Glycosylation is a global target for androgen control in prostate cancer cells

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Abstract
Changes in glycan composition are common in cancer and can play important roles in all of the recognised hallmarks of cancer. We recently identified glycosylation as a global target for androgen control in prostate cancer cells and further defined a set of 8 glycosylation enzymes (GALNT7, ST6GalNAc1, GCNT1, UAP1, PGM3, CSGALNACT1, ST6GAL1 and EDEM3), which are also significantly upregulated in prostate cancer tissue. These 8 enzymes are under direct control of the androgen receptor (AR) and are linked to the synthesis of important cancer-associated glycans such as sialyl-Tn (sTn), sialyl LewisX (sLeX), O-GlcNAc and chondroitin sulfate. Glycosylation has 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. Our results suggest that alterations in patterns of glycosylation via androgen control might modify some or all of these processes in prostate cancer. The prostate is an abundant secretor of glycoproteins of all types, and alterations in glycans are, therefore, attractive as potential biomarkers and therapeutic targets. Emerging data on these often overlooked glycan modifications have the potential to improve risk stratification and therapeutic strategies in patients with prostate cancer.

Introduction
The prostate gland is principally responsible for secreting fluid, which nourishes and protects sperm during reproduction. Both the development of the prostate gland and maintenance of these secretory functions depend on the actions of androgen steroid hormones (Livermore et al. 2016). Androgens also play a critical role in the development and progression of prostate cancer, and androgen deprivation therapy (ADT) is usually the first-line treatment for metastatic disease. Androgens control gene expression via the androgen receptor (AR) transcription factor. The AR is important for many signalling pathways and is essential for prostate cancer cell viability, proliferation and invasion (Haag et al. 2005, Hara et al. 2008, Snoek et al. 2009). Although ADT is usually initially effective, after 2–3 years, many patients develop castrate-resistant prostate cancer (CRPC), which is ultimately lethal. Progression to CRPC is believed to involve reprogramming of the AR transcriptional landscape and a selective pressure for cells to maintain AR activity even in lower concentrations of circulating androgens (Sharma et al. 2013, Mills 2014). AR signalling can be maintained through gene amplification (Visakorpi et al. 1995), activating mutations (Veldscholte et al. 1990, Steinkamp et al. 2009), AR splice

The AR classically acts to control the expression of target genes. Androgen binding promotes dimerisation of AR and its translocation to the nucleus where it can act as either a transcriptional repressor or activator (Cai et al. 2011, Karantanos et al. 2013, Munkley et al. 2014, 2015a,b). AR target genes can be classified based on the transcripts and proteins that respond to androgens. Numerous genomic and transcriptomic studies have been carried out to identify AR-binding sites and target genes; however, these have been limited by the genome coverage on microarrays (Massie et al. 2007, 2011, Wang et al. 2009, Rajan et al. 2011) or by the challenges of directly associating an AR-binding site with a given gene and its expression (Massie et al. 2011, Wu et al. 2011, Sharma et al. 2013, Barfeld et al. 2014b). To address these issues and to further understand the mechanisms by which androgens drive the development and growth of prostate cancer, RNA sequencing has been used to comprehensively profile how the prostate cancer transcriptome changes in response to androgens. We directly correlated gene expression data from LNCaP prostate cancer cells exposed to androgens (Munkley et al. 2016b) with data from 7 prostate cancer patients before and after ADT (Rajan et al. 2014). Our approach produced a comprehensive map of 700 genes controlled by the AR in clinical prostate cancer and provides a new window through which to understand the signalling pathways downstream of the AR (Munkley et al. 2016b). Gene set enrichment analysis of these 700 genes identified known AR-regulated processes including fatty acid metabolism (Swinnen et al. 1997a, 2000, Liu 2006), response to endoplasmic reticulum stress (Segawa et al. 2002, Sheng et al. 2015) and cholesterol and lipid biosynthesis (Swinnen et al. 1997b, Suburu & Chen 2012, Wu et al. 2014, Butler et al. 2016). In addition to these well-characterised AR-regulated pathways, our data highlighted ‘glycosylation’ as a previously unidentified global target for androgen control in prostate cancer cells (Munkley et al. 2016b). Gene ontology analysis identified 7 androgen-regulated processes containing the term ‘glycosylation’ (Fig. 1).

