

## IDENTIFICATION OF CD44-POSITIVE CELLS IN BLADDER CANCER CELL LINES AND THEIR CALCIUM SIGNALLING RESPONSES TO HYALURONIDASE STIMULATION

### Hypothesis / aims of study

Bladder cancer is the second most common urological malignancy and symptoms include frequency, urgency, haematuria and pain. Treatments include surgery, chemotherapy and radiation therapy – the latter may exacerbate urgency and lead to urinary incontinence. A recent study has demonstrated the existence of specialized tumour-initiating cells (T-IC) in bladder cancer (1) which may explain the frequent recurrence of urothelial cancers. One of the cellular markers of bladder T-IC was reported to be CD44. Developing the means to selectively target the expression/activity of bladder T-IC is currently an exciting area of research.

The aims of the present study were to identify CD44-positive cells in human bladder cancer cell lines at the gene and protein level and to investigate the Ca<sup>2+</sup>-signaling properties of bladder cancer cells in the absence and presence of CD44 stimulation by hyaluronidase.

### Study design, materials and methods

Bladder cancer cell lines T24 and HT1376 cells were used in this study. Cells were processed for flow cytometry and immunohistochemical analysis with CD44 antibodies (a T-IC marker). CD44 mRNA and protein analysis of these two cell lines were performed via qPCR and Western blot respectively. In order to analyse Ca<sup>2+</sup>-signalling properties, T24 and HT1376 cells were loaded with a Ca<sup>2+</sup>-indicator and imaged with real-time fluorescent microscopy.

### Results

Flow cytometry experiments (n=3) showed CD44-positive cells in both cell lines (85%-90% of the total population). T24 and HT1376 cells were cultured as monolayers and HT1376 cells formed spherical colonies when grown in soft agar. Immunostaining for CD44 revealed localisation of CD44 dominantly on the cell surface and to a lesser extent in the cytoplasm. CD44 was highly expressed on the cell surface in the majority of T24 cells while expression in HT1376 cells was more variable. HT1376 cells formed spherical colonies on soft agar with CD44<sup>+</sup> cells expressed only on the outermost layer (n=3). CD44 expression was demonstrated by qPCR (n=3) and Western blot (n=5) analysis in both cell lines.

In calcium imaging studies, cells were loaded with the calcium indicator, Fluo4 AM. Under control conditions, both cell lines exhibited spontaneous changes in [Ca<sup>2+</sup>]<sub>i</sub> (79 cells from 14 colonies). Application of hyaluronidase (HA, 50mg/ml) to HT1376 cells evoked increases in [Ca<sup>2+</sup>]<sub>i</sub> (20 cells from 5 colonies).

### Interpretation of results

CD44 is expressed in both cell lines at the mRNA and protein level. Flow cytometry and immunocytochemistry showed that majority of cells in T24 and HT1376 cell lines grown in monolayer are CD44<sup>+</sup>-cells. In HT1376 3-dimensional cell cultures, the CD44<sup>+</sup>-cells appear localised on the outer aspect of the colonies, indicative of a specialised role in colony formation. The bladder cancer cells exhibited spontaneous Ca<sup>2+</sup>-signalling properties which occasionally spread from cell-to-cell, indicative of functional intercellular communication. HT1376 cells response to HA stimulation indicated a Ca<sup>2+</sup>-dependent CD44 cellular transduction.

### Concluding message

We identified CD44<sup>+</sup>-cells in monolayers of T24 and HT1376 bladder cancer cell lines and in spherical colonies of HT1376 cells. Results indicate that Ca<sup>2+</sup>-signalling is spontaneous and suggest that it is involved in cell to cell communication. HA stimulation of the CD44 receptor plays a role in Ca<sup>2+</sup>-signalling. A comparison of Ca<sup>2+</sup>-signalling characteristics of both cell lines and the communication between cells in the HT1376 spherical colonies is ongoing.

### References

1. Chan KS, Espinosa I, Chao M, et al. (2009) Proc Natl Acad Sci U S A. 106(33):14016-21.

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*What were the subjects in the study?*

NONE

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