

Analysis of the role of ADAMTS-1 and -15 in Prostate Cancer Progression

Summary Report to the Education and Training Committee
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Introduction: Prostate cancer is a leading cause of cancer death in men. Death from prostate cancer is usually a consequence of tumour progression, metastasis and androgen independent tumour growth. Some metalloproteinases have been implicated in the process of cancer progression. ADAMTS proteases (A Disintegrin And Metalloproteinase with ThromboSpondin motifs) are metalloproteinases that play diverse roles in tissues. Prostate cancer cells express ADAMTS-1 and -15 but the role played by these proteases in prostate cancer progression is unknown. ADAMTS-1 is a proteoglycanase and is also known to have anti-angiogenic properties. ADAMTS-15 is predicted to be a proteoglycanase, and low tumour expression levels of ADAMTS-15 is associated with poor prognosis in breast cancer patients. This study was designed to determine whether ADAMTS-1 and -15 expression is regulated by androgen, and whether they play a role in prostate cancer progression.

Materials & Methods: Tumour spheroids were created using prostate cancer and stromal cells and grown in 3-dimensional culture in Matrigel containing quenched fluorescent gelatin (DQ-Gelatin). The tumour spheroids were examined under a fluorescence microscope for evidence of proteolytic activity. Androgen sensitive LNCaP prostate cancer cells were treated with dihydrotestosterone (DHT) and Flutamide and changes in expression were analysed by real time RT-PCR and western blotting. The *ADAMTS1* and *ADAMTS15* genes and promoter regions were screened for the presence of putative androgen response elements (AREs). ADAMTS-1 and -15 expression was knocked-down in PC3 prostate cancer cells using siRNA and the effect of knock-down on proliferation, migration and invasion was analysed.

Results: Fluorescence microscopy of PC3, LNCaP and stromal cell spheroids revealed proteolytic cleavage of DQ-Gelatin in Matrigel. DHT treatment in LNCaP cells had no effect on ADAMTS-1 expression, but down-regulated ADAMTS-15 mRNA and protein expression. The effect of DHT was not inhibited by Flutamide. There were no AREs associated with the *ADAMTS1* promoter and 2 AREs in the gene sequence. There was 1 ARE in the *ADAMTS15* promoter and 12 AREs in the gene sequence. An antibody directed against the ADAMTS-15 pro-peptide domain was validated. The anti-ADAMTS-15 antibody detected 50kDa bands, suggesting a novel cleavage site within the disintegrin-like domain of ADAMTS-15 that releases a C-Terminal fragment with potential anti-angiogenic properties. ADAMTS-1 and -15 knock-down had no effect on proliferation, migration or invasion under the experimental conditions of this study.

Conclusions: ADAMTS-15 expression is androgen-regulated, possibly via binding of activated androgen receptors to AREs associated with the *ADAMTS15* gene. Knock-down of ADAMTS-1 and -15 expression does not affect the proliferation, migration or invasive potential of PC3 cells in vitro. Cleavage of ADAMTS-15 in the disintegrin-like domain results in the release of a C-terminal fragment with potential anti-angiogenic properties. Down-regulation by DHT in prostate cancer cells suggests that ADAMTS-15 could be playing an anti-tumour role in prostate cancer progression.

Presentations:

- **Molokwu CN**, Adeniji OO, Hsu Y, Hamdy FC, Buttle DJ. *Regulation of ADAMTS15 by dihydrotestosterone in prostate cancer cells and identification of putative androgen response elements*. Poster & Oral Presentation, British Association of Urologic Surgeons Annual Meeting, Glasgow. June 2009
- **Molokwu CN**, Adeniji OO, Hsu Y, Hamdy FC, Buttle DJ. *Regulation of ADAMTS15 by Androgen and Identification of Putative Androgen Response Elements*. Poster Presentation, American Association of Cancer Research Centennial Conference, Colorado Convention Centre, U.S.A. April 2009
- **Molokwu CN**, Hamdy FC, Buttle DJ. *Regulation of ADAMTS15 by Androgen and Identification of Putative Androgen Response Elements*. Poster Presentation, Royal College of Physicians & Surgeons of Glasgow Triennial Conference, Glasgow. Nov 2008
- **Molokwu CN**, Adeniji OO, Waterman EA, Cross NA, Hamdy FC, Buttle DJ. *Evaluating the Role of ADAMTS Enzymes & TIMP-3 in Prostate Cancer Progression*. Poster Presentation, National Cancer Research Institute Conference, Birmingham. Oct 2006
- **Molokwu CN**, Hamdy FC, Buttle DJ. *Evaluating the Role of ADAMTS-1 in Prostate Cancer Invasion*. Poster Presentation, American Society for Clinical Oncology Prostate Cancer Symposium, San Francisco Marriott, U.S.A. Feb 2006

Abstracts:

- **Molokwu CN**, Adeniji OO, Chandrasekharan S, Hamdy FC, Buttle DJ. Regulation of ADAMTS15 by dihydrotestosterone in prostate cancer cells and identification of putative androgen response elements. *BJU Int* 2009; 103(S4):20
- **Molokwu CN**, Adeniji OO, Hamdy FC, Buttle DJ. Regulation of ADAMTS expression in prostate cancer and stromal cells by androgen and TNF: P040. *Int J Exp Pathol* 2007; 88(6):A95
- **Molokwu CN**, Adeniji OO, Hamdy FC, Buttle DJ. Imaging proteolysis in prostate cancer and stromal cells: P039. *Int J Exp Pathol* 2007; 88(6):A95
- Adeniji OO, **Molokwu CN**, Waterman EA, Cross NA, Hamdy FC, Buttle DJ. Evaluating the role of ADAMTS proteoglycanases and TIMP-3 in prostate cancer progression: P8. *Int J Exp Pathol* 2007; 88(4):A58

Prizes:

HRH The Princess Royal Prize for 2nd Best Presentation, Royal College of Physicians & Surgeons of Glasgow Triennial Conference, Nov 2008, SECC, Glasgow

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Full report available on request