AACR Poster #: 2115

An Authenticated *In Vitro* Model for Prostate Microenvironment Studies That Utilizes Prostate Epithelial Cells and Stromal-derived Cells

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Abstract

Prostate cancer remains one of the most common cancers diagnosed in men and one of the leading causes of cancer death in men. Tumor development and progression have been shown to be highly influenced not simply by the genetic makeup of a cell, but by its surrounding stroma (particularly fibroblasts). It has been demonstrated that prostate cancer–associated fibroblasts (CAFs, which are located marginal to the prostate tumor) differ from prostate normal–associated fibroblast (NAFs, which are located distal to the prostate tumor) with regard to their contribution to tumor progression. However, human prostate cancer *in vitro* model systems have focused largely on prostate cancer epithelial cells exclusively. A need exists for a more physiologically relevant human cell model system to study prostate cancer progression within the context of its tumor microenvironment.

In this study, we utilized prostate CAFs, prostate NAFs, and normal prostate epithelial (PrE) cells; all three lines were immortalized by human telomerase reverse transcriptase (hTERT) alone and then were cryopreserved, thawed, and continuously passaged without any indication of a decrease in growth rate. All cell lines expressed appropriate specific cell lineage markers for either fibroblasts or epithelial cells. Fibroblasts expressed TE7 and alpha smooth muscle actin (a-SMA), while PrE cells expressed cytokeratin 18, low levels of prostate specific antigen (PSA), and high levels of P63 throughout their passage; all characteristics were in accord with their primary cell counterparts. Next, cell proliferation was measured under the influence of CAFs and NAFs for various prostate-derived epithelial cells. The effects of stromal cells on prostate cell proliferation was cell line–dependent. CAF cells promoted the growth of PrE, DU145, and the unrelated epithelial cell A549, while NAF cells inhibited their growth. Meanwhile, CAF cells inhibited the growth of the viral transduced RWPE1 cell line, while NAF cells promoted the growth of the cancer-derived prostate epithelial cell LNCap. This study demonstrates that these three hTERT-immortalized cells from human prostate are a valuable model system for the study of prostate cancer cell progression and tumor microenvironment studies.

Introduction

Prostate cancer remains a common cancer with an estimated 161,000 new cases in 2017, representing about 10% of all new cancer cases in the United States in 2017. Over three million men are estimated to be living with prostate cancer in the United States. Mortality rates for prostate cancer have remained consistent over the past decade, accounting for 4.5% of all cancer deaths[1]. Animal models have provided some valuable insights into the biology of prostate cancer. In fact, a comprehensive analysis of genetically engineered mouse models identified that invasive prostate cancers are associated with the presence of fibroblast or myofibroblast cells. Additionally, these cells were later observed in a subset of human cancers[2]. It is known that prostate cancer progression results not only from genetic changes within a cell, but also by the tumor microenvironment[3], which includes the underlying connective tissue and fibroblasts, myofibroblasts, endothelial cells, and immune cells. However, the mechanisms behind prostate cancer progression remain poorly understood, in part due to a lack of human cellular models. Developing human cell models is difficult, particularly human prostate cells, which are difficult to grow continuously in culture. Thus, an *in vitro* human cell model that incorporates normal prostate cells and their surrounding stromal cells is vital to understanding prostate cancer progression mechanisms.

