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MINI-REVIEW

Sialic acids: biomarkers in endocrinal cancers

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Abstract Sialylations are post translational modification of proteins and lipids that play important role in recognition, signaling, immunological response and cell-cell interaction. Improper sialylations due to altered sialyl transferases, sialidases, gene structure and expression, sialic acid metabolism however lead to diseases and thus sialic acids form an important biomarker in disease. In the endocrinal biology such improper sialylations including altered expression of sialylated moieties have been shown to be associated with disorders. Cancer still remains to be the major cause of global death and the cancer of the endocrine organs suffer from the dearth of appropriate markers for disease prediction at the early stage and monitoring. This review is aimed at evaluating the role of sialic acids as markers in endocrinal disorders with special reference to cancer of the endocrine organs. The current study is summarized under the following headings of altered sialylations in endocrinal cancer of the (i) ovary (ii) pancreas (iii) thyroid (iv) adrenal and (v) pituitary gland. Studies in expression of sialic acid in testis cancer are limited. The future scope of this review remains in the targeting of endocrinal cancer by targeting altered sialylation which is a common expression associated with endocrinal cancer.

Keywords Sialic acids · Marker · Endocrines · Cancer · Sialylations · Sialoglycoconjugate

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Introduction

Sialic acids are negatively charged nine-carbon carboxylated monosaccharides on glycosylated proteins and lipids formed due to post translational modification. Located terminally on the cell surface they constitute the glycocalyx, forming receptors that contribute to the structure, function, recognition, signaling, and interaction of the host cell with other molecules and is vital for cell-cell and cell-pathogen interaction. A battery of enzymes including (i) sialyltransferases catalyzing their biosynthesis, (ii) sialidases also termed as neuraminidases, catalyzing their cleavage regulate their occurrence in the mammalian system [1]. Studies in infection by *Trypanosoma cruzi*, reveal that signaling by sialylated mucins *via* sialic acid-binding Ig-like lectin receptors enable inhibitory effects on CD4+ T cells causing G1 cell cycle arrest thereby modulating host immune responses [2]. Sialylation of Fc region of antibodies have been reported to show anti-inflammatory properties on one hand and on the other hand over-expression of sialylation affects antibody function by altering the antigen specific binding, decreased Fc mediated effector functions and increased protease sensitivity [3]. Polysialic acids (PSA) linked by (α2-8) glycosidic linkages by the enzyme polysialyltransferase-1 (ST8SiaIV) and sialyltransferase-X (STX or ST8SiaII) is a normal constituent of the human neuronal cell adhesion molecule (NCAM) that play role in neural development in processes including neuronal regeneration, neurite outgrowth, axonal growth, and neuronal sprouting [4]. Sialylations contribute to normal function of ion channels [5] and have been shown to vary in an "M" shaped manner during normal menstrual cycle of healthy women [6]. Upregulation of sialylation of apolipoprotein C-III (apoCIII) protein coded by APOC3 gene, which is a risk factor in cardiovascular diseases has been demonstrated for

its proinflammatory properties in type 2 diabetic patients [7] while enhanced sialylation have been reported during hypo and hyperthyroidism [8]. Sialylation of thyrotropin (TSH) hormone has been reported to increase the hormone bioactivity [9].

Altered sialylations and their modifications including acetylation of sialylated proteins [10–15], together with the altered sialyl transferases [16, 17] are associated with disorders and have been reported as disease markers and potential targets for therapeutic approaches. Lysosomal neuraminidase deficiency, including sialidosis and galactosialidosis leads to genetical storage disorders [18]. In cancer, altered sialic acid profile plays dominant role enhancing tumor growth, metastasis, evading immune surveillance, escaping the apoptotic pathways, leading to cancer cell survival and resistance to therapy [10–15]. Overexpression of *O*-acetylated sialoglycoconjugates in childhood acute lymphoblastic leukemia (ALL) has been documented in correlating the monitoring of cancer progression and enabling cancer cell survival [10, 11, 14, 15] establishing their potential biological role in cancer progression.