Glycosylation

Glycosylation is an enzymatic process that links glycan sugars to other glycans, lipids or proteins. The complete pattern of glycan modifications in a cell or tissue (known as the glycome) is assembled by the synchronised action

Figure 1
Glycosylation is a global target for androgen control in prostate cancer patients. RNA sequencing analysis of prostate cancer cell lines and patients identified a set of 700 androgen-regulated genes. Gene ontology (GO) analysis of these genes identified 72 terms with significant gene enrichment (P < 0.05) and further defined glycosylation as an androgen-regulated process in prostate cancer cells.

of numerous glycosylation enzymes and takes place in the Golgi apparatus and the lumen of the endoplasmic reticulum. 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, sugars are added sequentially to the hydroxyl oxygen of serine and/or threonine residues on the target protein, whereas in N-linked glycosylation, preassembled blocks of 14 sugars are transferred co-translationally via the amide group of an asparagine residue on the target protein (Schwarz & Aebi 2011, Kudelka et al. 2015) (Fig. 2). How much a given protein or lipid is glycosylated depends on the number of glycosylation sites present, as well as the expression and activities of specific glycosylation enzymes within the cell or tissue (Marth & Grewal 2008).

Aberrant glycosylation can play an important role in cancer progression. Glycans can have a key role in cell adhesion, migration, interactions with the cell matrix, immune surveillance, cell signalling and cellular metabolism (Munkley & Elliott 2016a). It is well documented that the development and progression of cancer results in fundamental changes to the glycome; these are associated with all the recognised hallmarks of cancer (Munkley & Elliott 2016a). Numerous studies have described aberrant glycosylation in prostate cancer; however, despite this, the study of glycans has lagged behind our characterisation of the genome and proteome (Munkley et al. 2016a). As mounting evidence links changes in the glycan composition of prostate cancer cells to disease progression (Munkley et al. 2016a), we further analysed our RNA-sequencing data to identify 25 genes with roles in the glycosylation process, which
are regulated by androgens in prostate cancer cells (Munkley et al. 2016b). Further analysis of these 25 genes indicated that androgens control the expression of enzymes and lectins operating at multiple steps within the glycosylation synthetic pathways. These include enzymes regulating the synthesis and processing of both O- and N-glycans, the hexosamine biosynthetic pathway (HBP) and the synthesis of chondroitin sulfate (Table 1). Importantly, and consistent with AR-dependent regulation of key glycosylation enzymes, androgens also control the synthesis of several cancer-associated glycans in prostate cancer cells (Fig. 3).

