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Distinct phases of human prostate cancer initiation and progression can be driven by different cell-types

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Abstract

The cells that initiate and propagate cancer are important therapeutic targets. However, the progression from cells of origin to tumor-propagating cells is poorly defined for most human cancers. Mouse models indicate that both basal and luminal cells can initiate prostate cancer, while studies with human prostate tissue have demonstrated a role for basal cells in transformation. Our recent study provides evidence that a common cell of origin can produce alternative variants of human epithelial cancer. Our findings also reveal that the cell of origin that initiates cancer is not continuously required to maintain and propagate the disease. Importantly, the cells responsible for initiating human prostate cancer can have a distinct cellular phenotype from the cells needed to maintain it.

Keywords

prostate cancer; tumor-propagating cells; epithelial cells; basal-like cells

Introduction

Since the cell-types driving cancer are important therapeutic targets, it is essential to determine whether the same cell population that initiates cancer is responsible for maintaining tumor progression. Understanding fundamental mechanisms of human cancer biology, including (1) whether different histological variants of cancer arise from common or distinct target cells and (2) as tumors progress whether the cell-type of origin is

continually required to maintain the disease, are critical for developing new effective therapeutic strategies against cancer.

Cell of origin for prostate cancer

The “cell of origin” or “target cell” refers to the normal cell-type in an adult tissue or organ that acquires the first tumor promoting characteristics [1, 2]. To investigate the contribution of distinct cell populations to the initiation and progression of human prostate cancer, our group developed a human tissue-regeneration assay [3–5]. This assay utilizes defined genetic alterations to initiate human prostate cancer in a purified subset of primary human prostate epithelial cells [3–5]. A combination of oncogenes commonly found altered in prostate cancer is introduced into primary human epithelial cells via lentiviral transduction. This assay allows for the isolation of distinct cell types from primary benign human tissues such as basal or luminal cells based on surface marker expression [3–5]. Using this approach we have previously demonstrated that primary human basal cells can serve as efficient target cells for prostate cancer initiation [4, 5]. In mouse models of prostate cancer both basal and luminal cells can serve as cells of origin for prostate cancer [6–8]. Several groups have utilized genetically engineered mice in which PTEN is deleted specifically in the basal or luminal cells using lineage-specific promoters to express the Cre recombinase. These studies demonstrate that basal and luminal cells can both initiate prostate cancer, with variations in aggressiveness of the resulting cancer depending on the mouse genetic background and incidence of *Pten* deletion by the basal or luminal lineage promoters used [6–8]. Recent studies also suggest that the microenvironment may influence the susceptibility of distinct prostate epithelial cell populations to cancer initiation [9, 10].

Tumor heterogeneity

Tumor heterogeneity poses a significant challenge to cancer treatment as distinct histological variants respond to treatments differently. Two models have been previously postulated that may explain the origins of tumor heterogeneity [1, 11]. First, different histological variants of cancer may arise from distinct target cells in the normal tissue, each giving rise to different tumor phenotypes [1, 11]. Alternatively, distinct genetic alterations may take place in a common target cell that is capable of multi-lineage differentiation or may change its phenotype over time as the tumor evolves to generate multiple histological variants of cancer [1, 11].

To investigate if histologically distinct phenotypes of human prostate cancer arise from common or distinct cells of origin, we introduced defined oncogenes found commonly altered in prostate cancer such as Myc and myristoylated AKT (myrAKT) into primary human basal cells via lentiviral transduction [5]. The recovered tumors contained features of both adenocarcinoma and a rarely observed histological variant of prostate cancer, squamous cell carcinoma, with each variant characterized by activation of distinct signaling pathways [5]. Although squamous cell carcinoma is not commonly found in clinical settings, it is associated with aggressive disease and resistance to androgen ablation, chemotherapy and radiation [12]. One of the advantages of the tissue recombination assay is that the oncogenes are introduced in primary human cells via lentiviral transduction which allows

clonality analysis based on identity of lentiviral integration sites within the genome. Therefore, the adenocarcinoma and squamous tumor phenotypes allow the opportunity to determine the origins of such heterogeneity. To address if histological variants arise from the same target cell or different cells, we performed laser capture microdissection of adjacent adenocarcinoma or squamous cell carcinoma regions. Lentiviral integration site analysis revealed that different histological variants of prostate cancer shared integration sites indicating they share a clonal origin [5]. These results demonstrate that distinct histological phenotypes of human cancer can be clonally-derived from a common cell of origin.

Relationship between the cells of origin and tumor propagating cells

The cancer stem cell model suggests the existence of cell populations within cancer that are preferentially responsible for tumor maintenance and propagation. Pioneering studies have established that some subtypes of human leukemia are hierarchically organized and that a subset of cells shares the critical properties of normal tissue stem cells: self-renewal and differentiation to generate mature cell lineages [13, 14]. These findings gave rise to the cancer stem cell concept, functionally defined as a cell that can propagate the disease into immune-compromised mice. The major clinical implication of the cancer stem cell concept is that elimination of all mature cancer cells will initially cause tumor regression, but over time, the cancer stem cells can self-renew and drive disease recurrence. Importantly, the frequency of cancer stem cell subsets varies greatly depending on the tumor genotype and site of origin and is not necessarily rare [15]. Subsequent studies showed that several regulators of growth and self-renewal including HoxA cluster transcription factors normally restricted to the hematopoietic stem cell compartment can be acquired by more mature leukemic subsets to confer cancer-propagating activity in a cell population with a distinct phenotype from hematopoietic stem cells [16]. Emerging evidence, first in breast cancer and later in a number of other epithelial cancers, suggests that solid tumors may also be maintained by tumor-propagating cancer stem-like cells [15]. But what is the relationship between the cells of origin and tumor propagating cells? Does the tumor propagating cell share the same phenotype with the cell of origin or can its appearance change over time?