Overexpression of α2,6 sialylation of glycoproteins due to expression of increased ST6GalI (β-galactoside-α2,6 sialyltransferase) have been reported to contribute to metastasis, increase migration by increased sialylation of integrin β1 and overexpression of CDw75 with Sia-α(2-6) Galβ(1-4) Glc-NAc sequence, leading to resistance in colorectal cancer [19, 20]. ST3GalI has been reported of promoting tumor in breast cancer [21].

Increased sialylations together with altered enzyme profile regulating sialylation has been reported in several cancers including childhood acute lymphoblastic leukemia [10–15], colorectal [19, 20], breast [21], colon [22–24], cervical [25], ovary [26–40], prostate cancer [41, 42], pancreatic cancer [43–54], multiple myeloma [55–57], hepatocellular carcinoma [58].

Deletion in the human cmah gene coding for the enzyme cytidine monophospho-*N*-acetyl-neuraminic acid hydroxylase (CMP-Neu5Ac hydroxylase) prevents them from synthesizing *N*-glycolyl-neuraminic acid (Neu5Gc). However, in malignant states, Neu5Gc-sialoconjugates, are reported in particular from the gangliosides. Therefore the impact of elevated sialylation in cancers can thus further be appreciated by the observation of occurrence of Neu5Gc in cancer cell lines although they are not reported from adult healthy individuals [59] inferring at the altered sialylation synthetic machinery associated with malignancy.

In the current review the scope of sialylation as disease marker for endocrinal disorders are evaluated and studied under the headings of sialylations in the disorders of the endocrine organs including (i) ovary (ii) pancreas (iii) thyroid (iv) adrenal gland (v) pituitary and (vi) hypothalamus. The future scope of this review remains in the targeting of endocrinal disorders by targeting altered sialylations, which is a common manifestation in endocrinal disorders.

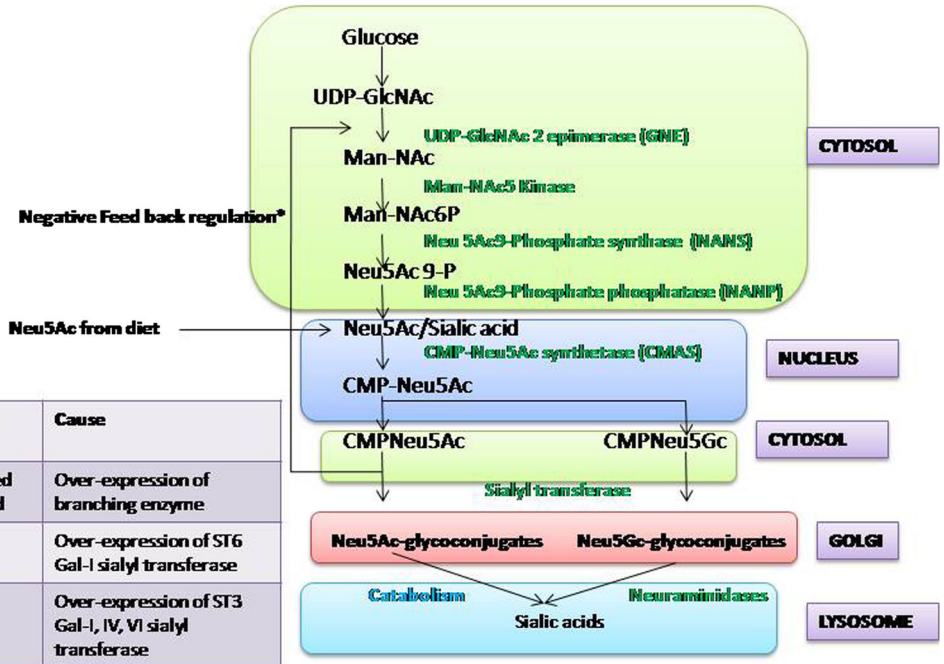
Sialylations in endocrinal disorders with special reference to cancers

