P057 Discovery of prognostic biomarkers from the prostate cancer membrane proteome using bioorthogonal chemistry

EUR Urol Suppl 2019;18(11):e3479

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Introduction & Objectives: The link between aberrant glycosylation and cancer progression has formed the basis for using glycans and glycoproteins in the clinical management of cancer. However, the utility of biomarkers, such as PSA, have been challenged in recent years due to low cancer specificity and high false-positive rates. The aim of this study was to use synthetic analogues of monosaccharides called azidosugars, to dynamically monitor changes in cell surface glycosylation in a model of prostate cancer progression, in order to identify novel biomarkers.

Materials & Methods: A panel of human prostate cancer cell lines was used, which represented the stages of prostate cancer progression. The characteristics of each cell line were investigated using scratch wound assays to compare migration, Boyden chambers to compare invasion and immunohistochemistry to compare the relative expression of known invasion biomarkers.
The cell lines were incubated with analogues of sialic acid and N-acetylgalactosamine that had been labelled with an azide group (N-azidoacetylmannosamine (Ac_4ManNAz) and N-azidoacetylgalactosamine (Ac_4GalNAz)). These are incorporated biosynthetically into cell surface glycans. The membrane proteome was isolated by detergent-based fractionation, the incorporated azide groups covalently ligated to Alkyne-biotin using copper-catalysed chemistry, and the azidosugar labelled proteins enriched using streptavidin-coupled beads. Proteins were resolved via gel electrophoresis and identified by mass spectrometry.

Results: The metastatic potential of the established cell lines used in the study was confirmed in the scratch wound and Boyden chamber assays and by demonstrating a correlation with increased expression of vimentin, loss of PTEN expression, and decreased E-cadherin expression. Collectively, the data validated the panel as a suitable *in vitro* model of prostate cancer progression.
Proteomic analysis revealed 8 proteins exhibiting an increase in Ac_4ManNAz and Ac_4GalNAz glycan labelling, which correlated with an increase in the metastatic potential of the cell lines. Normalised Spectral Abundance Factors, showed, as expected, a relative increase in the expression of total PSA, but also an increase in Ac_4ManNAz and Ac_4GalNAz glycan labelling, which correlated with an increase in the metastatic potential. More interesting was the case of Basigin (CD147), which showed a strong increase in Ac_4ManNAz and Ac_4GalNAz labelling, despite its total expression decreasing across the panel of cell lines.

Conclusions: We demonstrate the use of bioorthogonal chemistry in detecting the differential sialyation and N-acetylgalactosamine glycosylation of proteins from human cell lines representing the progression of prostate cancer. Our data suggest that differential glycosylation may be a more sensitive and specific marker for monitoring prostate cancer progression, than increases in total protein expression alone.