Phenotypic plasticity has been demonstrated in epithelial cancers. Studies in breast cancer suggest that basal-like tumors can arise from luminal progenitor cells carrying BRCA mutations rather than basal cell as was originally predicted [17, 18]. Moreover breast cancer contains multiple tumor propagating cell-types with discrete phenotypes [19]. In other tumors the cell population responsible for initiating cancer shares the same phenotype as the cell population that maintains and propagates cancer. Murine brain tumors can be initiated in and maintained by Nestin+ neural stem/progenitor cells [20, 21]. In the mouse intestine Lgr5+ intestinal cells can initiate and maintain adenomas [22, 23]. In murine skin cancer, tumor propagating cells resemble the cells in the hair follicle bulge that can serve as target cells for transformation [24–27]. However, in most human epithelial cancers, it has not been determined whether the cell-types that give rise to cancer are also capable of maintaining advanced disease.

Having initiated human prostate cancer in naïve benign epithelial cells using defined genetic alterations, we asked whether the cells that initiate cancer are continually required to sustain the disease. We previously described that human prostate epithelial cells can be separated into enriched fractions of basal or luminal cells based on cell surface expression of CD49f [4, 5]. Basal-like cells express high level of CD49f (CD49f^{hi}) and luminal-like cells are characterized by low levels of CD49f surface expression (CD49f^{low}) [4, 5]. We found that primary tumors initiated by oncogenes Myc and myrAKT contained distinct cancer stem cell populations: basal-like tumor propagating cells (CD49f^{hi}) with dual potential to propagate both adenocarcinoma and squamous phenotypes and luminal-like unipotent tumor propagating cells (CD49f^{low}) that serially propagate strictly adenocarcinoma. Our new findings indicate that prostate cancer can start in basal cells and then evolve to be maintained by self-renewing cells with a different phenotype demonstrating that the stem-like component driving prostate cancer can change over time. These results indicate that human prostate adenocarcinoma can be initiated in basal cells and maintained by luminal-like cells, demonstrating that the cell of origin is not continuously required for propagation of human prostate cancer.

Further studies will need to be conducted to evaluate pathways that regulate the self-renewal capacity of prostate tumor propagating cells *in vivo*. Given that the stem-like populations driving prostate cancer initiation and progression have distinct phenotypes, the major clinical challenge will be finding strategies to inhibit common growth promoting and survival mechanisms in order to effectively eliminate all tumor-initiating and propagating cells regardless of phenotype.

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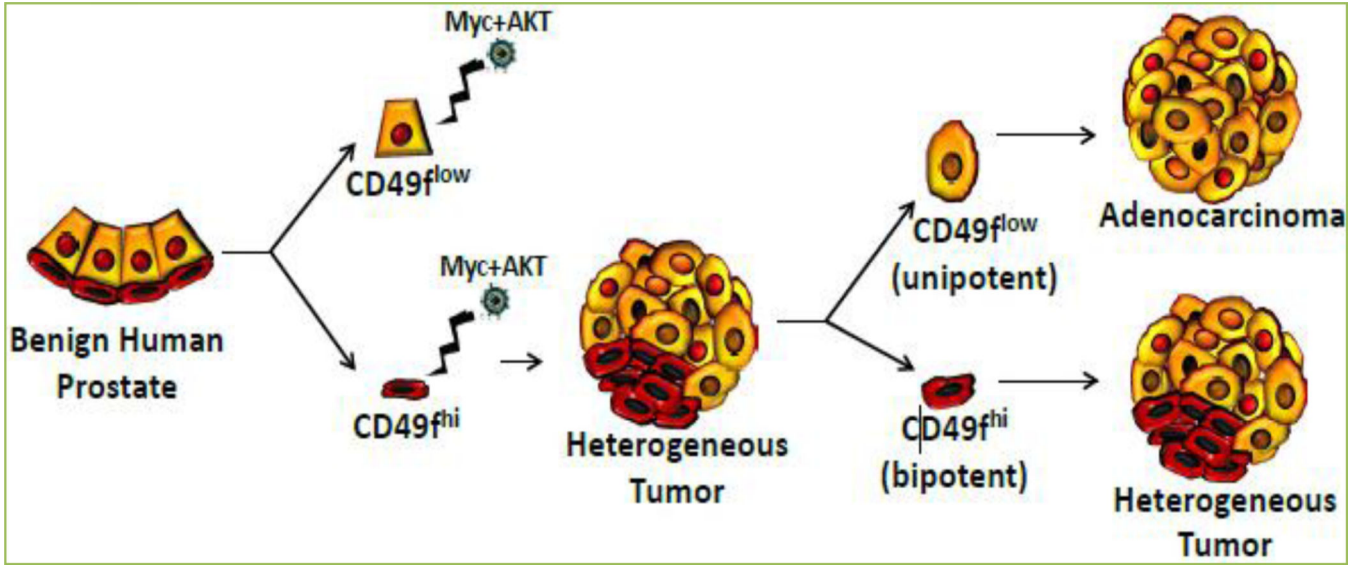


Figure 1. Cell populations with distinct phenotypes can initiate and propagate human prostate cancer

Model of transformation of epithelial subsets (basal-CD49^{hi} and luminal-CD49^{low}) with Myc and myrAKT resulting in tumors from the basal fraction but not from the luminal population. CD49^{hi} cells isolated from mixed tumors (containing both adenocarcinoma and squamous features) could propagate mixed tumors, while CD49^{low}K18⁺ luminal-like tumor cells propagate strictly adenocarcinoma in the absence of CD49^{hi} or K14⁺ p63⁺ basal-like cells.