**Androgens control O-glycan biosynthesis**

O-glycans consist of branched and linear arrangements of sugar groups that are transferred to glycoproteins by glycosylation enzymes in the Golgi apparatus. O-glycan synthesis depends on many factors, including the expression and localisation of glycosylation enzymes, the structure of the Golgi and the availability and levels of sugar donors (Kudelka et al. 2015). In O-linked glycosylation, sugars are added incrementally to the hydroxyl oxygen of serine and threonine residues on the target protein. O-glycans become altered in the early stages of cellular transformation and are important for cancer initiation, invasion and metastasis (Kudelka et al. 2015). We identified five androgen-regulated glycosylation enzymes (GALNT7, ST6GalNAc1, GCNT1, GCNT2 and FUT1) with roles in O-glycan biosynthesis (Munkley et al. 2016b). These enzymes control key steps in the initiation of O-glycosylation and core synthesis and have been linked to the production of several cancer-associated glycans. Initiation of O-glycosylation is carried out by a family of GALNT sialyltransferase enzymes, including GALNT7, which catalyse the transfer of GalNAc to serine/threonine residues on target proteins to produce the Tn antigen (GalNAc-O-Ser/Thr) (Ten Hagen et al. 2003). GALNT7 has been identified as oncogenic in several types of cancer (Gaziel-Sovran et al. 2011, Peng et al. 2012, Li et al. 2014b, Peng et al. 2016a).
Table 1  Overview of the glycosylation genes regulated by androgens in prostate cancer cells.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
<th>Role in cancer?</th>
<th>Previously linked to prostate cancer?</th>
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<tbody>
<tr>
<td><strong>O-glycan biosynthesis</strong></td>
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<tr>
<td>ST6GALNAC1</td>
<td>Sialylation of O-glycans to produce the cancer-associated sTn antigen (Munkley 2016)</td>
<td>Associated with poor prognosis in numerous cancer types (Munkley 2016). Promotes tumourigenicity and metastasis in breast and gastric cancer cells (Julien et al. 2006, Ozaki et al. 2012)</td>
<td>ST6GALNAC1 shown to synthesise sTn and reduce cell adhesion in prostate cancer cells (Munkley et al. 2015c, Munkley &amp; Elliott 2016b). sTn antigen expressed in high grade prostate tumours (Myers et al. 1994, Genega et al. 2000). Androgen regulated in prostate cancer cells (Munkley et al. 2015c, Munkley &amp; Elliott 2016b)</td>
</tr>
<tr>
<td>GALNT7</td>
<td>Initiation of O-glycosylation</td>
<td>Identified as oncogene in several types of cancer and controlled by micro-RNAs (Gaziel-Sovran et al. 2011, Peng et al. 2012, Li et al. 2014b, Nie et al. 2015, Lu et al. 2016)</td>
<td>Upregulated in malignant PCA as part of a glycosylation gene signature (Barfeld et al. 2014a). Androgen regulated and linked to prostate cancer cell viability (Munkley et al. 2016b)</td>
</tr>
<tr>
<td>GCNT1</td>
<td>Formation of core 2 branched O-glycans</td>
<td>Upregulated in endometrial carcinoma and associated with poor survival (Miyamoto et al. 2013)</td>
<td>Increased tumour growth in orthotopic mouse models (Hagisawa et al. 2005). Promotes resistance to NK cell immunity (Okamoto et al. 2013). Upregulated in prostate cancer and implicated in the synthesis SLex (Hagisawa et al. 2005, Chen et al. 2014). Role in synthesising the F77 antigen (Nonaka et al. 2014) which increases prostate cancer cell growth (Zhang et al. 2010). Correlates with aggressive disease (Kojima et al. 2015) and is a predictor of recurrence after radical prostatectomy (Sato et al. 2016). Detected in patient urine (Kojima et al. 2015). Androgen regulated and linked to prostate cancer cell viability (Munkley et al. 2016b)</td>
</tr>
<tr>
<td><strong>Hexosamine biosynthetic pathway (HBP)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>GCNT2</td>
<td>Formation of core 2 branched O-glycans</td>
<td>Suppression of GCNT2 linked to metastasis in colorectal cancer (Nakamura et al. 2015). Increased expression promotes EMT transition and metastasis in breast cancer (Zhang et al. 2011)</td>
<td>Role in synthesising the F77 antigen (Nonaka et al. 2014) which increases prostate cancer cell growth (Zhang et al. 2010)</td>
</tr>
<tr>
<td><strong>UAP1</strong></td>
<td>Final enzyme in the hexosamine biosynthetic pathway (HBP)</td>
<td>Established role in synthesising O-GlcNAc which is elevated in numerous cancer types and has itself been described as a hallmark of cancer (Fardini et al. 2013, Ma &amp; Vosseller 2014)</td>
<td>Over-expressed in prostate cancer and linked to increased synthesis of O-GlcNAc (Itkonen et al. 2014). Over-expression of O-GlcNAc linked to poor prognosis (Kamigaito et al. 2014). Promotes resistance against inhibitors of N-glycosylation (Itkonen et al. 2014). Detected in patient serum and urine (Ma et al. 2014, Albitar et al. 2016). HBP potential target in CRPC (Kaushik et al. 2016)</td>
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<thead>
<tr>
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<th>Function</th>
<th>Role in cancer?</th>
<th>Previously linked to prostate cancer?</th>
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</thead>
<tbody>
<tr>
<td>PGM3</td>
<td>Penultimate enzyme in the hexosamine biosynthetic pathway (HBP)</td>
<td>Established role in synthesising O-GlcNAc which is elevated in numerous cancer types and has itself been described as a hallmark of cancer (Fardini et al. 2013, Ma &amp; Vosseller 2014)</td>
<td>Androgen regulated and linked to prostate cancer cell viability (Lee et al. 2010, Munkley et al. 2016b). HBP is a potential target in CRPC (Kaushik et al. 2016)</td>
</tr>
<tr>
<td>GNPNAT1</td>
<td>Second enzyme in the hexosamine biosynthetic pathway (HBP)</td>
<td>Established role in synthesising O-GlcNAc which is elevated in numerous cancer types and has itself been described as a hallmark of cancer (Fardini et al. 2013, Ma &amp; Vosseller 2014)</td>
<td>GNPNAT1 is decreased in CRPC (Kaushik et al. 2016). Inhibition of HBP suggested as a potential therapy in CRPC (Kaushik et al. 2016)</td>
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### Chondroitin sulfate synthesis

