Isolation and analysis of discreet human prostate cellular populations

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ABSTRACT

The use of lineage tracing in transgenic mouse models has revealed an abundance of subcellular phenotypes responsible for maintaining prostate homeostasis. The ability to use fresh human tissues to examine the hypotheses generated by these mouse experiments has been greatly enhanced by technical advances in tissue processing, flow cytometry and cell culture. We describe in detail the optimization of protocols for each of these areas to facilitate research on solving human prostate diseases through the analysis of human tissue.

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1. Introduction

More than forty years ago it was discovered that embryonic mesenchyme orchestrated epithelial morphogenesis and differentiation in prostate (Cunha, 1972). There is now a greater appreciation of the diverse subpopulations of adult stroma and epithelia in prostate development and disease. This review will cover the technical advances in flow cytometry, organoid culture, and tissue regeneration with human cells that are used to address questions regarding the functional roles of epithelial and stromal subpopulations in prostate development and disease.

1.1. Mouse vs. human prostate

The human prostate is a major cause of both morbidity and mortality. Unfortunately, there are no good experimental animal models that mimic the human organ structure and disease profile. Chimpanzees have a prostate anatomy similar to that of humans,

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and indeed suffer from human-like benign prostatic hyperplasia (BPH) (Steiner et al., 1999). However in chimpanzees the disease progression is slow and stochastic, which, aside from the ethical issues incumbent to working with primates, makes the model impractical to use experimentally. The only other commonly encountered species that suffers from prostate cancer and hyperplasia is the dog, although the canine prostate structure and pathobiology have significant differences from humans (Berry et al., 1986; Isaacs, 1984; Teske et al., 2002). Again, disease occurrence is sporadic and is associated with aging, making the model impractical for most purposes. The prostatic structure and disease profile in the most common experimental animal models (rats and mice) is very different from humans, with a lobular rather than zonal anatomy, a very different stromal to epithelial cell ratio (with much more prominent stroma in the human), and important differences in the ratio of basal to luminal epithelial cells (El-Alfy et al., 2000; Price, 1963; Price and Williams-Ashman, 1961; Sugimura et al., 1986). Furthermore, stem cell surface marker profiles are distinctly found in the basal cells of human prostate epithelium while in mice they are also expressed in a subset of luminal epithelia (Leong et al., 2008; Missol-Kolka et al., 2011). The *ex vivo* culture conditions for mouse versus human epithelial cells also reveals different nutritional requirements (Hofner et al., 2015; Karthaus et al., 2014). Finally, rats can be induced by various means, notably by hormonal carcinogens to undergo malignant transformation in the prostate (Noble, 1977; Wang and Wong, 1998) while the mouse prostate is relatively resistant to malignant transformation (Shappell et al., 2003).

1.2. Human prostate diseases

Nodular expansion of the prostate transition zone resulting in benign prostatic hyperplasia (BPH) is the most common symptomatic condition in aging men. The typical human prostate transition zone expands from 15 g, in men in their 40's, to 45 g in men in their 60's (Roehrborn, 2005). Over the course of decades, the transition zone slowly expands with a doubling time estimated at 4.5 years in men 51 to 70 years old (Berry et al., 1984). The histological incidence of BPH and associated lower urinary tract symptoms increase dramatically with age (Platz et al., 2012). Patients are treated with α-adrenergic blockers and/or 5α-reductase inhibitors (5ARI) that respectively relax and shrink the gland. Baseline prostate volume and PSA levels are the best predictors of patients who will fail medical therapy, and almost 250,000 men per year undergo some form of surgery for benign prostatic hyperplasia/lower urinary tract symptoms (BPH/LUTS) (Roehrborn et al., 1999; Wei et al., 2008). The predominant medical therapy for an enlarged prostate is a 5-alpha-reductase inhibitor (5ARI), which reduces total prostate volume through apoptosis of both stroma and epithelium (Anon, 1993). The 5ARI, Finasteride reduces the risk of symptomatic progression of BPH by only 34%, and the drug must be taken continuously with undesirable side effects (McConnell et al., 2003). The cost of treating BPH has been steadily growing commensurate with our aging population and in 2005 was estimated at nearly $4 billion per year (Saigal and Joyce, 2005). In summary, the development of personalized medical interventions that reliably prevent prostatic enlargement or better reduce prostate volume with fewer side effects is needed.

