Sialyltransferase family members and cervix squamous cell carcinoma

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Summary

Sialic acids including a number of their derivatives are ubiquitous at the terminal positions of the oligosaccharides of glycoproteins. The transfer of sialic acids from cystidine-5-monophospho-N-acetylneuraminic acid (CMP-NeuAc) to the terminal position of the carbohydrate group of glycoproteins and glycolipids is catalyzed by a family of sialyltransferases (STs). There is a large body of evidence to suggest that tumor cells have altered surface properties from their normal counterparts, and that these changes are partially due to altered sialo-glycoconjugates expressed on the plasma membrane and that altered sialylation (change in glycoprotein expression), which occurs during certain pathological processes, such as oncogenic transformation, tumor metastases, and invasion, is associated with enhanced ST activity. In this report we attempt to review the important findings in studing sialyltransferases of cervix squamous cell carcinoma.

Key words: Prognosis; Sialyltransferase; Squamous cell carcinoma of the cervix.

Introduction

Cell surface oligosaccharides are major components of the outer surface of mammalian cells and these oligosaccharides are very often characteristic of cell types [1]. Oligosaccharide changes are often noted during mammalian development and specific types of oligosaccharides are expressed at different stages of differentiation, which result in expression of distinct carbohydrates eventually restricted to specific cell types. Aberrations in these cell surface oligosaccharides are associated with various kinds of pathological conditions, including malignant transformation [2-42]. Oligosaccharides are unique in the complexity of their structures because they are linked together in more than one form and result in almost unlimited variations in structure. Sialic acids include a number of derivatives of the nine-carbon amino acid sugar, neuraminic acid. The amino group of neuraminic acid may be substituted with an acetyl or glycoloyl moiety, while the hydroxyl groups can be methylated from the ester with acetyl, lactyl, sulfate or phosphate groups to form over 20 naturally occurring derivatives [2]. Sialic acids are widely distributed in nature as terminal sugars on oligosaccharides attached to protein or lipid moieties. Sialyltransferases (STs) are a family of glycosyltransferases, which catalyze the transfer of sialic acid from CMP-Neu5Ac (cytidine monophosphate N-acetylneuraminic acid) to non-reducing terminal positions on sugar chains of glycoconjugates (glycoproteins and glycolipids) [2-42]. So far, at least 17 distinct ST genes exist [2, 6], and all involve tumor-associated changes in the expression of cell-surface sialoglycoconjugates. Theses 17 STs can be divided into at least three subtypes according to involvement of different linkages-α2, 3-linkage, α2, 6-linkage and α2, 8-linkage. However, the overlapping function occurs often and because of this complexity, sialoglycoconjugates can provide almost unlimited variations in the structure. Thus, the study of ST function is complicated and difficult. For example, six human α2,3-sialyltransferase genes (hST3Gal I-VI) have thus far been cloned [6, 42-46]. In addition, the final sialyl-glycan structure is determined by the concerted action of all expressed STs; just as ST3Gal II [43-45], which shares almost identical specificity with ST3Gal I; moreover, ST3Gal VI shares acceptor specificity with ST3Gal III and IV and competes with ST6Gal I [42]. STs involve α2, 3-linkage including ST3Gal I, ST3Gal II, ST3Gal III, ST3Gal IV, ST3Gal V, and ST3Gal VI. STs involve α2, 6-linkage including ST6Gal I, ST6GalNAc I, ST6GalNAc II, ST6GalNAc III, ST6GalNAc IV, and ST6GalNAc V. STs involve α2, 8-linkage including ST8Sia I, ST8Sia II, ST8Sia III, ST8Sia IV, and ST8Sia V. Each enzyme has its favorite substrate as an acceptor. For example, ST3Gal I favors Gal β1,3GlcNAc and ST3Gal III favors Gal β1,3GlcNAc β1,3Gal β1,4GlcNAc as the best acceptor to study. Table 1 lists the different STs and the favoring acceptor.

In Taiwan, cervix carcinoma is still the most common female cancer with an incidence rate of 32.1 per 100,000 [47]. The prognosis is dependent on many factors, among which the majority are based on histopathological parameters. Many pathological factors including bulky tumor size, poor differentiation, presence of lymph-vascular space involvement, presence of parametrial invasion, and deep stromal invasion are related with lymph node meta-

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Table 1. — *Sialyltransferase family and the corresponding acceptor.*