<table>
<thead>
<tr>
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<th>Function</th>
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<th>Previously linked to prostate cancer?</th>
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<tbody>
<tr>
<td>CSGALNACT1</td>
<td>Initiation of chondroitin sulfate (CS) synthesis</td>
<td>CS is a key molecule in cancer progression (Theocharis et al. 2006). The large CS proteoglycan Versican is linked to poor prognosis in many different cancer types (Riciardielli et al. 2009b). CSGALNACT1 is upregulated by NEDD9 in breast cancer cells (Iida et al. 2015)</td>
<td>CSGALNACT1 is androgen regulated and required for prostate cancer cell viability (Munkley et al. 2016b). Versican is androgen regulated (Read et al. 2007) and is linked to disease progression in early stage prostate cancer (Riciardielli et al. 1998). CS levels can predict prostate cancer progression and are increased in metastatic disease (Riciardielli et al. 1997, 2009a). Targeting oncofetal CS using glycosaminoglycan binding malaria protein inhibits PC3 cell growth in mouse models (Salanti et al. 2015)</td>
</tr>
<tr>
<td>CHPF</td>
<td>Chondroitin sulfate synthesis</td>
<td>CS is a key molecule in cancer progression (Theocharis et al. 2006). The large CS proteoglycan Versican is linked to poor prognosis in many different cancer types (Riciardielli et al. 2009b). Differential methylation of CHPF is induced by FOXM1 (Hwang et al. 2013)</td>
<td>Not reported</td>
</tr>
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### Lectins

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
<th>Role in cancer?</th>
<th>Previously linked to prostate cancer?</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLEC</td>
<td>Mannose binding lectin</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>LMAN2</td>
<td>Lectin that binds high mannose glycoproteins</td>
<td>Over-expressed in the gastric cancer secretome (Marimuthu et al. 2013)</td>
<td>Not reported</td>
</tr>
<tr>
<td>ERLEC1 (CIM)</td>
<td>Lectin with role in N-glycan recognition</td>
<td>Critical for lung cancer metastasis (Yanagisawa et al. 2010)</td>
<td>Not reported</td>
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</table>

### OST complex

<table>
<thead>
<tr>
<th>Enzyme</th>
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<th>Role in cancer?</th>
<th>Previously linked to prostate cancer?</th>
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<tbody>
<tr>
<td>RPN2</td>
<td>Subunit of Oligosaccharyltransferase (OST) complex which catalyses central reaction in N-glycosylation</td>
<td>Linked to malignancy (Tominaga et al. 2014) and confers resistance to docetaxel in breast cancer (Honma et al. 2008). Potential biomarker in colorectal cancer (Zhang et al. 2015). Linked to survival and invasion in lung cancer (Fujita et al. 2015)</td>
<td>Not reported</td>
</tr>
<tr>
<td>STT3A</td>
<td>Subunit of Oligosaccharyltransferase (OST) complex which catalyses central reaction in N-glycosylation</td>
<td>Not reported</td>
<td>Not reported</td>
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(Continued)
Nie et al. 2015, Lu et al. 2016) and is upregulated in malignant prostate cancer as part of a glycosylation gene signature (Barfeld et al. 2014a). As GALNT7 initiates O-glycosylation, upregulation of this enzyme could be linked to a range of changes to O-glycans in prostate cancer cells.

We also identified the sialyltransferase ST6GalNAc1 as being directly regulated by androgens in prostate cancer cells (Munkley et al. 2015c, 2016b). ST6GalNAc1 adds sialic acid to the Tn antigen to produce the cancer-associated onco-foetal sialyl-Tn (sTn) antigen, which is linked to poor prognosis in numerous cancer types (Munkley 2016). S'Tn is a truncated O-glycan containing a sialic acid α-2,6 linked to GalNAc α-O-Ser/Thr and has been shown to promote tumour growth and metastasis in mouse models (Julien et al. 2006, Ozaki et al. 2012). In prostate cancer, the sTn antigen is detected in up to half of all high-grade tumours (Myers et al. 1994, Genega et al. 2000), and

