Munkley J, Mills IG, Elliott DJ.

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***Nature Reviews Urology* 2016**

DOI: http://dx.doi.org/10.1038/nrurol.2016.65

The role of glycans in prostate cancer development and progression

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Abstract

Prostate cancer is a unique and heterogeneous disease and there is a key clinical need to develop biomarkers to help distinguish indolent from aggressive disease. The prostate is an abundant secretor of glycoproteins of all types, and as such alterations in glycans are attractive potential biomarkers and therapeutic targets. Aberrant glycosylation is frequently observed in cancer but despite recent progress profiling the genome and proteome, the glycoproteome remains relatively understudied. Emerging data show a wide range of alterations to glycoproteins in prostate cancer, which include increased sialylation and fucosylation, increased O-GlcNAc, the emergence of cryptic and high mannose N-glycans and alterations to proteoglycans. Glycosylation can alter the functional activity of proteins and play a key role in many important biological processes in cancer including cell adhesion, migration, interactions with the cell matrix, immune surveillance, cell signalling, and cellular metabolism, and may modify these processes in prostate cancer. Recently glycosylation-specific antibodies have proven to be powerful tools in classifying prostate cancers based on immunohistochemistry, and glycosylation gene signatures have been reported which achieve this at the transcript level. Consequently it is timely to review emerging data that highlight the enormous potential of these relatively under-studied modifications in prostate cancer to improve risk stratification and therapeutic strategies.

The authors declare there are no competing interests as defined by Nature Publishing Group, or other interests that might be perceived to influence the interpretation of the article.

Key Points

- Glycosylation is a key cellular mechanism regulating many important biological processes in cancer. Alterations in glycoproteins and glycans are common features of cancer cells.
- The prostate is a secretory gland involved in male reproduction, and as such is an abundant secretor of glycoproteins of all types.
- A wide range of alterations to glycoproteins have been observed in prostate cancer including: increased sialylation and fucosylation, increased O-GlcNAcylation, the emergence of cryptic and high mannose N-glycans and alterations to proteoglycans.
- Glycosylation specific antibodies and glycosylation gene signatures have proven to be powerful tools in classifying prostate cancers.
- Glycans have major potential applications in prostate cancer as predictive and prognostic biomarkers and therapeutic targets.

Jennifer Munkley is a Prostate Cancer UK funded postdoctoral research associate within David Elliott's group at the Institute of Genetic Medicine, University of Newcastle, UK. Jennifer completed her PhD in Cancer Research at the University of York. She then moved into the field of prostate cancer research, focussing on androgen-mediated mechanisms of prostate cancer cell growth. She has a particular interest in how androgen-mediated changes in glycosylation influence prostate cancer progression.

Ian G. Mills is a Reader within the Centre for Cancer Research and Cell Biology, Queens University, Belfast, UK and is involved in the recently formed Prostate Cancer UK/ Movember Centre of Excellence for Prostate Cancer Research. He is also a group leader within the Centre for Molecular Medicine Norway (NCMM), Oslo, Norway. He is also a visiting scientist fellow at Cancer Research UK and an honorary senior visiting research fellow at the University of Cambridge, UK. His early career involved characterizing multi-protein complexes in endosome fusion and subsequently in clathrin-coated vesicle formation in Liverpool and Cambridge. He then began to research prostate cancer, focusing on transcriptional regulation and the interplay between transcription factors and co-regulators in prostate cancer, as well as the feedback relationship between metabolic stress pathways and gene expression. He has become interested in glycosylation as an important mediator of metabolic feedback affecting cancer progression

David J. Elliott is professor of Genetics at Newcastle University, where he is part of the Institute of Genetic Medicine. He carried out postdoctoral training in Boston and Edinburgh before taking a faculty position in Newcastle University. David's research has examined gene expression control in health and disease.

Introduction

The normal function of the prostate is to act as a secretory gland involved in male reproduction, and as such it is an abundant secretor of glycoproteins of all types. An important feature of prostate cancer is prognostic heterogeneity: while some prostate cancers can remain indolent for many years others can become much more rapidly aggressive. Active surveillance, rather than prostatectomy is an option for men with indolent cancers [1], however it is currently very difficult to distinguish between indolent (i.e. not necessarily lethal) and aggressive disease. The Gleason grading system is an established prognostic indicator in prostate cancer and is largely based on the size, morphology and structural characteristics of the prostate epithelial glands [2]. In a normal prostate, glands are large and irregularly shaped. However as tumours develop and progress, these glands become smaller and more rounded or oval. This change in shape and size could potentially drastically impact the normal secretory pathway where proteins and lipids are often glycosylated to produce glycoconjugates to be either secreted or exposed at the cell membrane.

