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Introduction

AR mediates transcriptional activation through a series of events including ligand binding, binding to cognate androgen response elements (AREs) and interaction with various coactivators, resulting in transcriptional initiation of AR target genes by the general transcriptional machinery¹ (Fig.2). The mechanism responsible for the switch of AR mediated growth promotion and inhibition could be due to the different set of AR target genes activated. Recent efforts in identifying AR target genes in prostatic cell lines and human cancer using DNA microarrays have resulted in the identification of several AR target genes²⁻⁷. It is of particular interest of this proposal to determine whether any of these are also expressed in prostate stromal cells and whether their expression contribute to racial disparity of prostate cancer. In an effort to understand their role in prostate cancer proliferation and differentiation, we have also identified several genes as AR target genes including cellular caspase-8 (FLICE)-like inhibitory protein, c-FLIP⁸ by deletional, mutational and chromatin immunoprecipitation analysis of the promoter region of the gene of interest. c-FLIP is an androgen receptor target gene, affecting survival and apoptosis of prostate cells⁸. LNCaP cells infected with a retrovirus harboring c-FLIP lead to increased levels of c-FLIP protein and accelerated the progression to androgen independence. In addition to its increased expression in human prostate cancer cells, we found that there are an increased number of c-FLIP positive stromal cells surrounding prostate cancer.

Towards the goal of better understanding the complex function and role(s) of stromal cFLIP in human prostate tumorigenesis and racial disparity of prostate cancer, we performed IHC to determine stromal cFLIP expression on 56 androgen dependent prostate cancer cases including 27 AA and 29 Caucasian patients. Further, we established an immortalized prostate stromal cell line mimicking the behavior of primary stromal cells and showed that the immortalized stromal cells, while not oncogenic, can induce the proliferation and invasion of prostate cancer cells. We have studied stromal cFLIP expression and function in prostate cancer growth and invasion.

BODY:

B.3.1. Specific Aim 1. To test the hypothesis that altered expression of AR target genes is associated with racial difference in prostate cancer patients.

We have generated cFLIP polyclonal antibodies in large scale and are in the process to determine stromal cFLIP expression in prostate cancer by immunohistochemical tests.

To determine the association between altered stromal cFLIP expression in prostate cancer racial disparity and progression, we have selected androgen dependent cases from AA (n=27) and Caucasian (n=29) as well as androgen independent (n=25) prostate cancer patient. The grade and stage was matched and confirmed independently by two Pathologists according to newly adapted Modified Gleason Grading System⁹.

We showed that there is a statistically significant increase of stromal c-FLIP expression ($p < 0.001$) within the neoplastic gland stroma as compared with benign areas of prostate distant from the cancer foci (Fig.1 and 2). There is an association between increased tumor stromal c-FLIP expression and patients PSA, tumor grade and stage, but not patients' age and race (Fig.3). These results indicate that stromal c-FLIP expression promotes prostate cancer growth and invasion.

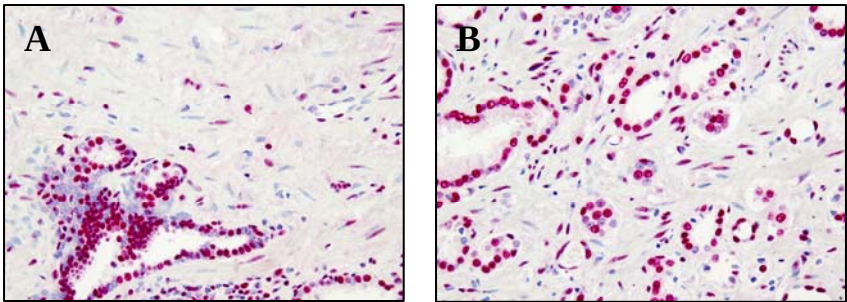


Figure 1. Increased expression of stromal cFLIP in the areas of prostate cancer (B) compared to benign prostate tissue (A).

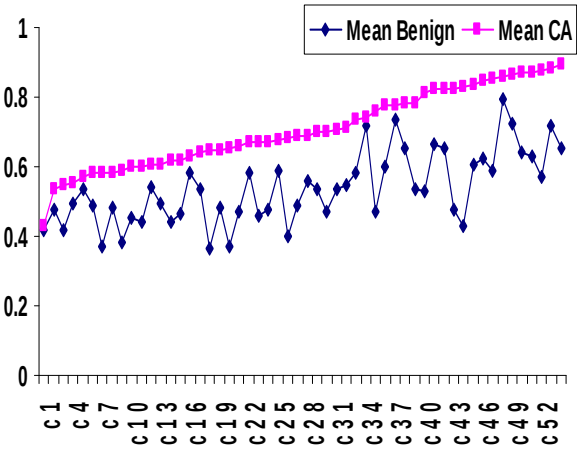


Figure 2. Increased stromal cFLIP expression in prostate cancer is statistically significant.