Ovary

Ovarian cancer being lethal when diagnosed late suffers from the limitation of suitable sensitive biomarkers for early tumour detection, inflammation and progression. Increased sialylation ([26–40], Fig. 1) and branching of sialylation mediated by over-expression of sialyl transferase branching enzyme MGAT5 [27], alterations in DNA methylation [28], differential overexpression of sialoglycoproteins in serum [29] and overexpression of ST6GalI (β-galactosamide α2,6-sialyltranferaseI) gene, and overall increased sialylations in the proximal fluid from patients suffering from ovarian cancer [30], altered mRNA expressions of α2,3-sialyltransferase ST3GalI, ST3GalIII, ST3GalIV, ST3GalVI, and α2,6-sialyltransferase ST6GalI together with increased expression of ST3GalI leading to increased α(2-3)-linked sialylation [31] causing increase in tumour sizes, metastasis and resistance to therapy thereby indicating the role of sialic acid as biomarker for ovarian cancer detection and monitoring [26, 29, 30]. Modification of kallikrein 6 (KLK6) with α(2-6)-linked sialic acid in ovarian cancer patients further correlates with the fact that sialic acid could be a potential marker in ovarian cancer [32]. Over expression of sialylation and its branching correlates with inflammation and progress of ovarian tumour [27]. Ascites, plasma and serum from ovarian cancer patients reveal increased sialic acids [33], disialic acids [34], gangliosides [35], altered sialylation of alpha-1 protease inhibitor [36], increased lipid associated sialic acid (LSA, [38–40]) and increased sialylation in the cyst and peritoneal fluids of patients with ovarian cysts and tumors [40] are reported features in ovarian tumours.

Pancreas

Studies with pancreatic adenocarcinoma cell lines reveal that overexpression of α(2-6)-sialic acids that mediate increased adhesion to extracellular matrix (ECM) while overexpressed α(2-3)-sialic acids contribute to increased migration of cells thus overall leading to metastatic effects in cancer [43]. The contributory factors to increased sialylation and sialic acid in pancreatic carcinoma have been attributed to major factors including altered enzymes for biosynthesis of sialic acids [44], overexpression of GD3 synthase thereby inhibiting survival and angiogenesis of pancreatic cancer cells through cell cycle arrest [44], increased serum sialic acids [45] increased cell surface expression of sialic acid modulating migration and matrix adhesion in pancreatic adenocarcinoma [46], increased



Altered Sialylation in endocrinal cancer	Cause
Increased β 1-6 N linked branching of Sialic acid	Over-expression of branching enzyme
Increased α 2,6-sialylation	Over-expression of ST6 Gal-I sialyl transferase
Increased α 2,3 sialylation	Over-expression of ST3 Gal-I, IV, VI sialyl transferase
Overall increased sialylation	1.Over-expression of sialyl transferase 2. Downregulation of sialidase/neuraminidase
*Negative Feed back regulation of UDP-GlcNAc 2 epimerase by cytosolic CMP-Neu5Ac	Status not known in cancers of endocrine