Both the development of the prostate gland and maintenance of these secretory functions are dependent upon the androgen receptor (AR). Classically, androgen binding is thought to induce AR dimerization and translocation to the nucleus. Once in the nucleus, the AR binds to DNA sequences termed androgen response elements (AREs) within the promoter regions of a number of androgen-regulated genes, including genes encoding cell cycle regulators and regulators of central metabolism and biosynthesis [3]. Androgen deprivation therapy (ADT or chemical castration) is an effective first-line treatment for prostate cancer which is often initially effective. However recurrence of castrate-resistant form of the disease within 2-3 years can subsequently occur which is thought to involve persistence of AR signalling and reprogramming of the AR transcriptional landscape [4,5]. As castrate resistant prostate cancer is ultimately lethal, there is an urgent need to identify new diagnostic and therapeutic targets.

The two most common mechanisms by which glycans are linked to proteins are O-linked glycosylation and N-linked glycosylation (Figure 1). In O-linked glycosylation sugars are added incrementally to the hydroxyl oxygen of serine and threonine residues, whereas in N-linked glycosylation preassembled blocks of 14 sugars are transferred cotranslationally via the amide group of an asparagine residue. Hence glycans are attached to proteins via an oxygen atom of a serine or threonine (O-linked glycan) or a nitrogen atom of an asparagine (N-linked glycan). These posttranslational modifications occur as a consequence of the synchronised action of glycosyltransferases and glycosidases and take place in the Golgi apparatus and the lumen of the endoplasmic reticulum. In mammals there are nine sugar-nucleotide donors for glycosyltransferases; uridine diphosphate (UDP)-glucose, UDP-galactose, guanosine diphosphate (GDP)-mannose, GDP-fucose, UDP-xylose, UDP-glucuronic acid, cytidine monophosphate (CMP)-sialic acid, UDP-N-acetyl-galactosamine (UDP-GalNAc) and UDP-N-acetyl-glucosamine (UDP-GlcNAc) [6]. The expression and activity of the glycosyltransferase and glycosidase enzymes determine the cellular glycome which adds intrinsic diversity to the information encoded by the genome, transcriptome and proteome.

Glycosylation alterations in cancer were first described more than 60 years ago [7,8], and were later confirmed by the development of monoclonal antibodies against oncofetal antigens present on tumour glycoproteins [9-12]. Cancer cells display numerous glycosylation alterations compared to normal tissue, which can be due to altered expression or mislocalisation of glycosyltransferases, and differences in the availability of acceptor substrates, sugar nucleotide donors and cofactors in cancer cells. The most frequently observed cancer-associated glycosylation changes are in sialylation, fucosylation, truncated O-glycans and glycan branching. As cancer progression leads to dramatic changes to the glycoproteome, tumour-secreted glycoproteins may reflect the altered glycosylation pattern of cancer cells, and so are potential candidates for biomarkers.

Glycosylation of PSA

One of the principal functions of the prostate is to produce prostate specific antigen (PSA).PSA is a glycoprotein with a long history of being used as a prostate cancer biomarker [13,14], however, recent work has suggested that levels of PSA in serum do not effectively distinguish between prostate cancer and other prostatic conditions such as benign prostate hyperplasia (BPH) [1,15]. PSA is a serine protease that liquefies semen and enhances sperm motility [16]. During the early stages of prostate cancer, disruption of the prostate epithelium allows PSA to leak into circulating blood.