Prostate cancer is the most common non-skin malignancy in Western males and the second leading cause of cancer-related mortality averaging more the 27,000 deaths per year in the U.S. (Siegel et al., 2015). The widespread use of PSA testing means that prostate cancer is overdiagnosed, while our inability to predict which tumors will remain indolent and which will progress to lethal disease means that patients are steered towards more aggressive approaches. This combination gives rise to an epidemic of

overtreatment (Klotz, 2013; Vickers et al., 2014). Our current treatment paradigm of depriving aggressive tumors of androgens typically extends lifespan by 1–3 years, but ultimately fails due to a number of tumor compensatory mechanisms (Antonarakis et al., 2014; de Bono et al., 2011; Scher et al., 2012). Accordingly, the most important clinical problems in prostate cancer treatment include the development of biomarkers of lethal disease and the treatment of castration-resistant tumors. The evolution of castration-resistant tumors and 5ARI-resistant BPH is the result of dynamic multicellular interactions among epithelia, stroma and inflammation; therefore, the development of novel therapeutic strategies requires a more detailed understanding of the functional contribution of each cell type to disease progression.

In addition to BPH and cancer, the prostate is also the center for prostatitis, inflammation resulting from chronic or acute infections, or from other irritants in the organ. Prostatitis has been linked to chronic pelvic pain syndrome, but this major clinical problem does not seem to involve novel prostatic cells and is outside of the scope of this review.

1.3. Prostate cellular hierarchy

The necessity of tissue interactions for organ development and function also extends to the patterning of tissues into cellular hierarchies. Similar to other organs, the prostate is organized with mesenchymal support of parenchymal epithelial tissue. A detailed view of the mechanisms governing these interactions in development and disease is emerging (Strand et al., 2010). In particular, identification of stem cell markers and the use of cellular lineage tracing have uncovered a cellular hierarchy of epithelium during development and in response to injury (Bonkhoff and Remberger, 1996; Choi et al., 2012; Chua et al., 2014; Collins et al., 2001; Garraway et al., 2010; Goldstein et al., 2010; Goldstein et al., 2008; Karthaus et al., 2014; Leong et al., 2008; Luo et al., 2013; Ousset et al., 2012; Richardson et al., 2004; Stoyanova et al., 2013; Vander Griend et al., 2008; Wang et al., 2009, 2013, 2014a, 2014b; Xin et al., 2003, 2005, 2007). A number of distinct stromal cell lineages have also been identified (Peng et al., 2013). The hope is that a detailed characterization of how these stromal and epithelial subpopulations are altered in disease will aid our understanding of the cell(s) of origin in the pathogenesis of prostate diseases for the rational design of targeted therapeutics.

The hierarchy of prostate epithelium has been extensively reviewed (Shen and Abate-Shen, 2010; Wang et al., 2001). In brief, there are three main epithelial lineages: neuroendocrine, basal and luminal. The emergence of a neuroendocrine phenotype in advanced prostate cancer necessitates a deeper understanding of what is normally a tiny subpopulation of epithelium (Beltran et al., 2014). The basal and luminal epithelial lineages have recently been subdivided into multiple subpopulations suggesting these positional terms simply reflect their orientation in a pseudo stratified cellular bilayer. Finally, a transitory intermediate epithelial lineage with characteristics of both basal and luminal cells is observed only in development, regeneration following castration and subsequent androgen replacement, disease and cellular culture (Hudson et al., 2001; van Leenders and Schalken, 2003; van Leenders et al., 2003). Human prostate stroma is normally predominantly smooth muscle with occasional fibroblastic cells, blood vessels, nerves and infiltrating immune/inflammatory cells. In BPH and cancer the form of the stroma can become more reactive with increased levels of collagen, less well differentiated smooth muscle, increased angiogenesis and often increases in leukocytic infiltrate. The cellular arrangement of the prostate is shown stylistically in Fig. 1.