Results

1. The hTERT-Immortalized Prostate-derived Cells Passage Continuously

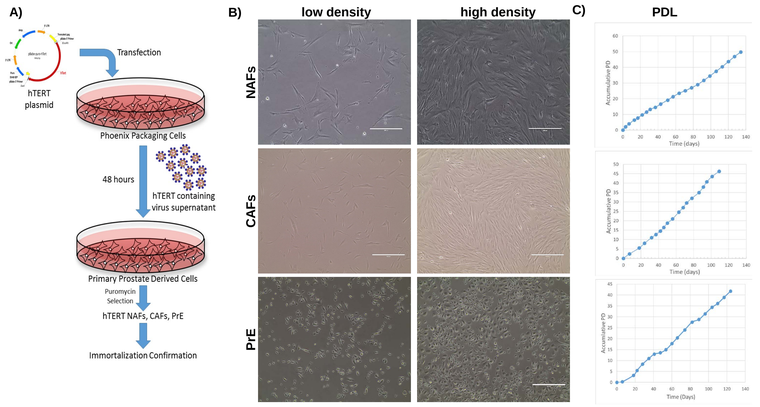

Figure 1. Prostate-derived fibroblast and epithelial cells grow continuously in culture. (A) NAFs (ATCC® CRL-3291™), CAFs (ATCC® CRL-3290™), and PrE (ATCC® CRL-3289™, coming soon) cells were validated after immortalization with hTERT and selection with puromycin[4]. The presence of hTERT was confirmed with both TeloTAGGG™ telomere length assay and telomeric repeat amplification protocol (TRAP) assays adopted from Koogan, et. al. Cancer Res 2006. (B) The selected clones grew continuously in culture for more than 40 population doublings (PDL). (C) CAFs grow faster than NAFs, reaching 40 population doublings in under 100 days. Scale bar, 400 μm.

2. Normal Prostate Epithelial Cells Maintain Epithelial-specific Markers

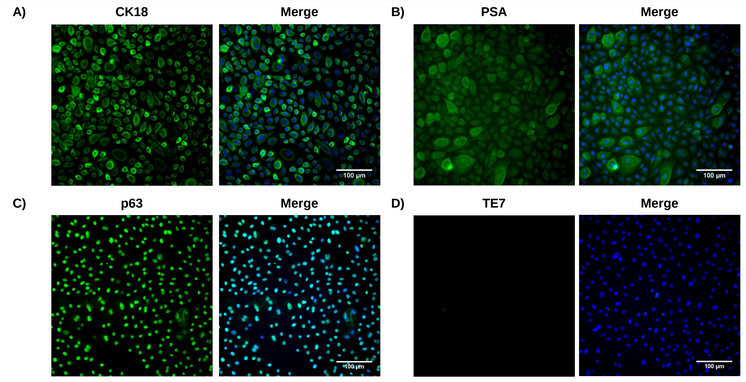

Figure 2. PrE cells express epithelial markers, but do not express a fibroblast marker. PrEs were fixed using 4% paraformaldehyde, then immunostained with primary antibodies to (A) cytokeratin 18 (CK18), (B) prostate-specific antigen (PSA), (C) p63, and (D) anti-human fibroblast (TE7), followed by staining with a secondary fluorescent antibody (green). The nuclei were stained with DAPI (blue). Cells were imaged with a fluorescent high-content screening system, and a composite image was generated (merge). PrEs show expression of CK18, PSA, and p63, but do not express TE7, suggesting that PrEs are of prostate origin. Scale bar, 100 μm.

3. Prostate-associated Fibroblasts Maintain Fibroblast-specific Markers

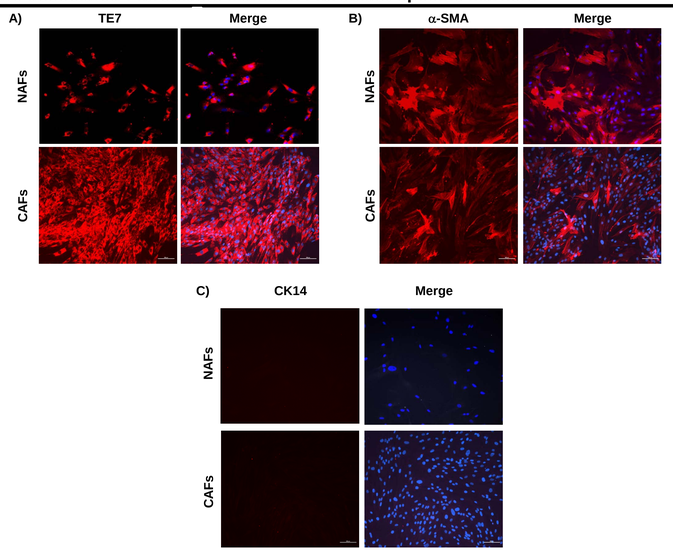

Figure 3. NAFs and CAFs express fibroblast-specific markers. NAFs and CAFs were fixed using 4% paraformaldehyde, then immunostained with primary antibodies to (A) anti-human fibroblast (TE7), (B) alpha-smooth muscle actin (α-SMA), and (C) cytokeratin 14 (CK14), followed by staining with a secondary fluorescent antibody (red). The nuclei were stained with DAPI (blue). Cells were imaged with a fluorescent microscope and a composite image was generated (merge). Both cell lines positively stained for TE7 and α-SMA, but not for CK14, suggesting CAFs and NAFs are mesodermal-derived human connective tissue. Scale bar, 100 μm.

