

The glycosylation landscape of pancreatic cancer (Review)

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Abstract. Pancreatic adenocarcinoma is a lethal disease with a 5-year survival rate of <5%, the lowest of all types of cancer. The diagnosis of pancreatic cancer relies on imaging and tissue biopsy, and the only curative therapy is complete surgical resection. Pancreatic cancer has the propensity to metastasise at an early stage and the majority of patients are diagnosed when surgery is no longer an option. Hence, there is an urgent need to identify biomarkers to enable early diagnosis, and to develop new therapeutic strategies. One approach for this involves targeting cancer-associated glycans. The most widely used serological marker in pancreatic cancer is the carbohydrate antigen CA 19-9 which contains a glycan known as sialyl Lewis A (sLe^A). The CA 19-9 assay is used routinely to monitor response to treatment, but concerns have been raised about its sensitivity and specificity as a diagnostic biomarker. In addition to sLe^A, a wide range of alterations to other important glycans have been observed in pancreatic cancer. These include increases in the sialyl Lewis X antigen (sLe^x), an increase in truncated O-glycans (Tn and sTn), increased branched and fucosylated N-glycans, upregulation of specific proteoglycans and galectins, and increased O-GlcNAcylation. Growing evidence supports crucial roles for glycans in all stages of cancer progression, and it is well established that glycans regulate tumour proliferation, invasion and metastasis. The present review describes the biological significance of glycans in pancreatic cancer, and discusses the clinical value of exploiting aberrant glycosylation to improve the diagnosis and treatment of this deadly disease.

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1. Introduction

Pancreatic adenocarcinoma is one of the worlds' most aggressive malignancies with a five year survival rate of less than 5%, the worst prognosis among all cancers (1). The poor survival rate is mostly due to the lack of a reliable early detection method, a tendency to metastasise at an early stage and resistance to available therapeutic options (2,3). In addition, there is often an absence of symptoms in early disease and established disease can have clinical similarities to benign conditions, making it difficult to diagnose (4). The diagnosis of pancreatic cancer relies on imaging and tissue biopsy, and the only curative therapy is complete surgical resection. Non-invasive biomarkers, such as those from serum, could provide a useful complement to imaging and cytology diagnostic methods and have the potential to aid clinical decisions as part of a routine blood test. Currently, the only clinical biomarker used in the management of pancreatic cancer is the serum marker CA19-9, which although used widely for disease monitoring, does not provide adequate accuracy for early detection and diagnosis. Given the usually late diagnosis of pancreatic cancer, highly specific circulating biomarkers for cancer detection and screening are urgently needed, and would be a major breakthrough allowing treatment for more patients.

Glycosylation is an enzymatic process that links glycan sugars to other glycans, lipids or proteins. Glycosylation takes place in the Golgi apparatus and endoplasmic reticulum and occurs as the consequence of the synchronised action of glycosylation enzymes. The two most common mechanisms by which glycans can be linked to lipids and proteins are O-linked and N-linked glycosylation. In O-linked glycosylation glycans are added sequentially to the hydroxyl oxygen of serine/threonine residues on target proteins and extended to produce various core and terminal structures that can be sialylated and/or fucosylated. In N-linked glycosylation 14 sugar preassembled blocks are transferred co-translationally to the amide group of an asparagine residue. N-glycans contain a common pentasaccharide core region consisting of three mannose and two N-acetylglucosamine (GlcNAc) subunits. This can be further

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modified by the addition of terminal Gal (galactose), GlcNAc, fucose and sialic acid moieties.

Aberrant glycosylation in cancer was first described nearly 50 years ago (5), and since then it has been well documented that the development and progression of cancer results in fundamental changes in the glycosylation patterns of cell surface and secreted glycoproteins (6). Many of the first cancer-specific antibodies identified were directed against oncofetal antigens expressed on embryonic and tumour cells but not in adult tissues (7) and growing evidence supports crucial roles for glycans during all steps of tumour progression. Glycans can regulate tumour proliferation, invasion, metastasis and angiogenesis (8), and aberrant glycosylation has been proposed as a general hallmark of all cancers (6).

