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 cancer-associated fibroblasts (CAFs), which are located marginal to the prostate tumor, offer prostate cancer cells growth signals that are distinct from those normally found in fibroblast and epithelial cell interactions. There is evidence that CAFs and fibroblasts are mesodermal-derived human connective tissue and that tumor progression may be linked with how these cells communicate with each other.

### 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. Animal models have provided some valuable insights into the biology of prostate cancer progression and tumor microenvironment studies. However, the mechanisms behind prostate cancer progression remain poorly understood, in part due to a lack of human cellular models.

### Results

#### 1. The hTERT-immortalized Prostate-Derived Cells Progress Continuously

- Normal prostate epithelial cells do not express a fibroblast marker. hTERT-immortalized prostate epithelial cell lines maintain epithelial-specific markers, while hTERT-immortalized prostate fibroblasts express fibroblast-specific markers.

#### 2. Normal Prostate Epithelial Cells Maintain Epithelial-specific Markers

- PrE cells express epithelial markers, but do not express a fibroblast marker. They 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 microscopy system, and a composite image was generated (merge). PrE cells express CK18, PSA, and p63, but do not express TE7, suggesting that PrE cells are of prostate origin.

#### 3. Prostate-associated Fibroblasts Maintain Fibroblast-specific Markers

- NAFs and CAFs express fibroblast-specific markers. NAFs and CAFs were fixed using 4% paraformaldehyde, then immunostained with primary antibodies to (A) alfa smooth muscle actin (α-SMA), and (C) cytokeratin 14 (CK14), followed by staining with a secondary fluorescent antibody (green). The nuclei were stained with DAPI (blue). Cells were imaged with a fluorescent microscopy and a composite image was generated (merge).

#### 4. NAFs and CAFs Promote Epithelial Cell Growth Differently

- NAFs and CAFs promote epithelial cell growth differently. Normal prostate epithelial cell LNCaP (PrE) cells on microporous inserts were 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 replaced and mixed with fresh normal epithelial cell LNCaP (PrE) 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. A representative image of growth and staining of PrE cells in the presence of NAFs or CAFs was acquired. Nuclei were stained green. Scale bar, 500 μm. (C) Data were analyzed for percent change in growth of the experimental cells based on cell densities (cells/mm²) between the cells in the presence and absence of fibroblast cells. Data were normalized to the cells in the presence of NAFs or CAFs and the change in growth rate was estimated using a linear fit to the data. The data demonstrates that PrE growth is positively influenced by the presence of NAFs, while being negatively influenced by NAFs.

### References