Fig. 1 Sialic acid metabolism and cancer of the endocrine organs. Sialylated glycoconjugates are synthesized from sialic acid/Neu5Ac. It starts with (i) conversion of glucose to UDP-*N*-acetylglucosamine (UDP-GlcNAc) (ii) conversion of UDP-GlcNAc to *N*-acetylmannosamine (ManNAc) by the enzyme UDP-GlcNAc2 epimerase (GNE) (iii) phosphorylation of ManNAc to ManNAc-6-phosphate by the enzyme ManNAc5 kinase. (iv) ManNAc-6-P is then converted into NeuAc-9-phosphate by the enzyme sialic acid 9-phosphate synthase (NANS) (v) NeuAc-9-P converts into Neu5Ac mediated by sialic acid 9-phosphate phosphatase (NANP). The formed *N*-acetyl neuraminic acid (Neu5Ac)/ sialic acid formed in the cytosol then moves into the nucleus through a nuclear pore and is converted into CMP-Neu5Ac by the enzyme CMP-Neu5Ac synthase (CMAS). The enzymatic activity of UDP-GlcNAc2 epimerase is negatively regulated by feedback inhibition by cytosolic CMP-Neu5Ac in normal individuals. However the status of this regulation in not known in endocrinal cancers. CMP-Neu5Ac enters the cytosol from the nucleus by means of nuclear pores entering the Golgi apparatus where they are catalysed by sialyltransferases (ST) enabling their linkage to glycoconjugated moieties leading to the formation of sialoglycoconjugates. *N*-glycolyl neuraminic acid (Neu5Gc) absent in adult human is, however, observed in cancer mostly entering the body by dietary intake [59]. Neuraminidases/Sialidases catalytically removes the sialic acid from the sialoglycoconjugates. In the endocrine cancer biology a common pattern of overexpression of sialyltransferases, and downregulation of sialidases are reported leading to overall increase in sialylations [1–77] while the status of negative feed back regulation of GNE producing UDP-GlcNAc2 epimerase by CMP-Neu5Ac, in the sialic acid biosynthesis pathway reported from all eukaryotes remains unknown in cancers of the endocrine

expression of α2,3 sialyltransferase ST3GalIV promoting migration and metastasis in pancreatic adenocarcinoma [46], overall increased sialic acid conjugated N-glycans [47], sialylation of MUC1 mucin are reported from benign pancreas and pancreatic duct adenocarcinoma [48]. Alpha2,8-polysialyltransferase enzymes namely ST8Sia IV, ST8Sia II, and ST8Sia III are reported to be capable of incorporating both oligosialic and polysialic acid on various sialylated *N*-acetyllactosaminyl oligosaccharides, including NCAM of which ST8Sia IV and ST8Sia II were reported to efficiently catalyze polysialylation of NCAM as compared to ST8Sia III [4, 49]. Highly polysialylated neural cell adhesion molecule (NCAM) termed as PSA-NCAM have been reported to be a key molecule initiating metastatic pathway leading to peri neural invasion (pni) [49, 50], observed specially in pancreatic cancer mediated by the cancer cell invasion of nerves. However the complete mechanism of pni remains yet to be deciphered [50]. Down-regulation of two enzymes in the biosynthetic pathway of sialic acid biosynthesis including UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE) and *N*-acetylneuraminic acid 9-phosphate synthase by tumour suppressor, p16(INK4a) in pancreatic carcinoma model indicates further the correlation of sialylation with cancerous state in pancreatic cancer [51], increased metabolic influx of sialic acids [52], alteration in gene expression [53], overexpression of alpha ST3Gal III [54] are phenotypic

expression in pancreatic cancer with biological function in pancreatic cancer cell progression and metatstasis. Bulk metabolic flux through the sialic acid pathway causing about 40 % of proteins with doubled sialylation has been shown to correlate with the altered adhesive properties of pancreatic cancer cells implicating their role in metastasis [52], thus highlighting the role of sialic acid as diagnostic marker in pancreatic cancer.

Diabetes mellitus patient are reported to reveal increased plasma sialic acid content [60] correlating with marked insulin deficiency, increased sialylation of apolipoprotein E [61], glycosylation and sialylation of insulin-like growth factor-binding protein (IGFBP-3). This increases the affinity of IGFBP-3 for IGF-I while sialylation decreases it leading to complications [62]. Sialidase activity of peripheral mononuclear leukocytes in diabetic individuals has been reported to be significantly decreased as compared to normal individuals [63] and decreased glycophorin sialylation leads to increased erythrocyte aggregation that in turn leads to pathogenesis of vascular disease in diabetes [64]. Individuals with gestational diabetes mellitus (GDM), revealed higher placental sialidase activity accounting for reduced sialic acid content of glycodelin-A(GdA) leading to defective immunomodulatory effects in GDM pregnancies [65].