Several studies have investigated whether a tumour specific glycan signature on PSA can be utilised to distinguish between prostate cancer and BPH with the goal of developing a more reliable diagnostic test than determining serum PSA [17,18]. Initial studies compared the glycans detected on PSA from seminal fluid of healthy donors to PSA purified from the LNCaP cell line [19-21]. PSA glycans from LNCaP cells were found to have decreased sialic acid content and increased fucose and N-acetylgalactosamine [21]. Numerous other studies compared seminal plasma derived PSA obtained from patients with different disease states [22-25]. Serum PSA from patients with prostate cancer has been found to have increased levels of α2,3-linked sialic acid when compared to serum from patients with BPH [21,23,26]. Studies have also found an increase in O-linked glycosylation with the sialyl lewis X (SLe^x) antigen in PSA from malignant compared to adjacent normal prostate tissues [27].

PSA has a single site of N-glycosylation at Asn-69, which has been the target of multiple characterisation studies [17]. In 2013 the results of a comprehensive multi-laboratory study looking at Asn-69 N-glycosylation in healthy donors was reported [28]. The participating laboratories used three main approaches, which included determining glycoforms on intact PSA glycoproteins, a PSA glycopeptide analysis to determine glycans attached to a protease digested preparation, and analysis of glycans after PNGaseF digestion. These studies cumulatively reported 61 possible glycan structures attached to PSA, the most

abundant of which were four biantennary glycans, estimated as approximately 80% of the total. These four abundant glycans all include biantennary Hex5 (3-mannose;2-galactose)/HexNAc4 (4 GlcNAc) composition, either one or two sialic acid residues and the presence or absence of core fucose (Figure 2). Studies analysing the crystal structure of PSA in clinical samples from prostate cancer patients have also identified larger triantennary and tetraantennary glycans [19,22,29]. These larger glycans may reflect the disease status of the prostate cancer patients. However, as shifts in N-glycan profile have been linked to human ageing [30], it will be important to control for donor age in any future studies.

Another prostate cancer biomarker, prostatic acid phosphatase (PAP), is abundant in prostate tissue, and was used to monitor and assess the progression of prostate cancer before being replaced by PSA. Studies have found that PAP from prostate cancer patients has increased non-fucosylated structures, decreased high mannose chains [31], and increased core 2 linked SLe^x[27]. Both PSA and PAP are attractive targets for glycopathology, however for this to be confirmed there is a need to increase the number of clinical samples analysed, and to ultimately determine structural information for the cancer associated glycopeptides.

Sialyl-Tn can affect adhesion of prostate cancer cells

Prostate cancer cells display cancer-associated glycans associated with both incomplete synthesis and neo-synthesis processes. In general, incomplete synthesis occurs more often in the early stages of cancer, whereas neo-synthesis is more commonly observed in more advanced cancer stages[32]. The incomplete synthesis process is due to the impairment of normal complex glycan synthesis and leads to the biosynthesis of truncated structures such as the Tn and the sialyl-Tn (sTn) antigen [33] (Figure 3). sTn has been linked to cancer progression and survival in breast and colon cancer [34-36], and in prostate cancer sTn-MUC1 has been correlated with survival outcome and higher serum PSA levels [37]. The formation of the sTn antigen is catalysed by the sialyltransferase ST6GalNAc1, an enzyme which

catalyses transfer of a sialic acid in α2-6 linkage onto the Tn antigen (GalNAc1-OSer/ Thr) [34,35]. Increased expression of sialylated antigens can inhibit cell adhesion and promote cell detachment from the tumour due to electrostatic repulsion of negative charges. Recent work has demonstrated that expression of both ST6GalNAc1 and the sTn antigen are induced by androgens in prostate cancer cells (Figure 4), and can influence cell adhesion and transition to a more mesenchymal cell phenotype [38]. These results are important, as the sTn antigen is detected in up to half of high grade prostate cancers[39,40]. Although in breast and colon expression of ST6GalNAc1has been shown to increase tumour growth and metastasis, in DU145 cells ST6GalNAc1 dramatically hindered the formation of stable tumour masses in vivo and did not influence metastasis[38]. Interestingly, although expression of ST6GalNAc1 is upregulated in primary prostate tumours, it is relatively downregulated in metastatic tissue. These results are consistent with a 'reprogramming' of the genomic actions of AR during prostate cancer progression [5], and indicate that ST6GalNAc1 may have a transient role in tumour progression[38]. As is seen for other types of cancer, prostate cancer cells also bear the Tn antigen (GalNAc-O-Ser/Thr) on their cell surface. Although the role of this glycosylation in prostate cancer is unknown it has been a proposed target for immunotherapy in other cancers[41].

