The Identification and Characterization of Androgen-Insensitive Cells Prior to Androgen Deprivation Therapy in Prostate Cancer

Introduction and Objectives: The mechanisms contributing to castration-resistant progression in prostate cancer (PC) are not fully elucidated. PC lesions are heterogeneous and thus, it is important to understand whether among the heterogeneous collection of cell types, androgen-insensitive cells exist prior to androgen deprivation therapy. The identification of these cells may predict patient prognosis as well as provide a better understanding of treatment resistance.
Materials and Methods: LNCaP cells (10,000 cells in 10cm dish) were grown for 40 days in a normal medium. Fifty colonies were selected and 22 subclones were established. In five of these clones, expression levels of androgen receptor (AR) and prostate specific antigen (PSA) were examined by western blotting and *in vitro* androgen-sensitivities were evaluated to be grown in normal and androgen-depleted medium. Between an androgen-sensitive and -insensitive clone cell invasiveness was examined by Matrigel invasion assays and *in vivo* tumor growth potential was evaluated by xenograft tumor formation assays. Between these clones gene copy number and gene expression were compared using Human SNP array 6.0 and Human Genome U133 Plus 2.0, respectively. Two of the genes differentially expressed between these clones were knocked down by in LNCaP or PC3 cells and the influences on AR activation, cell proliferation, cell invasion and *in vivo* tumor formation were evaluated. The expression levels of these genes in PC tisssue samples were examined by real-time PCR.
Results: LNCaP subclones with different androgen-sensitivity were established at the same passage numbers. Their androgen-insensitivities were correlated with their PSA expression levels. LNCaP-cl1 had higher PSA expression and androgen-insensitivity but lower invasiveness and tumor growth potential than LNCaP-cl5. In these clones DNA copy numbers were significantly different in several regions, indicating that these clones contain genetically different cells. The expression levels of Sprouty1 (SPRY1) and Jagged1 (JAG1) were significantly lower in LNCaP-cl1 than in LNCaP-cl5. Both genes are regulated by miR-21, an androgen-regulated microRNA. SPRY1 knockdown in LNCaP cells enhanced PSA expression and cell proliferation under epidermal growth factor stimulation without androgen. JAG1 protein administration in LNCaP cells enhanced cell invasion and JAG1 knockdown in PC3 cells suppressed cell invasion and tumor formation by down-regulating N-cadherin expression. In PC tissue samples SPRY1 expression levels were significantly lower in patients with PSA recurrence after surgical treatment ($p=0.0076$) and JAG1 expression levels were significantly higher in Gleason sum (GS) 8-9 disease than in GS 5-6 ($p=0.0121$).
Conclusions: A random population of LNCaP cells comprises a heterogeneous group of cells with different androgen-sensitivities and invasiveness. The expression differences in SPRY1 and JAG1 caused the phenotypic differences between these clones and they may represent novel biomarkers for differentiating aggressive PC cells.