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RESEARCH HIGHLIGHT

Sugars and cell adhesion: the role of ST6GalNAc1 in prostate cancer progression

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O-linked glycans become altered in cancer cells, leading to changes in cell adhesive properties and contributing to metastasis. But the mechanisms driving these changes and how these carbohydrate groups are involved in tumour spread remain poorly understood. We recently identified the sialyltransferase gene *ST6GalNAc1* as a novel androgen-regulated gene in prostate cancer [1]. Expression of ST6GalNAc1 in prostate cancer cells induced expression of the cancer-associated sTn antigen, reduced cell adhesion, and dramatically inhibited the formation of stable tumour masses in vivo after orthotopic transplantation experiments in mice. Although *ST6GalNAc1* is significantly upregulated in primary prostate carcinoma tissue, there is a striking downregulation of this gene in metastatic prostate tissue, suggesting an important yet transient role for ST6GalNAc1 in prostate cancer progression. Here we discuss mechanistically how changes in sialylation could alter prostate tumour cell behaviour and contribute to cancer cell dissemination.

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Metastasis remains the principal cause of mortality from solid tumours. There is a key clinical need to develop biomarkers indicative of metastatic disease to help stratify patients towards personalised therapy. Cancer cell dissemination from the primary site and establishment at a secondary site occurs through a complex multistep process. Key questions are how tumour cells within the primary tumour overcome the cell-cell adhesion forces which normally hold tissue in place, and then reattach themselves at a new site. Alterations in cell surface glycosylation are closely linked with the malignant properties of cancer cells. Cell surface glycans can alter cell-cell and cell-matrix adhesion and can alter adhesive properties leading to tumour spread. Studies strongly suggest that truncation of O-glycans directly induces oncogenic features of growth and invasion [2, 3]. The most frequently observed aberrant glycophenotype is expression of the most immature truncated O-glycans Tn and sTn [2, 3]. However, the mechanisms driving the expression of these glycans and they are involved in tumour spread remain poorly understood.

Mucin (GalNAc) -type O-glycosylation of proteins is an abundant and diverse form of post-translational modification that is initiated by a family of enzymes called GalNAc-transferases. These enzymes modify proteins by transferring GalNAc residues, and in subsequent steps these GalNac residues are further elongated, branched and capped to result in O-glycosylation. Whereas in normal cells O-glycosylation produces mature elongated and branced O-glycans capped by sialic acid, many cancer cells express

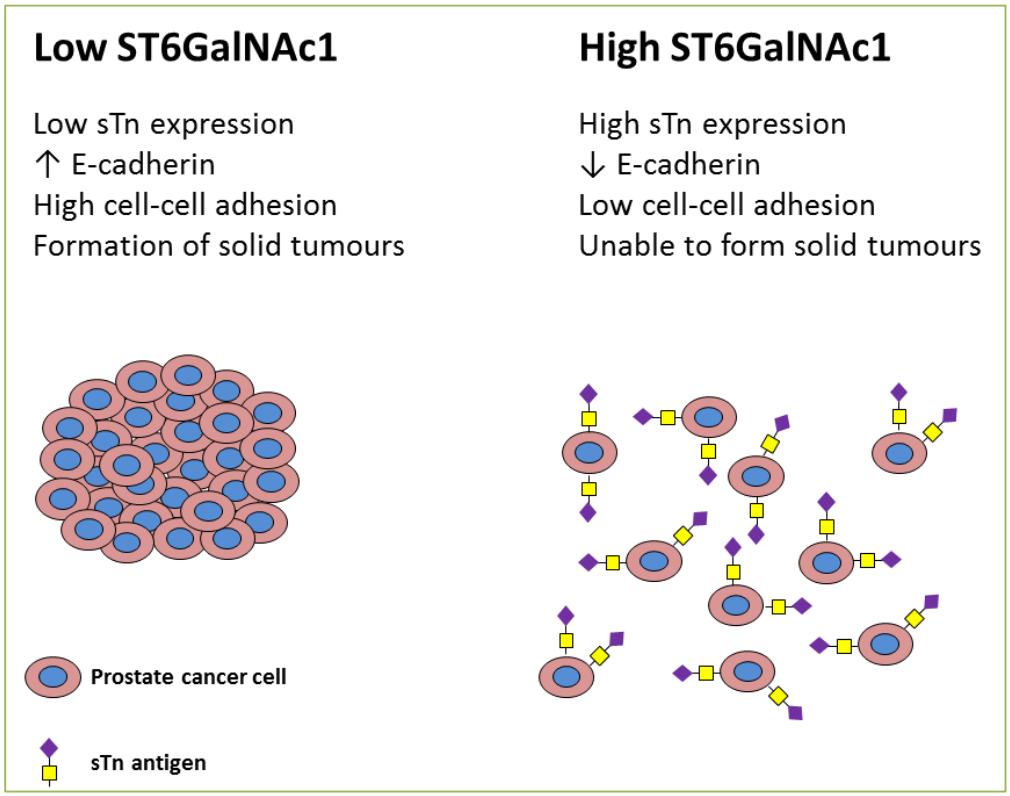

Figure 1. Model: ST6GalNAc1 inhibits the formation of solid tumours in prostate cancer cells. Prostate cancer cells with low ST6GalNAc1 do not express the sTn antigen and are able to form solid tumours in vivo. In contrast, in prostate cancer cells expressing the ST6GalNAc1 enzyme there is an induction of the sTn antigen, a reduction of E-cadherin expression, reduced cell adhesion, and the cells are unable to form solid tumours.