Sialyl Lewis X

The neo-synthesis process is the cancer-associated induction of genes linked to the expression of certain antigens such as sialyl lewis A (SLe^A) and sialyl lewis X (SLe^X) in many cancers. Following formation of a core 2 branch, sequential addition of sialic acid and fucose results in the formation of SLe^A and SLe^X (Figure 3). SLe^A and SLe^X have been demonstrated to be highly expressed in many malignant cancers, and high SLe^X expression levels are associated with poor survival in cancer patients [42,43].

Numerous studies have examined the role of SLe^A and SLe^X in prostate cancer, with particular focus on metastatic disease [27,44-47]. Upregulation of SLe^X has been associated with poor prognosis and with hormone resistant aggressive prostate cancer [46]. In prostate cancer cells PSA, MUC1 and PAP have increased core 2 O-linked SLe^X in malignant relative to normal prostate tissue [27]. SLe^X could influence prostate cancer development and progression through several mechanisms. Particularly, these structures are recognised by selectins expressed on different tissues involved in immune cell binding and binding to cells in distant organs and sites. For example, core 2 O-linked glycosylation of MUC1 on prostate cancer cells can allow them to evade immune destruction by NK cells [48].

Fucosylation

Fucosylation comprises the attachment of a fucose residue to glycans, and can be divided into terminal fucosylation which gives rise to specific blood group antigens such as SLe^A and SLe^X , and core fucosylation which creates a non-extendable modification [49]. Increased core fucosylation has also been detected in the serum of patients with prostate cancer compared to controls [50 51 52], and fucosylation is increased in the androgen-independent PC3 cell line relative to androgen dependent LNCaPs [53]. Fucosylated glycans are synthesised by a range of fucosyltransferases. *FUT8*, *FUT7* and *FUT3* gene expression levels are increased in prostate cancer which may influence cell trafficking [54], and upregulation of *FUT8* is associated with aggressive prostate cancer [55].

Branched N-glycans

β1,6GlcNAc tri and tetra branched N-glycans are frequently over-expressed in prostate cancer [56]. MGAT5 which initiates β1,6GlcNAc branching has been suggested to play a role in metastasis [57]. High throughput, comprehensive serum N-glycan profiling has been used to

investigate the use of serum N-glycans as a predictive biomarker. Castrate resistant prostate cancer patients have been found to have both increased transcription levels of N-glycan branching enzymes and increased tri and tetra-antennary N-glycans [52,58] (Figure 5A).

Cryptic N-glycans

Cryptic N-glycans are a result of alterations in the normal biosynthesis and processing of glycans [59] and represent high mannose structures which would normally be processed to more complex structures, N-Glycan precursors, or complex N-glycans which do not get further modified with galactose or sialic acid [60]. Cryptic N-glycans are normally masked by other sugar moieties, and as such are normally hidden. Cryptic N-glycans may be relevant in prostate cancer disease progression. The presence of cryptic N-glycans has been detected in advanced prostate tumours, with a high abundance of autoantibodies to the cryptic N-glycan Man9 in serum from prostate cancer patients [61](Figure 5B). Further development of assays to detect Man9 antibodies in serum samples will likely be a useful clinical tool in the future.

Glycosphingolipids

Many of the core-O-glycan structural motifs found on glycoproteins are also found on glycoshingolipds, which are glycans linked to a lipid ceramide. Glycosphinolipids that contain sialic acid are known as gangliosides. Ganglioside GD1a has been reported to be abundantly produced in castrate resistant prostate cancer [62,63], however further analyses of GD1a in prostate cancer tissue and model systems are needed. The F77 antigen has been linked to prostate cancer progression and was recently characterised as a glycolipid with α1,2 fucose linkages [64,65]. The F77 antibody was initially isolated from mice after injection of PC3 tumour cells and provoked great interest based on its potential diagnostic and

therapeutic properties [66,67]. The F77 antibody was found to stain 112 of 116 primary prostate tissues and 29 of 34 metastatic tissues with minimal staining of non-tumour prostatic tissue [67], and to inhibit the growth of PC3 or DU145 tumour xenographs in nude mice [67]. The glycosyltransferases responsible for the synthesis of the F77 antigen have been identified as FUT1, GCNT1, GCNT2 and GCNT3, as well as a possible hypoxia induced mechanism [64,65]. Notably the core 2 glycosyltransferase GCNT1 is upregulated in prostate cancer tissue and has been linked to disease progression [27,68].