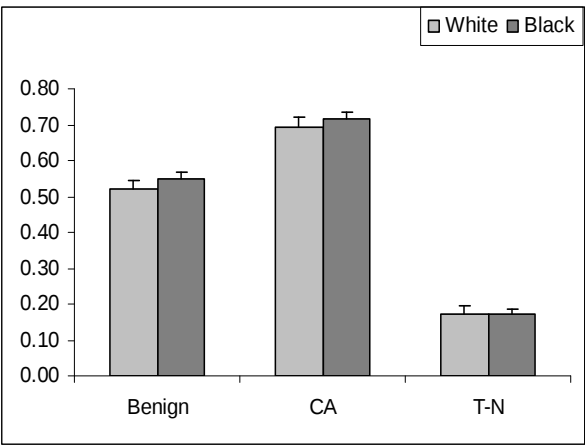


Figure 3. There is no difference in stromal cFLIP expression between AA and Caucasian patients.

B.3.2. Specific Aim 2. To test the hypothesis that altered expression of AR target genes promotes cell growth, invasion and metastasis in prostate cancer cell lines and nude mice xenografts.

We have established stromal cell line with and without cFLIP overexpression. Co-culture experiments with c-FLIP overexpressing stromal cells and PC3 cells significantly increased cell proliferation of androgen-independent OC3 prostate cancer cells than cancer cells co-cultured with control stromal cells (Figure 4).

We further investigated the invasion ability of PC3 cell under co-culture condition with stromal cells overexpressing cFLIP. c-FLIP overexpressing stromal cells significantly increased cell invasion of androgen-independent prostate PC3 cancer cells than cancer cells co-cultured with control stromal cells (Figure 5).

We are in the process confirming these observations in other prostate cancer cell lines including androgen dependent LNCaP cells and elucidating the mechanism involved.

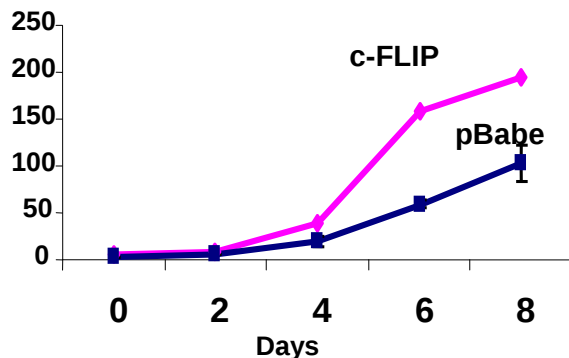


Figure 4. Stromal cFLIP overexpression promotes PC3 cell growth in cocultured system.

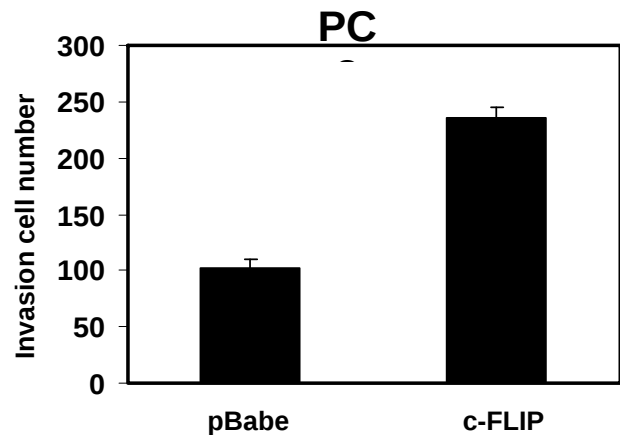


Figure 5. Stromal cFLIP overexpression enhanced PC3 prostate cancer cell invasion in cocultured system.

KEY RESEARCH ACCOMPLISHMENTS

B.3.1. Specific Aim 1. To test the hypothesis that altered expression of AR target genes is associated with racial difference in prostate cancer patients.

- Performed IHC to analyze the expression of AR target cFLIP in stromal cell between normal and cancerous prostate
- Performed IHC to analyze the expression of AR target cFLIP in stromal cells between AA and Caucasian prostate cancer patients.

B.3.1. Specific Aim 2. To test the hypothesis that altered expression of AR target genes promotes cell growth, invasion and metastasis in prostate cancer cell lines and nude mice xenografts.

- Established stromal stable cell lines overexpressing full-length cFLIP
- Performed cell proliferation assays in cocultured system with stromal cells overexpressing AR target gene cFLIP and LNCaP or PC3 cells
- Performed Matrigel invasion assays in cocultured system with stromal cells overexpressing AR target gene cFLIP and LNCaP or PC3 cells

REPORTABLE OUTCOMES

- Abstract accepted for DOD IMPACT meeting in Washington DC in September 2007
- Abstract presented for United State and Canadian Academic of Pathology meeting at San Diego in March 2007

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