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</tr>
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<tbody>
<tr>
<td>EDE3</td>
<td>Mannose trimming of N-glycans</td>
<td>Not reported in other cancers</td>
<td>Upregulated in malignant PCa as part of a glycosylation gene signature (Barfeld et al. 2014a). Androgen regulated and linked to prostate cancer cell viability (Munkley et al. 2016b). May play a role in creating these aberrant N-glycans (Munkley et al. 2016b)</td>
</tr>
<tr>
<td>ST6GAL1</td>
<td>Sialylation of terminal N-glycans</td>
<td>Over-expressed in many types of cancer (Swindall et al. 2013). Expression reduced by epigenetic inactivation in bladder cancer (Antony et al. 2014). Linked to EMT transition and malignancy (Lu et al. 2014)</td>
<td>Androgen regulated and linked to prostate cancer cell viability (Munkley et al. 2016b)</td>
</tr>
<tr>
<td>UGGT1</td>
<td>Recognizes and reglucosylates N-glycans with minor folding defects.</td>
<td>Binds to mutation specific isoform of EGFR (Erdem-Eraslan et al. 2015)</td>
<td>Not reported</td>
</tr>
<tr>
<td>ALG2</td>
<td>Mannosyltransferase with role in N-glycan biosynthesis.</td>
<td>Role in TRAIL-induced apoptosis (Ovcharenko et al. 2007)</td>
<td>Not reported</td>
</tr>
<tr>
<td>NEU1</td>
<td>Sialidase that cleaves terminal sialic acid from glycoproteins and glycolipids</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>NANS</td>
<td>Sialic acid biosynthesis (sialic acid synthase)</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>SERP1</td>
<td>Stabilizes membrane proteins during stress and facilitates subsequent glycosylation</td>
<td>Involved in tumour cell survival under stress – correlates with survival in glioblastoma patients (Mucaj et al. 2015)</td>
<td>Not reported</td>
</tr>
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The 25 androgen-regulated genes have roles in O-glycan biosynthesis, the hexosamine biosynthetic pathway (HBP), chondroitin sulfate (CS) synthesis, N-glycan processing and the oligosaccharyltransferase (OST) complex.
modification of the cell surface adhesion protein MUC1 with sTn is positively correlated with prostate-specific antigen (PSA) levels and negatively correlated with survival outcome (Arai et al. 2005). Increased expression of sialylated glycans creates negative charges and can inhibit the cell adhesion through electrostatic repulsion (Munkley & Elliott 2016b). We found previously that in prostate cancer cells, the androgen receptor controls the expression of the cancer-associated sTn antigen and cell adhesion through induction of ST6GalNAc1 (Munkley et al. 2015c) (Fig. 3). Induction of ST6GalNAc1 was found to promote a more mesenchymal cell phenotype in prostate cancer cells and to inhibit the formation of stable tumour masses in mouse models. Further analysis of prostate cancer tissue showed that although ST6GalNAc1 is increased in primary tumours, expression is dramatically downregulated in metastatic disease, indicating that ST6GalNAc1 may have a transient role in prostate cancer progression (Munkley et al. 2015c).

Our analysis of the prostate cancer transcriptome also identified the core 2 branching enzyme GCNT1 as an AR target gene in prostate cancer patients (Munkley et al. 2016b). GCNT1 is upregulated in prostate cancer in which its expression correlates with aggressive disease and is a predictor of recurrence after radical prostatectomy (Hagisawa et al. 2005, Kojima et al. 2015, Sato et al. 2016). GCNT1 can be detected in patient urine where in combination with PSA it is a reliable indicator for extracapsular extension of prostate cancer (Kojima et al. 2015). Upregulated GCNT1 in prostate cancer cells increases cell adhesion and dramatically increases tumour growth in orthotopic mouse models (Hagisawa et al. 2005). GCNT1 is implicated in the synthesis of the cancer-associated Sialyl-LewisX (SLeX) antigen, which is formed by sequential addition of sialic acid and fucose to the core to branch structure (Pinho & Reis 2015). SLeX is also regulated by androgens in prostate cancer cells (Munkley et al. 2016b) (Fig. 3). Consistent with this, in prostate cancer tissue, upregulated GCNT1 is associated with higher levels of SLeX in PSA, PAP and MUC1 proteins (Chen et al. 2014). SLeX is highly expressed in many malignant cancers, where it is associated with reduced rates of survival (Amado et al. 1998, Baldus et al. 1998). In prostate cancer, upregulation of SLeX is associated with hormonal-resistant, aggressive disease (Jorgensen et al. 1995). Increased SLeX could influence prostate cancer progression through a number of mechanisms including immune recognition by selectins; modification of the MUC1 protein with SLeX may enable prostate cancer cells to evade destruction by NK cells (Okamoto et al. 2013).
The core 2 branching enzyme GCNT2 is also directly regulated by androgens in prostate cancer cells (Munkley et al. 2016b). Although GCNT2 is not well studied in prostate cancer, increased GCNT2 has been shown to promote an epithelial-to-mesenchymal transition (EMT) and metastasis in breast cancer cells (Zhang et al. 2011). Similarly, the fucosyltransferase FUT1 is also androgen regulated in prostate cancer cells (Munkley et al. 2016b) and can act as an oncogene in several cancer types (Hao et al. 2008, Zhang et al. 2008, Yan et al. 2010, Milde-Langosch et al. 2014). GCNT1, GCNT2 and FUT1 are believed to play a role in synthesising the prostate cancer-associated F77 antigen (Nonaka et al. 2014). The F77 antibody was isolated from mice in 2010 after the injection of PC3 tumour cells and provoked great interest based on its diagnostic and therapeutic potential (Zhang et al. 2010). The F77 antibody was found to stain 141 out of 150 prostate cancer tissue samples with minimal staining of non-malignant prostate tissue and to inhibit the growth of DU145 and PC3 tumour xenografts in mouse models (Zhang et al. 2010). The F77 antigen was later determined using an O-glycome array and characterised as a glycolipid with α1,2-fucose linkages (Gao et al. 2014). Expression analysis of glycosyltransferase genes later identified the branching enzymes (including GCNT1 and 2) and FUT1 as essential for F77 antigen formation (Nonaka et al. 2014). Taken together, these findings suggest that androgen-mediated upregulation of GCNT1, GCNT2 and FUT1 may have a role in the production of F77.