Proteoglycans

Proteoglycans are glycoconjugates which contain glycosaminoglycans (GAGs) such as chondroitin sulfate, heparin suphate and keratin sulphate [32]. In the prostate gland proteoglycans make up important components of the tissue stroma and extracellular matrices [69]. Proteoglycans can act as key molecular effectors of the cell surface and pericellular microenvironment, and play a role in cancer progression through their ability to interact with both ligands and receptors [70 71]. Studies suggest that alterations to proteoglycan composition and function can promote prostate cancer cell survival and metastasis[72]. In prostate cancer some proteoglycans can be both pro- and anti-tumourigenic. Proteoglycans studied in prostate cancer include the extracellular proteoglycans decorin, perlecan and versican, and the cell surface proteoglycan syndecan-1[72,73].

The small leucine rich proteoglycan decorin has been shown to suppress prostate tumour growth through inhibition of both AR and phosphatidylinositol-3 kinase (PI3K) signalling. Decorin has been found to be reduced in prostate cancer stroma and is a promising target for future therapy for PTEN negative prostate cancers [74 75]. Conversely, the basement membrane proteoglycan perlecan is associated with disease progression and might be a tumour promoter through upregulation of sonic hedgehog signalling [76]. Increased levels of the large chondroitin sulphate proteoglycan versican have also been associated

with poor outcome in several types of cancer, including prostate cancer [77-80]. Versican can modulate binding to various components of the ECM and enhance the motility of prostate cancer cells [69,70]. Specific alterations to chondroitin sulphate side chains may be crucial for disease progression in prostate cancer, and may have important prognostic implications for disease progression when combined with Gleason score and PSA levels[81 82]. The cell surface proteoglycan syndecan-1 may also play a role in cancer progression. Syndecan-1 has been identified as a key molecule in maintaining prostate tumour initiating cells[83], and may play a role in the epithelial-to-mesenchymal transition and prostate cancer progression [84,85]. Further study of proteoglycans in prostate cancer will help improve our knowledge of both the prostate tumour cell microenvironment and the role of epithelial-stromal cell interactions in prostate cancer progression. Proteoglycan expression patterns in prostate tumours may also be useful as predictive and prognostic biomarkers.

Upregulated HBP in prostate cancer

The hexosamine biosynthesis pathway (HPB) (Figure 6) is sustained by metabolites sourced from the major metabolic processes in the cell, and has emerged as a major metabolic integration point influencing cell cycle, growth, metabolism and stress [86,87]. HBP produces an amino-sugar conjugate UDP-N-acetylglucoamine (UDP-GlcNAc), which provides a substrate

for posttranslational modification of plasma membrane and secretory proteins. UDP-GlcNAc can be utilised by 1) O-GlcNAcylation catalysed by O-GlcNAc transferase (OGT) in the cytoplasm, nucleus and mitochondria [88], and 2) O- and N-linked glycosylation occurring in the Golgi apparatus and endoplasmic reticulum (ER) leading to complex sugar conjugates on target proteins [89]. Expression of OGT in prostate cancer correlates with Gleason score and biochemical recurrence [90,91], and elevated O-GlcNAc is associated with poor prognosis of patients [92]. OGT glycosylates and therefore modifies the function of a range of target proteins. In prostate cancer key targets of OGT include transcription factors such as c-MYC and FOXM1 [90]. Hence upregulation of OGT activity in prostate cancer may promote metabolic reprogramming of tumour cells in combination with *MYC* copy number gain and has significant effects on transcription [90,91].

The last enzyme in the HBP pathway, UDP-N-acetylglucosamine pyrophosphorylase 1 (UAP1), is highly over-expressed in prostate cancer. UAP1 expression is regulated by AR activity and has been shown to correlate with Gleason score and expression of the AR [93]. High levels of UAP1 expression and/or HBP activity in prostate cancer appears to be protective against inhibitors of N-linked glycosylation [93].