Enzyme	Acceptor
α 2, 3 linkage	
ST3Gal I	Gal β 1,3GalNAc
ST3Gal II	Gal β 1,3GalNAc- Obzl
ST3Gal III	Gal β 1,3GlcNAc β 1,3Gal β 1,4GlcNAc
ST3Gal IV	Gal β 1,4GlcNAc
ST3Gal V	GM3
ST3Gal VI	NLC6
α 2, 6 linkage	
ST6Gal I	Asialo-α 1 acid glycoprotein
ST6GalNAc I	Asialo-bovine submaxillary mucin
ST6GalNAc II	Asialo-fetuin
ST6GalNAc III	GM1b, fetuin
ST6GalNAc IV	Neu5Ac α 2, 3 Gal β 1,3GalNAc
ST6GalNAc V	GM1b
α 2, 8 linkage	
ST8Sia I	GD3
ST8Sia II	NCAM
ST8Sia III	Fetuin
ST8Sia IV	NCAM
ST8Sia V	GT1b

stases and closely respond to disease-free survival [48-54]. Besides these conventional pathological parameters for predicting prognosis in an early stage of cervical cancer [55], there are many biological factors (cellular molecules) which show a close correlation with aggressive and invasive behaviors in tumors [56-60]. Among these biological factors, sialic acids are one of the most promising molecules because they involve cell-cell and cell-matrix interactions and cellular recognition. In addition, it has been demonstrated that hypersialylation, which has been observed in certain biological and pathological processes, goes along with enhanced ST activity, such as development, differentiation, oncogenic transformation, tumor metastasis, invasion, and others [4-40]. In this review we attempted to summarize our recent studies of altered ST expression of cervix squamous cell carcinoma.

Materials and Methods

Altered activity and surface amount of sialyltransferase in cervical cancer cell lines [14]

We successfully detected the activities of each subtype of sialyltransferases using Gal_1,3GalNAc-acetyl-lactosamine)-Obzl (acceptor for ST3Gal I), Galβ1, 3GlcNAcβ1, 3Galβ1, 4GlcNAc (acceptor for ST3Gal III), Galβ1, 4GlcNAc (acceptor for ST3Gal IV), asialo-bovine submaxillary mucin (acceptor for ST6GalNAc I), asialo-fetuin (acceptor for ST6GalNAc II), and fetuin (acceptor for ST6GalNAc III), respectively; we also successfully detected the amounts of sialic acids using fluorescein-conjugated *Sambucus nigra agglutinin* (SNA) specific for α2,6-sialic acids and fluorescein-conjugated *Maackia Amurensis agglutinin* (MAA) specific for α2,3-sialic acids. We found that increasing enzyme activity of the ST3Gal group and ST6Gal group might be important in various kinds of gynecological cancers. More specifically, enhanced activity of sialyltransferases involving α2,6-sialic acid sugar chains might be more important in cancer development. In the cell line study, we could suspect that altered α2,6-sialo-glycoconjugates are very important in malignant transformation. Future studies will investigate whether the enzyme expression of these sialyltransferases can be helpful for clinical practice.

Enhanced ST6Gal I mRNA expression in cervical squamous cell carcinoma of the cervix without extracervical invasion

Based on the findings in the study of gynecological cell lines, we directly studied the difference of mRNA expression of 4-type sialyltransferases (ST3Gal I, ST3Gal III, ST3Gal IV and ST6Gal I) between cervical squamous cell carcinoma tissue and normal cervical tissue using a semiquantitative reverse transcription-polymerase chain reaction method and found that sialyltransferase ST6Gal I expression was enhanced in squamous cell carcinoma of the cervix, but mRNA expression from the other three sialyltransferases (ST3Gal I, ST3Gal III, and ST3Gal IV) was significantly down-expressed in squamous cell carcinoma of the cervix compared to the normal cervix. Further evaluation of the relationship between ST6Gal I and other conventional clinico-pathological prognostic factors of cervix squamous cell carcinoma was done, and we found that high ST6Gal I expression was associated with more invasive properties of cervical cancer, such as deep stromal invasion, lymph-vascular space involvement, and poor differentiation. This correlation points out the important role of enhanced ST6Gal I expression in malignant transformation of cervix tissue and enhanced ST6Gal I expression and might also contribute to the aggressive behavior of the tumor because the more increasing invasion potential of the cancer found, the more increasing ST6Gal I expression is suspected. The role of ST6Gal I in other cancers was reviewed as follows.

Sialyltransferase ST6Gal I is responsible for the addition of sialic acid in the α2,6-linkage to Galβ1,4GlcNAc (N-acetyl-lactosamine), a sequence commonly found in N-linked chains of glycoproteins. Much evidence supports the theory that enhanced ST6Gal I expression is possibly important in cancer development and progression [18, 31-32, 39-40]. The majority of the studies showed that high ST6Gal I expression was associated with poor histopathological parameters, especially in solid tumors, such as grade III-poor differentiation [18], absence of progesterone receptors [18], and invasive potential [31-32, 39-40]. In studying ST6Gal I expression in cervix squamous cell carcinoma, we also conclude that enhanced sialyltransferase ST6Gal I mRNA expression might be important processes in cervical cancer [15].