N-glycan biosynthesis (OST complex, N-glycan processing)

In addition to the O-glycosylation enzymes discussed previously, our study also identified a set of androgen-regulated enzymes with roles in the synthesis and processing of N-glycans (Table 1). N-glycosylation begins with a common precursor consisting of 14 sugars, which is added to target proteins in the endoplasmic reticulum (ER) (Taniguchi & Kizuka 2015). Three androgen-regulated glycosylation enzymes (RPN2, STT3A and TUSC3) are subunits of the oligosaccharyltransferase (OST) complex, which is the central enzyme of N-linked protein glycosylation. OST transfers the 14-sugar preassembled oligosaccharide to selected asparagine residues within the consensus sequence asparagine-X-serine/threonine (Mohorko et al. 2011). Emerging evidence links both the OST complex and its individual subunits to cancer progression. In non-small-cell lung cancer cells, the OST complex is essential for EGFR localisation and signalling, and therapeutic inhibition of OST has been shown to induce senescence (Lopez-Sambrooks et al. 2016). Upregulation of the RPN2 subunit is linked to malignancy and confers resistance to docetaxel in breast cancer (Honma et al. 2008, Tominaga et al. 2014). In contrast, the TUSC3 subunit has been identified as a tumour suppressor in several cancer types (Kratovichilova et al. 2015, Fan et al. 2016, Jiang et al. 2016). Although there is no reported evidence to date linking RPN2 or STT3A to prostate cancer, TUSC3 has been shown to act as a tumour suppressor in prostate cancer cells and is downregulated in prostate tumours (Horak et al. 2012, 2014).

After initiation of N-glycosylation by the OST complex, N-glycans are then further processed by specific glycosylation enzymes. N-glycan structures frequently change in cancer and are linked to disease progression and metastasis (Taniguchi & Kizuka 2015). Increased levels of branched and cryptic N-glycans are common in prostate cancer, have been linked to EMT and metastasis and are currently being investigated as potential diagnostic markers (Kyselova et al. 2007, Newsom-Davis et al. 2009, Pinho et al. 2012, Wang et al. 2013, Ishibashi et al. 2014, Li et al. 2014a, Taniguchi & Kizuka 2015). The expression of six enzymes with roles in N-glycan processing (EDEM3, ST6GAL1, B4GALT1, UGGT1, ALG2 and NEU1) is controlled by the AR in prostate cancer cells (Munkley et al. 2016b). Three of these enzymes (UGGT1, ALG2 and NEU1) have not been previously linked to prostate cancer (Table 1). Here, I discuss the potential roles of the other three enzymes (EDEM3, ST6GAL1 and UGGT1) in cancer and in prostate cancer. The EDEM3 enzyme is upregulated in prostate cancer tissue as part of a glycosylation gene signature (Chen et al. 2014, Munkley et al. 2016b) and is believed to stimulate mannose trimming of N-glycans from total glycoproteins (Hirao et al. 2006). Elevated levels of EDEM3 may play a role in creating aberrant branched and cryptic N-glycans, which occur frequently in malignant transformation (Munkley et al. 2016b). EDEM3 also enhances endoplasmic reticulum-associated degradation (ERAD) of misfolded glycoproteins, which is particularly important in the context of cancer where increased metabolic needs lead to the accumulation of faulty proteins (Hirao et al. 2006, Olvari & Molinari 2007, Olzmann et al. 2013).

ST6GAL1 plays a role in terminal sialylation of N-glycans and is upregulated in many types of cancer (Hedlund et al. 2008, Schultz et al. 2013, Swindall et al. 2013). Expression of ST6GAL1 is linked to EMT and
malignancy, but the mechanisms driving this transition remain to be explored (Lu et al. 2014). Increased sialylation can influence cancer cell metastasis, invasion and survival (Munkley 2016). In prostate cancer cells, increased sialylation of PSA is linked to more aggressive disease (Llop et al. 2016, Munkley et al. 2016a). Our analysis suggested that the role of ST6GAL1 in prostate cancer is likely to be influenced by cell background in context-dependent manner (Munkley et al. 2016b). B4GALT1 transfers a galactose residue to terminal N-acetylgalcosamine and is involved in the late processing of N-glycans. Upregulated B4GALT1 can predict adverse outcome in patients with non-metastatic clear cell renal cell carcinoma (Xie et al. 2016) and is associated with multi-drug resistance in leukaemia (Zhou et al. 2013). In prostate cancer cells, B4GALT1 can be upregulated by TNFα and is thought to have a role in cell motility and invasion (Radhakrishnan et al. 2011). Taken together, these findings suggest that both the initiation and processing of N-glycosylation is under extensive control of androgens in prostate cancer cells. A wide range of changes to N-glycans have been observed in prostate cancer (Munkley et al. 2016a), and it is likely that AR regulation of N-glycosylation enzymes plays a role in driving these changes during disease progression.

