate (NaBT), while NEU1 was up-regulated. Transfection of the NEU3 gene into colon cancer cells inhibited apoptosis, accompanied with increased Bcl-2 and decreased caspase expression. Compared to non-tumor mucosa, colon cancer tissues exhibited a marked accumulation of lactosylceramide (Lac-cer), a possible NEU3 product, and in fact, addition of the glycolipid to the culture reduced apoptotic cells during NaBT treatment. These results indicate that high expression of NEU3 in cancer