Upregulation of glycosylation enzymes in prostate cancer

One of the primary factors contributing to aberrant glycosylation in cancer is the differential expression of glycosyltransferases and glycosidases involved in the synthesis and catabolism of glycans [94]. There is growing evidence linking differential expression of glycosylation enzymes to prostate cancer progression [27,38,90,93,95,96]. A recent meta-analysis of clinical prostate cancer gene expression data identified a novel discriminatory signature enriched for 4 glycosylating enzymes: *ST6GalNAc1*, glucosaminyl (N-acetyl) transferase 1, core 2 (*GCNT1*), UDP N-acetylglucosamine pyrophosphorylase 1 (*UAP1*) and beta-1,3-glucuronyltransferase 1 (*B3GAT1*) [95].

As discussed above, the sialyltransferase enzyme gene *ST6GalNAc1* is upregulated in prostate cancer[38]. ST6GalNAc1 synthesises the sTn antigen and has been shown to reduce prostate cancer cell adhesion [38]. UAP1 is the last enzyme in the HBP pathway and is highly over-expressed in prostate cancer [93]. GCNT1 catalyzes the formation of core 2 branched O-linked glycans, and has previously been shown to be an independent predictor for prostate cancer recurrence, and to correlate positively with aggressive potential [68]. GCNT1 is also implicated in the production of the F77 prostate cancer antigen [64,65] and with altered O-glycosylation of PSA, PAP, and MUC1 [27]. Upregulation of GCNT1 in prostate cancer tissue frequently co-occurs with upregulation of *ST6GalNAc1* [38] and has been seen as part of a glycosylation signature with 6 other glycosylation enzyme genes, *GALNT7*, *ST6GalNAc1*, *GALNT3*, *B4GALT3*, *MOGS* and *EDEM3* [27]. These results suggest that it will be important to understand how changes in glycosylation enzymes function co-ordinately with each other rather than in isolation to modify the cellular behaviour of prostate cancer cells.

Future Perspectives

Although patterns of glycosylation change in many cancers, emerging studies suggest that the functional and prognostic relevance of some glycans may be unique to prostate cancer relative to other human adenocarcinomas. Positive binding of HPA lectin is associated with disease progression and metastasis in a range of cancer types [97-100], however in prostate cancer binding of HPA is reduced in metastatic disease and HPA negative tumours have poorer overall survival and faster progression. Studies suggest that expression of the ST6GalNAc1 enzyme in breast and colon cancer cell lines increases tumour growth, but that in prostate cancer cells ST6GalNAC1 expression actually inhibits the formation of solid tumour masses, [38,101,102]. Together with the abundance of glycosylation changes detected in prostate cancer, this data suggests that there is some yet undefined feature of glycosylation

that is unique to prostate tissue. One potential explanation is the emerging direct link between the androgen receptor and the expression of key glycosylation enzymes. Expression of the HBP pathway enzyme UAP1 has recently shown to be directly regulated by the androgen receptor in prostate cancer cells [93]. Similarly, expression of ST6GalNAc1, which is responsible for synthesising the sTn antigen, is also a direct transcriptional target of the androgen receptor [38]. It is interesting to speculate that some of the unique features of glycans observed in prostate cancer may be due at least in part to androgen receptor mediated changes to the prostate cancer glycoproteome. An alternative explanation is that the overall change in the glycoproteome is multi-factorial and we need to evaluate the impact of each glycosylation enzyme in a context in which multiple other enzymes are also dysregulated.

Current methods to evaluate glycosylation changes in prostate tissue usually involve homogenisation and extraction of glycans and glycoproteins for analysis by mass spectrometry or antibody / lectin array, meaning that any spatial or histopathology information is lost. Broad affinity carbohydrate binding lectins can be used to examine glycan structures in prostate tissue; however this does not determine individual glycan species. Recently an approach to profile N-linked glycans directly on Formalin-fixed, Paraffin-embedded (FFPE) tissues and tissue microarrays has been developed[18,103,104]. A molecular coating of PNGaseF is sprayed directly and the released glycans are analysed using a high resolution MALDI-FTICR instrument. Ion intensities of each released glycan at each tissue location are determined and converted to a heat map for each glycan. As FFPE tissues are usually readily available in pathology labs this technique holds particular promise. This approach has been used to detect cryptic glycan species, high mannose and glycans attached to PSA, and has the potential to identify novel biomarker panels of individual glycan species for prostate cancer detection and prognosis [18,103,104].

