Development and Characterization of an Androgen Receptor-siRNA- scAAV to Study Mechanisms in Evasion of Androgen Deprivation Therapy

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The androgen receptor (AR) is the key mediator of androgen signaling. AR acts as a transcription factor by binding to androgen responsive elements of downstream target genes. Recent studies suggest that the AR mediates androgen dependent growth and contributes to androgen independent proliferation of prostate tumor cells. We have developed and characterized a self-complementary adeno-associated virus (scAAV) that expresses a DNA cassette encoding RNA polymerase III promoter-driven hairpin siRNA to target and inhibit AR protein expression in the recurrent prostate cancer cell line CWR-R1. We have previously shown that epidermal growth factor (EGF) increases androgen-dependent transactivation of the AR through post-transcriptional modification of the SRC/P160 coactivator family member TIF2. AR protein knockdown, in the presence and absence of dihydrotestosterone (DHT) and EGF, resulted in significant cell growth inhibition *in vitro*. Loss of the viral vector expression, as determined by flow cytometry, correlated with re-expression of AR and subsequent increase in cell proliferation rates. Inhibition of cell growth induced by EGF only by the AR-siRNA-scAAV provided evidence for an androgen independent activation mechanism involved in AR induced cell proliferation. Our results suggest that the AR plays a critical role during prostate cancer recurrence and that there are key autocrine pathways involved in AR activity even after hormone deprivation. Treatment of CWR-R1 cells with the MAPK (ERK1/2) inhibitor U0126 (+/- DHT, EGF and DHT+EGF) also resulted in significant inhibition of cellular growth suggesting that the MAPK axis is, at least in part, involved in autocrine signaling that drives proliferation in CWR-R1 cells.