Chondroitin sulfate synthesis

Our study also detected androgen regulation of the pathway producing chondroitin sulfate (CS), as well as CS itself and the large CS proteoglycan Versican (Read et al. 2007, Munkley et al. 2016b) (Fig. 3). CS is a type of glycosaminoglycan (GAG) found in the extracellular matrix and on the cell surface of many cell types (Gandhi & Mancera 2008) and is a key molecule in cancer progression (Theocharis et al. 2006). CS is believed to play a role in cellular proliferation and differentiation (Hardingham & Fosang 1992), can be used to predict disease progression in early stage prostate cancer (Ricciardelli et al. 1997), is linked to poor prognosis (Ricciardelli et al. 1999) and is increased in metastatic prostate cancer (Ricciardelli et al. 2009a). Versican is linked to poor prognosis in many different cancer types (Ricciardelli et al. 2009b) and is associated with disease progression in early-stage prostate cancer (Ricciardelli et al. 1998). CS is synthesised as GalNAc-containing galactosaminoglycan polymers alternating with glucuronic acid. Chondroitin glycosaminoglycans are covalently attached to core proteins and can be sulfated to varying degrees depending on the tissue source. Initiation of CS begins with the addition of xylose to serine residues of core proteins in the ER and is followed by the addition of two galactose residues in the Golgi (Silbert & Sugumar 2002). Two enzymes with roles in CS synthesis (CSGALNACT1 and CHPF) are regulated by androgens in prostate cancer cells. CSGALNACT1 is required for the initiation and elongation processes (Sakai et al. 2007, Watanabe et al. 2010), whereas CHPF plays a role in polymerisation (Kitagawa et al. 2003, Mikami & Kitagawa 2013). The CSGALNACT1 enzyme is upregulated in prostate cancer cells and has been shown to be essential for prostate cancer cell viability, making it an attractive therapeutic target (Munkley et al. 2016b). Recent data suggest that targeting oncofoetal CS using glycosaminoglycan-binding malaria protein can inhibit the growth of PC3 prostate cancer cells in mouse models (Salanti et al. 2015) (as PC3 cells are an AR-negative prostate cancer cell line, it will be interesting to explore this finding further in a range of AR-negative and -positive cell types).

Hexosamine biosynthetic pathway (HBP)

The hexosamine biosynthetic pathway (HBP) is a major metabolic pathway, which produces uridine diphosphate-N-acetylgalcosamine (UDP-GlcNAc), an essential building block for glycan biosynthesis. The pathway is well positioned to affect glycans that accelerate cancer progression and is becoming of increasing importance in cancer biology (Vasconcelos-Dos-Santos et al. 2015). HBP is sustained by metabolites produced by metabolic processes and is a major metabolic integration point influencing cell growth, metabolism, cell stress and epigenetics (Hanover et al. 2012, Fardini et al. 2013, Bond & Hanover 2015). HBP produces an amino sugar conjugate, UDP-GlcNAc, which acts as a substrate for the post-translational modification of a wide range of proteins (Schwarz & Aebl 2011) (Fig. 4). UDP-GlcNAc can be added to target proteins in the cytoplasm, nucleus or mitochondria by the enzyme OGT (O-GlcNAc transferase) (Butkinaree et al. 2010) or as a modification to glycoproteins in the Golgi apparatus or ER (Schwarz & Aebl 2011). Both OGT and the O-GlcNAc glycan are elevated in patients with localised prostate cancer and associated with poor prognosis (Lynch et al. 2012, Kamigaito et al. 2014). OGT can modify a range of target proteins including the transcription factors FOXM1 and cMYC and may promote metabolic reprogramming in tumours cells (Itkonen et al. 2013).