Increased ST6Gal I and ST3Gal III mRNA expressions in cervix squamous cell carcinoma with pelvic lymph node metastases

Because we found that enhanced ST expression is associated with cancer transformation, we wanted to investigate whether the expression of these STs could be helpful for prognostic purposes. This study further investigated the changes in mRNA expression of the four STs in FIGO stage IB1 squamous cell carcinoma to assess the extent of sialylation associated with lymph node metastases using the same strategy – RT-PCR detection of the expression of STs. In addition, we also used substrate-enzyme assay methods to evaluate the alterations in ST activity in FIGO IB1 cervical squamous cell carcinomas. We found that both ST6Gal I mRNA and ST3Gal III mRNA expres-

sions were significantly increased in patients with lymph node metastases compared to those without lymph node metastases. Using receiver operating characteristic (ROC) curves of the ST ratio index for accurate comparison of lymph node metastases, ST3Gal III and ST6Gal I were observed to be fairly interchangeable. We further evaluated the relationship between ST6Gal I expression and other clinico-pathological prognostic factors, and we found that high ST6Gal I expression was associated with other invasive properties of cervical cancer, such as deep stromal invasion and lymph-vascular space involvement. ST6Gal I expression seemed to be more enhanced in larger tumors. In contrast, although ST3Gal III expression is enhanced in cervix squamous cell carcinoma with pelvic lymph node metastases, ST3Gal III expression does not appear to be associated with other clinico-pathological prognostic factors. This means that ST3Gal III over-expression might be an independent event and a late event when the tumor initiates its lymph metastases. In this section, we would like to give only a brief review on the role of ST3Gal III in cancer because it has been extensively reviewed in a previous report [16].

ST3Gal III is involved in the biosynthesis of $sLe^{(x)}$ and $sLe^{(a)}$ which are known selectin ligands and tumor-associated carbohydrate structures [61, 62] that play an important role in tumor metastases. In the beginning of haematogeneous or lymphatic metastasis, malignant cells have to first invade the blood or lymphatic vessels. After their dissemination via circulation, they may adhere to and penetrate through the vascular endothelium, and move into the surrounding tissue to form metastatic colonies [63-65]. The E-or P-selectin expressed on the surface of vascular endothelial cells interacts with sialyl Lewis antigens, such as $sLe^{(x)}$, $sDLe^{(x)}$, and $sLe^{(a)}$, expressed on the surface of malignant cells, and mediates the adhesion of malignant cells to the vascular endothelium [66, 67]. Since the expression of ST3Gal III mRNA involves the adhesion of the surrounding tissue, reduced cancer cell attachment to the surrounding stroma of the cervix might increase the ability of "drop of the cancer cells" or "escape of the cancer cells" from the surrounding tissue when down-regulation of ST3Gal III mRNA expression occurs in oncogenesis (earlier stage of cancer development), which is likely to help their release into the peripheral blood or lymphatic circulation. After dissemination, adhesion to the endothelial cells becomes more important, thus increased expression of ST3Gal III mRNA might enhance the interaction between cancer cells and vascular endothelium to help establish successful metastases. We suspect that increased expression of ST3Gal III might be a late event in cancer development, at least followed by increased expression of ST6Gal I mRNA [16].

Conclusion

Based on our limited studies, we have highlighted the important role of STs in cervix squamous cell carcinoma. So far, at least two of the sialyltransferase family members are known to play an important role in cervix squamous cell carcinoma; the first is ST6Gal I because its overexpression not only occurs in early-stage cervical cancer but is also associated with aggressive behavior and the second is ST3Gal III because its overexpression is associated with pelvic lymph node metastases although its expression is not prominent in early-stage cervical cancer. ST6Gal I overexpression is a very important process during the establishment of cervix SCC. With progressive growth and invasive process, enhanced ST6Gal I expression becomes more important. When entering the metastatic status, initiated overexpression of ST3Gal III should occur, because overexpression of both ST6Gal I and ST3Gal III could predict pelvic lymph node metastases accurately in FIGO IB1 squamous cell carcinoma of the cervix.

We highlight the vision that, with advancing biotechnology, more specific molecules against different kinds of STs might be found in the near future, just like soyasaponin I, which is a potent and specific sialyltransferase inhibitor for ST3Gal I and was first identified in our laboratory [17]. Using this strategy might provide a vision of possible synergistic therapy in cervical cancer patients or offer an effective tool in cancer prevention, especially for cervix squamous cell carcinoma.

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