4. NAFs and CAFs Promote Epithelial Cell Growth Differently

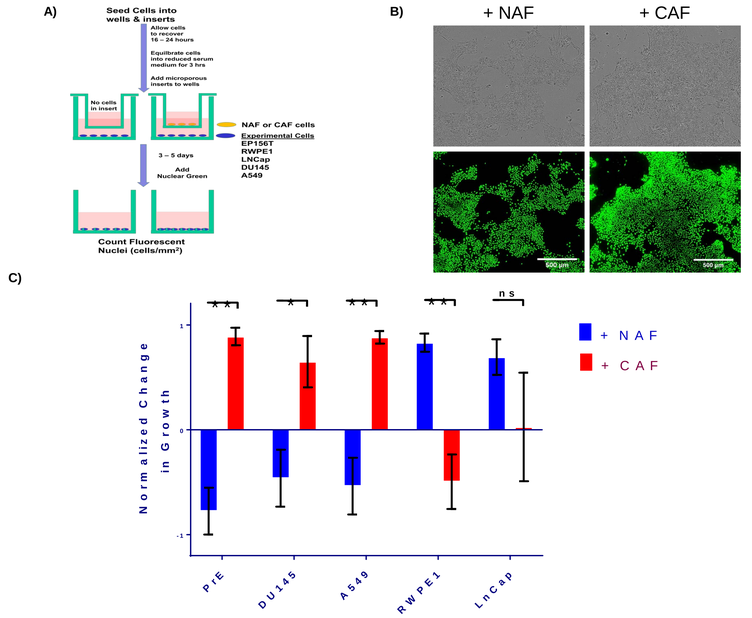

Figure 4. Growth of epithelial cells in the presence of NAFs and CAFs. (A) Experimental cells were seeded in triplicate into the wells of a multiwell plate, while NAF or CAF cells were seeded into 0.4 μm microporous inserts. After recovery, fibroblast cells were incubated in a reduced-serum medium for 3 hours. Experimental cells were incubated with a mixture of cell line–specific medium or reduced-serum medium (depending on the cell type) combined with fibroblast reduced-serum medium (mixed medium). Inserts with or without NAFs or CAFs were placed into the wells with the experimental cells. The insert medium was removed and replaced with mixed medium. Cells were allowed to grow for 3 to 5 days. After incubation, inserts were removed and nuclei were stained with a live cell DNA binding dye. Cell densities were determined by using the object counting analysis feature of an Incucyte® FLR system. (B) A representative image of growth and staining of PrE cells in the presence of NAFs (+NAF) and CAFs (+CAF). Nuclei are stained green. Scale bar, 500 μm. (C) Data were analyzed for percent change of growth of the experimental cells based on cell densities (cells/mm^2) between the cells in the presence and absence of fibroblast cells. Data were normalized for the cells in the presence of NAFs (+NAF) and CAFs (+CAF) utilizing a feature scaling set to -1 and 1. The data demonstrates that PrE growth is positively influenced by the presence of CAFs, while being negatively influenced by NAFs (N=3, Students t-test, ns = not significant, * P<0.05, ** P<0.005).

Summary

- Normal-associated fibroblasts, cancer-associated fibroblasts, and normal prostate epithelial cells derived from a single donor tissue were immortalized with hTERT.
- hTERT-immortalized prostate fibroblasts and prostate epithelial cells grow continuously without a change in growth rate or senescence.
- Normal prostate epithelial (PrE) cells continue to express epithelial-specific markers, but do not express a fibroblast specific marker.
- Normal-associated and cancer-associated fibroblasts continue to express fibroblast-specific markers, but do not express an epithelial-specific marker.
- Cancer-associated fibroblasts enhanced the growth of normal prostate epithelial cells derived from a single donor and of two other cancer epithelial cells.
- Cancer-associated fibroblasts did not enhance the growth of an HPV-immortalized prostate cell line, nor a hypotetraploid prostate cancer cell line. Surprisingly, normal-associated fibroblasts enhanced their growth.
- Together, these three characterized cell lines provide a unique human cell model to study prostate cancer progression in a tissue/tumor micro-environment.

References

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