Our recent study showed that the HBP is under extensive androgen control in prostate cancer cells.
Fructose-6-P → GFPT1 → Glucosamine-6-P → GNPNAT1 → N-acetyl glucosamine-6-P → PGM3 → N-acetyl glucosamine-1-P → UAP1 → UDP-GlcNAc → UDP → O-GlcNAc modification of proteins

Figure 4

The hexosamine biosynthetic pathway (HBP). The HBP is sustained by metabolites produced by metabolic processes and is a major biological integration point in the cell. HBP produces an amino sugar conjugate, UDP-GlcNAc that serves as a major sugar donor for O-GlcNAcylation and for classical N-linked and O-linked glycosylation. O-GlcNAc transferase (OGT) catalyses the transfer of GlcNAc from the sugar donor UDP-GlcNAc to serine and/or threonine residues. O-GlcNAcase (OGA) carries out the reverse reaction. The enzymes GNPNAT1, PGM3 and UAP1 (shown in red) are directly controlled by androgens in prostate cancer cells, as is the O-GlcNAc modification itself.

(Munkley et al. 2016b). The final enzyme in the HBP, UAP1 (UDP-N-acetylglucosamine pyrophosphorylase 1), is regulated by AR activity and is highly overexpressed in patients with prostate cancer (Itkonen et al. 2014). Our data showed that in addition to UAP1, both the second enzyme (GNPNAT1) and the penultimate enzyme (PGM3) in the pathway are also controlled by androgens in prostate cancer patients, as is the O-GlcNAc modification itself (Munkley et al. 2016b) (Fig. 3).

Although HBP is regulated by the AR and is upregulated in localised hormone-sensitive prostate cancer where it has a positive influence on disease progression, in the castrate-resistant state, downregulation of HBP appears to enhance tumour growth and is a key biochemical mediator of CRPC progression (Kaushik et al. 2016). The transcript levels of HBP genes are downregulated in CRPC tissue compared with localised hormone-sensitive tumours, and loss of GNPNAT1 in CRPC cells increases tumour growth and aggressiveness in mouse models in cells containing either AR-full length of AR-V7 (Kaushik et al. 2016). In castrate-resistant disease, the HBP can be targeted therapeutically by treatment with UDP-GlcNAc, which remarkably increases the efficacy of the anti-androgen enzalutamide also (Kaushik et al. 2016). Increased glycolysis is one of the main metabolic adaptations found in CRPC (Biernacka et al. 2013). Hence, although HBP is increased in localised prostate cancer, metabolic rewiring during disease progression is believed to promote a reduction in HBP in castrate-resistant disease. This could offer a selective adaptation to the bioenergetics demands of the tumour and the increased need for glycolysis (Kaushik et al. 2016).

Conclusions and future perspectives

The mechanisms driving the growth and spread of prostate cancer are complex and not fully understood, yet, are crucially important for disease management. We recently identified glycosylation (the enzymatic attachment of glycan sugar moieties to lipids and proteins) as a novel androgen-regulated process in prostate cancer patients (Munkley et al. 2016b). Our study identified 25 glycosylation enzymes and lectins, which are regulated by androgens and respond to androgen deprivation in prostate cancer patients. These glycosylation enzymes operate at multiple steps within the glycosylation synthetic pathways, including the synthesis and processing of both O- and N-glycans, control of the hexosamine biosynthetic pathway (HBP) and the synthesis of chondroitin sulfate. A wide variety of changes to the glycoproteome are frequently observed in prostate cancer. Glycans can influence cell survival, proliferation and metastasis and likely play a key role in these processes in prostate cancer (Munkley et al. 2016a). Androgens control key steps in glycan synthesis and are linked to several cancer-associated glycans (including sTn, SLeX, OGlcnAc, chondroitin sulfate, branched and cryptic N-glycans, and potentially, the F77 antigen).

Prostate cancer is a unique disease characterised by prognostic heterogeneity, and there is an urgent clinical need to identify biomarkers to help distinguish indolent from aggressive disease and to develop new treatments for castrate-resistant disease. The identification of
glycosylation as a global target for androgen control in prostate cancer cells has important clinical implications in terms of both diagnosis and treatment. Glycoproteins are the most commonly used serological biomarkers for cancer diagnosis, and monitoring the glycan composition of current biomarkers (such as PSA) can dramatically improve their specificity (Reis et al. 2010, Gilggun et al. 2013). Glycans also likely play roles in all aspects of cancer progression and are therefore attractive targets for therapeutic intervention (Pinho & Reis 2015, Munkley et al. 2016a). Glycans that hold particular promise as therapeutic targets in prostate cancer include chondroitin sulfate (Salanti et al. 2015), the F77 antigen (Zhang et al. 2010), SLeα (Hagisawa et al. 2005) and STn (Munkley & Elliott 2016b). An increased understanding of how glycosylation modulates the biological function of prostate cancer cells will allow the development of a relatively unexploited field of drugs based on inhibitors, glycan antagonists and glycan function modulators.

**Declaration of interest**
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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