only biosynthetic intermediates [3, 4]. Although the mechanism and biological significance of the truncated O-glycophenotype remains to be widely addressed, the expression of truncated O-glycans in cancer is thought to result from several different mechanisms, including altered expression, localisation or topology of glycosyltransferases [5-8], and fluctuations in cellular pH [9, 10]. O-glycosylation is an abundant protein modification; More than 80% of the human proteome that enters and passes through the secretory apparatus is predicted to be O-glycosylated [11]. The site-specific O-glycosylation of proteins can have a dramatic effect on protein function, including pro-protein processing, modulation of ligand binding and regulation of cell signalling [12-15].

We recently showed that expression of the sialytransferase ST6GalNAc1 is directly regulated by androgens in prostate cancer cells, and its encoding gene is upregulated in primary prostate cancer tissue relative to the normal prostate gland [1]. ST6GalNAc1 protein is responsible for catalysing synthesis of the truncated cancer-associated sialyl-Tn antigen (sTn) which is elevated in several cancer types and correlates with metastasis and poor prognosis in breast and colon cancer [16-23]. In prostate cancer, the sTn antigen is detected in up to half of high grade prostate carcinomas [16, 24], and the levels of sTn MUC1 have been correlated with worse survival outcomes and higher serum PSA levels in prostate cancer patients [25]. Androgen steroid hormones are key molecular drivers in prostate cancer, and our recent study showing that sTn expression is induced by androgens in prostate cancer cells, provides a link between the androgen receptor and expression of this cancer-associated antigen.

The truncation of O-glycans has previously been reported to play a role in cell invasion through the disruption of contact between the epithelium and the dermal compartment and loss of cell-cell adhesion [2]. To establish the role of ST6GalNAc1 in prostate cancer we used engineered DU145 and PC3 cells, which normally express low endogenous levels of ST6GalNAc1, to overexpress this sialytransferase and demonstrate that this induces expression of the sTn antigen in prostate cancer cells [1]. Prostate cancer cells expressing ST6GalNAc1 and sTn antigen have reduced cell adhesion onto uncoated plates and plates coated with collagen I or fibronectin, increased cell motility, and switch towards a more mesenchymal cell phenotype. The increased expression of sialylated antigens can create negative charges which can promote the detachment of cells from the tumour mass through electrostatic repulsion. Consistent with this, prostate cancer

cells expressing ST6GalNAc1 and the sTn-antigen are unable to form stable tumour masses in vivo within orthotopically transplanted mice, thus suggesting a role for ST6GalNAc1 in prostate cancer cell dissemination (Figure 1).

A recent study used quantitative phospho-proteomics to examine the main signalling pathways affected by truncation of O-glycans in pancreatic cancer cells [2]. The analysis revealed altered regulation of proteins involved in cell adhesion [2]. Similarly, we found that prostate cancer cells expressing ST6GalNAc1 have decreased expression of the epithelial cell-cell adhesion molecule E-cadherin and increased expression of migration-associated N-cadherin and vimentin proteins [1]. The epithelial to mesenchymal (EMT) transition has a role in the progression of prostate cancer [26-29]. A switch from E-cadherin to N-cadherin expression is of strong independent importance for the progress of prostate cancer [26-29], and up-regulation of vimentin expression is linked to prostate cancer invasion [29]. Our findings add to the growing evidence linking glycosylation and the EMT transition [30-33], and provide important insight into understanding how O-linked glycans can influence this process. Further dissection of the interplay between sTn and oncogenic features will require deciphering the effects of sTn on additional specific O-glycoproteins.

Alterations in O-glycans in cancer can alter ligands responsible for interactions between cancer cells and their environment and have many biological and pathological consequences. This can influence the growth and survival of cancer cells, the ability to metastasise, and interactions with cell surface receptors, lectins, and cells of the immune system. The patterns of ST6GalNAc1 expression in prostate cancer are consistent with a potentially transient role for ST6GalNAc1 in prostate cancer progression: the ST6GalNAc1 gene is up-regulated in primary prostate cancer tissue, but then strikingly down-regulated in metastatic prostate tissue relative to the normal prostate gland [1]. While ST6GalNAc1 protein could have a role in facilitating dissemination from the primary tumour, these dynamic gene expression patterns suggest that ST6GalNAc1 must subsequently be lost in order to form stable metastases.

Our findings in prostate cancer are in contrast to studies in breast and colon cancer cells where the ST6GalNAc1 enzyme was found to be increased in metastatic tissue and to increase primary tumour growth and the formation of metastatic foci [20, 23]. This suggests that the roles of ST6GalNAc1 will need to be analysed individually in different cancers. The differences in ST6GalNAc1 function observed between breast/colon and prostate cancer could be due to differences in cellular background or due the influence of other co-expressed glycosylation enzymes. In future studies it will be important to understand how changes in glycodynamics function co-ordinately to influence specific stages of cancer progression.

Conflicting interest

The authors have declared that no conflict of interests exist.

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