Glycomic analysis of serum and plasma from patients with prostate cancer has produced promising results in terms of distinguishing prostate cancer from non-tumour

samples [52,105]. Evaluation of changes in the glycosylation of exosome glycoproteins isolated from prostate cancer serum or expressed-prostatic secretions (EPS) fluids has also shown promise as an alternative approach [106]. In the future it is expected that the potential approaches described will be evaluated further using a large number of clinical samples.

An important clinical challenge of prostate cancer is prognostic heterogeneity: it is currently very difficult to determine between indolent (i.e. not necessarily lethal) and aggressive disease. We still do not understand what makes some prostate cancers slow growing relative to other aggressive tumours, or why prostate cancer tends to metastasise to bone. As glycoproteins and glycan composition likely play important roles in these processes, understanding their function and composition in both the normal prostate and in prostate cancer will be vital in terms of disease management.

Figure Legends

Figure 1. Representative O- and N-linked glycans in mammalian cells. Proteins can be glycosylated by N-linkage to Asp or O-linkage to Ser/Thr. O-glycans can be extended to produce different cores and terminal structures which are usually sialylated and fucosylated. N-glycans contain a common pentasaccharide core region that can be further modified by terminal structures.

Figure 2. The four most common biantennary N-linked glycan structures of PSA Asn-69. The PSA protein is glycosylated at Asn-69. The four most abundant PSA glycans are all composed of biantennary Hex5 (3-mannose;2-galactose)/HexNAc4 (4 GlcNAc), with either one or two sialic acid residues and the presence or absence of core fucose.

Figure 3. Schematic representation of glycan structures produced by incomplete and neo-synthesis. The incomplete synthesis process is due to the impairment of normal complex glycan synthesis and leads to the biosynthesis of truncated structures such as the Tn and the sialyl-Tn (sTn) antigen. The neo-synthesis process is the cancer-associated induction of genes linked to the expression of certain antigens such as Sialyl Lewis A (SLe^A) and Sialyl Lewis X (SLe^X). in many cancers. Following formation of a core 2 branch, sequential addition of sialic acid and fucose results in the formation of SLe^A and SLe^X .

Figure 4. Induction of the cancer-associated sTn antigen by androgens in prostate cancer cells. (A) The formation of the sTn antigen is catalysed by the sialyltransferase ST6GalNAc1, an enzyme which catalyses transfer of a sialic acid in α2-6 linkage onto the Tn antigen (GalNAc1-OSer/ Thr). (B) Immunofluorescent images of LNCaP prostate cancer cells grown in the presence or absence of 10nM of the synthetic androgen analogue R1881

(androgen) for 72 hours. In the presence of androgens expression of both ST6GalNAc1 and the sTn antigen are induced. ST6GalNAc1 and the sTn antigen were detected as described previously [107]. The antibodies used were: anti-ST6GalNAc1 C-term (Rb pAb, 15363-1, Proteintech) and anti-Sialyl Tn (Ms mAb, Abcam 115957), The scale bar is 10μM.

Figure 5. Structural examples of branched and cryptic N-glycans in prostate cancer. (A) Representative tri and tetra β1,6 GlcNAc branched N-glycans. β1,6 GlcNAc branching is initiated by the MGAT5 enzyme. (B) Cryptic N-glcans detected in prostate cancer include high mannose (Man9) clusters. Crypric N-glycans share the N-glycan mannose cores, but unlike fully glycosylated cellular N-glycans which are normally capped by sialic acid, these targets expose internal sequences.

Figure 6. The Hexosamine biosynthetic pathway (HBP). HBP metabolises glucose to produces UDP-GlcNAc. UDP-GlcNAc serves as a major sugar donor for O-GlcNAcylation and for classical N- and O-glycosylation. O-GlcNAc transferase (OGT) catalyses the transfer of GlcNAc from the sugar donor UDP-GlcNAc to the hydroxyl group of Ser and Thr residues. O-GlcNAcase (OGA) carries out the reverse reaction and hydrolyses the sugar from proteins. Expression of the rate limiting enzyme UDP N-acetylhexosamine pyrophosphorylase is upregulated in prostate cancer cells and has been linked to disease progression.

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