Glycans can control cell identity and cell environment interactions. Changes in the glycosylation modification of proteins that are expressed on the cell surface, or secreted by cancer cells, are promising sources of potential biomarkers (9,10). Glycoconjugates with altered glycosylation are often shed into the circulation, allowing the distinction between patients with and without cancer (11-13). Recent research has uncovered new ways that glycosylation can contribute to cancer biology, as well as new strategies to improve treatment by exploiting glycans (14). This review discusses the changes in glycosylation involved in pancreatic cancer, their role in disease development and progression, and the huge potential to exploit glycans to improve diagnosis and treatment.

2. Aberrant glycosylation in pancreatic cancer

In the normal pancreas glycosylated proteins have important functions, including protection and lubrication of the pancreatic ducts (15). In pancreatic cancer glycosylation of proteins becomes deregulated, and the aberrant expression of specific glycans is associated with disease progression and poor prognosis. Changes to the glycome in pancreatic cancer include increases in the sialyl Lewis antigens (sLe^A and sLe^X), an increase in truncated O-glycans (Tn and sTn), increased branched and fucosylated N-glycans, upregulation of specific proteoglycans and galectins, and increased O-GlcNAcylation (some of these alterations are summarised in Fig. 1 and Table I).

3. The Sialyl Lewis antigens (sLe^A and sLe^X)

The most widely used serological assay used in the management of pancreatic cancer detects a cancer associated carbohydrate antigen, called CA 19-9, that contains a glycan known as sialyl Lewis A (sLe^A) (16-20). sLe^A is part of the Lewis family of blood group antigens, named after the discoverer of a series of antigens found on red blood cells. Studies have shown that sLe^A is found at low levels in normal tissue, higher levels in embryonic tissue (21), and is overexpressed in epithelial cancers (22). In the normal pancreas sLe^A is found on the epithelial surfaces of the ducts, whereas in pancreatic cancer sLe^A can be heavily secreted into the lumen of proliferating ducts and pass into the bloodstream (23).

The CA 19-9 assay detects the sLe^A glycan motif, along with the additional glycans, lipids and proteins to which it is attached. sLe^A has been found on numerous proteins including

mucins, carcinoembryonic antigen and circulating apolipoproteins (24). The CA 19-9 assay is widely used to monitor response to treatment in patients already diagnosed with pancreatic cancer (25,26), but concerns have been raised about its sensitivity and specificity as a diagnostic biomarker, and it is not used in screening (22,27-29). Mucin glycoproteins have multiple roles in pancreatic cancer and are major carriers of glycans including CA 19-9 (15). Altered mucin glycoforms have been observed in both the early stages of pancreatic cancer, and in late stage metastatic disease (30). It has been suggested that measuring the CA19-9 antigen on specific protein carriers (such as mucins), and detecting additional related glycans may improve the performance of the CA19-9 assay (28,31,32). Targeting mucin glycosylation may also limit pancreatic cancer growth (33).

In addition to sLe^A, other members of the Lewis antigens also have roles in pancreatic cancer. An isomer of sLe^A (known as sialyl Lewis X (sLe^X)) is also upregulated in some pancreatic cancers, and can be detected in the blood of many patients (34-37). The sialyl Lewis antigens are the minimal recognition motif for ligands of selectins, a family of lectins with roles in leukocyte trafficking with roles in tumour extravasation and cancer metastasis (38). In pancreatic cancer sLe^X has been found on migrating lymphocytes and linked to invasion (39). Increased sLe^X antigen on the glycoprotein ceruloplasmin has been described in pancreatic malignancy (37), and numerous proteins implicated in pancreatic cancer (including Kras, SPARC and Wnt7b) have been found to express sLe^X glycans (40). Tang *et al* (2016) profiled the levels of multiple glycans in the plasma of 200 patients with either benign pancreatic disease or pancreatic cancer (32), and showed increased levels of CA19-9, sLe^X and also in sialylated type 1 LacNAc (also known as Dupan-2). Dupan-2 has previously been associated with pancreatic cancer and is increased in some patients (41,42). Each of the three glycans (CA19-9, sLe^X and Dupan-2) are increased in some pancreatic cancer patients but not in others, leading the authors to suggest the use of a three glycan panel to improve diagnosis and facilitate pancreatic cancer sub-classification (32).