Thyroid

Increased mRNA level expression and activity of thyroid sialyltransferase-I are reported in Graves' disease [66] with increased expression of α(2-3)-sialic acids as compared to α(2-6)-sialic acids in autoimmune thyroiditis [67]. Sialylation has been reported to modulate the bioactivity of thyroid hormones. The impaired thyroid hormone action, and secretion of high amounts of sialylated TSH isoforms in hypothyroid or fetuses showing resistance to thyroid hormone (RTH), has been attributed to early expression of α-2,6-sialyltransferase activity thereby increasing terminal sialylation within thyrotropes [68].

Contrasting reports of sialylation profile exists for malignancy of the thyroid [69, 70]. While some studies have revealed correlation of increased level of α(2-3)-sialic acids with malignant transformation in the thyroid gland [71], decreased sialylation has also been reported to be correlate with the thyroid malignancy [72]. The increased expression of sialylated fibronectin [71] sialic acid masked Lewis(a) antigen [72], serum and overall total sialic acid [73] are reported in human thyroid cancer.

Adrenal cancer

Very few reports exist over the expression of total sialic acid in adrenal cancer. Adrenal cancer reveals overall increase in sialylation as revealed from its total cellular and cytoplasmic sialic acid content [74] and in plasma bound form [75]. No reports on the sialylation content individually on the glycoproteins and glycolipids are known in studies from adrenal cancer.

Pituitary

Few studies on pituitary and brain cancer reveal altered sialylation, polysialylation of neural cell adhesion molecule [76, 77], serum sialic acids [78, 79] and finds importance as prognostic markers in pituitary cancer [78].

Discussions

Endocrinal disorders and cancers are life threatening. Sialic acids have been reported to be markers in cancer [10–81]. Sialic acid with modification like *O*-acetylations has been reported to play a dominant role in the biology of cancer metastasis and progression. [10–15, 80, 81]. *O*-acetylation of sialic acids have been reported to enable survival of lymphoblasts in childhood acute lymphoblastic leukemia (ALL, 10). 9-*O*-acetylated sialic acids are reported as immunological tool for monitoring pediatric patients suffering from acute lymphoblastic leukemia (ALL, 15). A decrease in the level of *O*-acetylated sialic acids leading to an increased level of expression of sialyl Lewis(X), a tumor-associated antigen have been reported to be associated with cancer metastasis in colorectal carcinoma, [80, 81].

An overall alteration in the sialic acid synthesis machinery has been reported from different endocrinal cancers [26–40, 44–54, 69–79]. However, the status of negative feed back regulation of GNE producing UDP-GlcNAc2 epimerase by CMP-Neu5Ac, in the sialic acid biosynthesis pathway (Fig. 1) reported from all eukaryotes [59] remains yet to be investigated in cancers of the endocrine.

Based on onging researches across the globe, the current review highlights that increased sialylation due to altered genetic and metabolic events is a (i) hall mark in the biology of endocrinal cancer and finds application as a marker for detection, monitoring and progress of endocrinal cancer, which suffers from the current limitation of sensitive markers for early detection of the disease and (ii) is a strong indicator of endocrinal disorders. Although reports of sialylation in endocrinal cancers are reported, no reports of additional modifications like *O*-acetylations exists from cancers of the endocrines. This review is the first of its kind to study the increased sialylation in endocrinal disorder with special reference to cancers of the endocrine. This finding has a major impact on the diagnosis, therapeutics and targeting of the endocrinal cancer. The significance of the findings remains that although non specific, but an overall rise in the sialic acid level and sialylated moieties and their regulating enzymes and genes,

in the body, could be early indications of endocrinal cancer, thus strongly suggesting the role of sialic acid in endocrinal cancer prediction, diagnosis and prognosis. The future scope of this review remains that since sialic acid is a common marker observed to be expressed in different types of endocrinal cancer, is it possible to designate sialic acids as a marker to endocrinal cancer and employ it in clinics for early detection of the disease and secondly to target the sialic acid in such cancers thereby proving promising as therapeutic targets in endocrine cancers.

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