4. Truncated O-glycans

Immature, truncated O-glycans are characteristic of virtually all epithelial cancer cells (43). In pancreatic cancer, the expression of the truncated cancer-associated O-glycans Tn and sialyl-Tn (sTn) are linked to poor patient outcome (11), and associated with cancer cell growth and metastasis (44,45). The normal pancreas does not express Tn or sTn (46), but levels are high in pancreatic cancer (30,45). Specifically, truncated O-glycans have been detected on Nucleolin, EGFR and Her2 (45,47). COSMC is a molecular chaperone that is essential for correct protein O-glycosylation (48). Knockdown of COSMC promotes aberrant O-glycosylation in pancreatic cancer, and this is linked to anti-apoptotic and pro-metastatic cell behaviour, reduced proliferation and increased migration (45). In addition to COSMC, the GALNT3 enzyme is also linked to the aberrant production of tumor-associated O-glycans in pancreatic cancer. GALNT3 is increased in well/moderately differentiated pancreatic cancer, but lost in poorly differentiated tissues (47,49).

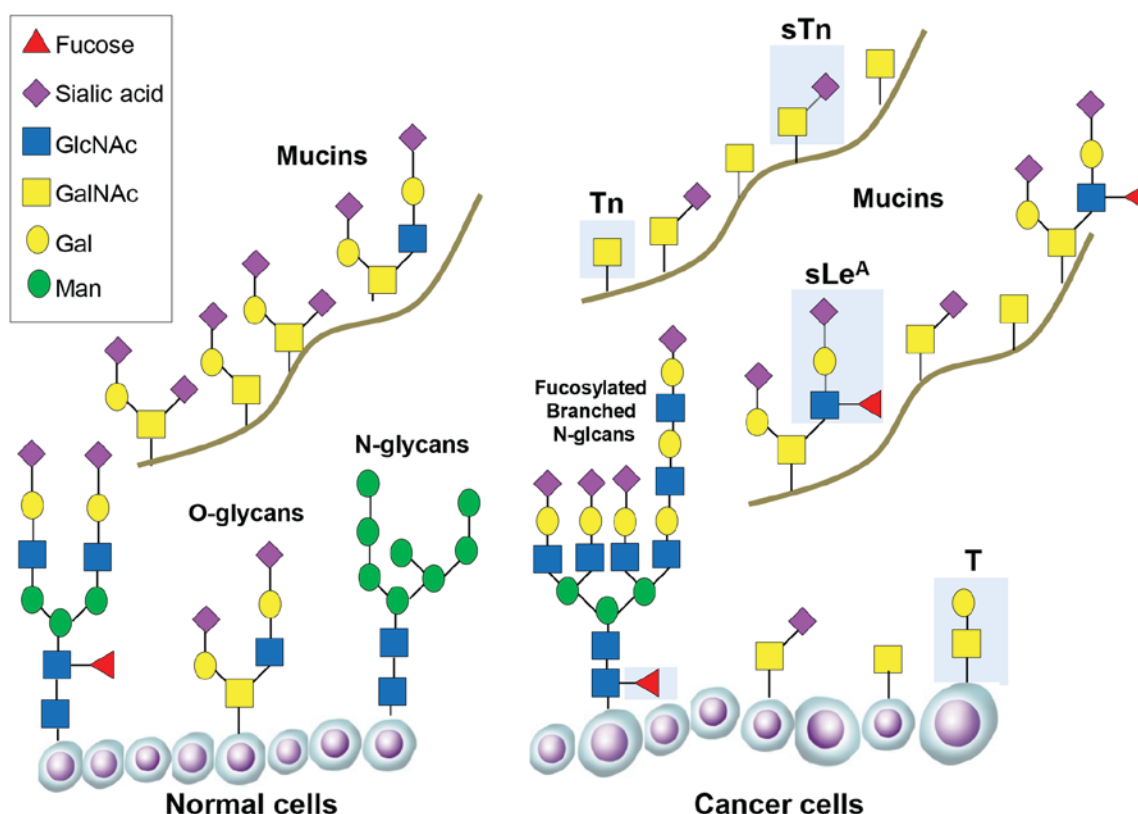


Figure 1. Changes in glycosylation during cancer progression. Representative O-glycans and N-glycans are shown attached on the surface of normal cells and cancer cells. O-glycans are also shown attached to mucin glycoproteins. Important tumour-associated glycans are shown in the blue boxes, including truncated O-glycans (Tn and sTn) and fucosylated branched N-glycans (sLe^A and sLe^X). For more information about the structure of each glycan see Table I.

5. N-glycans

Aberrant N-linked glycosylation is common in pancreatic cancer. In particular, pancreatic cancer cells frequently display increased levels of highly branched N-glycans, and alterations to N-glycan sialylation or fucosylation. Increased levels of N-glycosylation has been found on integrins and ECM adhesion proteins (50) and in proteins involved in pathways important in pancreatic cancer such as TGF- β , TNF, and NF-kappa-B signalling (51). N-glycosylation can also influence the surface expression of receptor tyrosine kinases and enhance the chemosensitivity of drug resistant pancreatic cancer cells (52). N-glycans have shown promise as biomarkers in pancreatic cancer. The sialyltransferase enzymes ST6Gal1 and ST3Gal3 are overexpressed in pancreatic tissue and this is linked to invasive potential (53-55). It is also possible to detect changes to N-glycans in patient blood. Increased fucosylation can be detected in serum from patients with pancreatic cancer (56), and highly branched N-glycans are increased in the blood of patients with aggressive disease (57,58). Fucosylated epitopes occur on specific proteins such as haptoglobin and ribonuclease 1 (RNASE1) and these are currently being explored for use diagnostically (59,60).

6. The HBP pathway

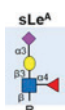
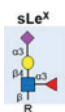


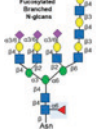



The hexosamine biosynthetic pathway (HBP) produces the amino sugar conjugate O-linked N-acetylglucosamine (O-GlcNAc). Addition of O-GlcNAc to proteins (known

as O-GlcNAcylation) can alter key hallmarks of cancer including transcription, cell signalling metabolism and epigenetics (61,62), and may impact cell survival and resistance during chemotherapy (63). O-GlcNAc is added to and removed from proteins by the O-GlcNAc cycling enzymes OGT and OGA. Both these enzymes are dramatically elevated in pancreatic cancer relative to normal pancreas, as are the overall levels of protein O-GlcNAcylation (64). In the normal pancreas OGT allows cells to dynamically respond to glucose levels by modulating O-linked protein glycosylation (65). When pancreatic cancer develops increased O-GlcNAcylation may block cancer cell apoptosis and lead to oncogenic activation of NF- κ B signalling (66). Several proteins with defined roles in pancreatic cancer have been shown to be modified by O-GlcNAc including the heat shock protein HSP70 (67), the transcription factor Sp1 (68), the Wnt signalling proteins β -catenin and LRP6 (69), and more recently the transcription factor Sox2 that determines self-renewal in pancreatic cancer and is responsible for tumour initiation (70). Inhibiting O-GlcNAcylation can reduce pancreatic tumour growth and progression suggesting HBP is promising potential therapeutic target (66,71,72).

7. Proteoglycans

In addition to aberrant protein glycosylation, cancer cells can also have alterations in proteoglycans (73). Proteoglycans are heavily glycosylated glycoproteins with attached glycosaminoglycans (GAGs) such as chondroitin sulphate and heparin

Table I. Summary of glycan alterations in pancreatic cancer.

Glycan	Structure	Change	(Refs.)
sialyl Lewis A (sLeA)		Upregulated Detected by CA 19-9 assay Found on various protein carriers including mucins	(23) (16-20) (15,28,31,32)
sialyl Lewis X (sLeX)		Upregulated Linked to invasion Found on numerous proteins implicated in pancreatic cancer	(34-37) (39) (32,40-42)
Tn antigen		Increased Linked to poor prognosis & metastasis	(30,45) (11,44,45)
sTn antigen		Increased Linked to poor prognosis & metastasis	(30,45) (11,44,45)
Fucosylated & branched N-glycans		Highly branched N-glycans increased in aggressive disease Increased fucosylation Found on numerous proteins implicated in pancreatic cancer	(57,58) (56,59,60) (50-52)
O-GlcNAcylation		Increased Inhibition can reduce tumour growth and progression	(64) (66,71,72)
Proteoglycans		Numerous proteoglycans are overexpressed in pancreatic cancer e.g., the heparin sulphate proteoglycan glypican-1 is linked to disease progression and expressed by exosomes	(74-82) (83,84)
Galectins		Galectin-1 & Galectin-3 overexpressed	(87-90)

sulphate that are located on the cell surface or secreted. Numerous proteoglycans have been found to be overexpressed in pancreatic cancer, including syndecan-1, versican, decorin, lumican and biglycan (74-81). Of particular interest, the heparin sulphate proteoglycan glypican-1 is overexpressed in pancreatic cancer cell models and patient tumours (82) and has been shown to contribute to pancreatic cancer progression using mouse models (83,84). A recent study found that glypican-1 is specifically expressed by circulating cancer exosomes, and may serve as non-invasive diagnostic and screening tool to enable early diagnosis of pancreatic cancer (85).

8. Galectins

As well as changes in glycosylation patterns cancer cells may also display altered expression of proteins that interact with glycans. An important example of such proteins is the galectins, which are a group of glycan binding proteins with an established role in cancer biology (86). In pancreatic cancer, Galectin-1 (GAL1) and Galectin-3 (GAL3) are overexpressed (87-90). This is important for cancer progression since GAL1 can induce stroma remodelling, tumour cell proliferation, invasion, angiogenesis, inflammation, and metastasis (91,92), and GAL3 can activate pancreatic cancer cells to produce inflammatory cytokines (88). It is likely that

galectin specific targeting will have a broad therapeutic potential in pancreatic cancer, either alone or in combination with other therapies (88,93).

9. Conclusions and future perspectives

The survival rates for pancreatic cancer have remained dismal for many years, and as such there is an urgent need to improve diagnosis and treatment. A wide range of alterations to glycans have been detected in pancreatic cancer, and these show promise as both potential circulating biomarkers and as targets for glycan specific therapies. The expression of specific glycans within pancreatic tumours, their presence in patient serum, and their possible ability to facilitate metastases, suggests glycans could help guide precision medicine strategies. Recent profiling has defined 4 molecular subtypes of pancreatic cancer (94), and it likely that diversity exists between pancreatic cancers in the variety and type of glycans made and secreted into the blood (24). To fully exploit glycans clinically it will be vital to fully profile the pancreatic cancer glycome and determine how this varies between different tumour types.

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JM conceived the review, researched the literature and wrote the manuscript.

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Competing interests

The author declares that there are no competing interests.

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