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In vitro modeling of the prostate cancer microenvironment ☆,☆☆

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ABSTRACT

Prostate cancer is the most commonly diagnosed malignancy in men and advanced disease is incurable. Model systems are a fundamental tool for research and many *in vitro* models of prostate cancer use cancer cell lines in monoculture. Although these have yielded significant insight they are inherently limited by virtue of their two-dimensional (2D) growth and inability to include the influence of tumour microenvironment. These major limitations can be overcome with the development of newer systems that more faithfully recreate and mimic the complex *in vivo* multi-cellular, three-dimensional (3D) microenvironment. This article presents the current state of *in vitro* models for prostate cancer, with particular emphasis on 3D systems and the challenges that remain before their potential to advance our understanding of prostate disease and aid in the development and testing of new therapeutic agents can be realised.

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1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer and second leading cause of cancer death in men throughout the Western world. Although this represents a significant and growing problem with an aging population, the mechanisms of PCa disease initiation, progression and metastasis remain poorly understood. Research towards this has been particularly hampered by the lack of robust and biologically relevant model systems. In particular, the lack of reliable *in vitro* models that accurately recapitulate the complex three-dimensional (3D) microenvironment of the prostate has been a major impediment to furthering our understanding of prostate disease, as well as the development and testing of new therapeutic agents.

Animal models have been the foundation of PCa research, however, these typically bear limited relevance to human disease and are hampered by additional significant limitations. The only nonhuman mammals known to develop prostate cancer naturally are nonhuman

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primates and dogs [1,2]. Both models, however, are highly limited due to significant expense and long tumour latencies. Experimental rodent models have also been developed and used extensively to elucidate discrete mechanisms of prostate carcinogenesis. These include a variety of transgenic mouse (eg, TRAMP, LADY, myc, Pten, and more) [3–7] and rat models (spontaneous, hormone or chemically induced) [8–10]. Ultimately, and without exception, each of these animal models is universally limited by its nonhuman origin, significantly restricting its relevance and application to human disease.

In vivo prostate cancer models of human origin typically consist of primary cell or tissue slice grafts [11], multiple cancer cell lines as grafts [12–15], as well as cancer tissue xenografts [16–19]. While these models address the nonhuman nature and fundamental limitation of animal models, they suffer from limitations relating to expense as well as experimental and tumour latency. The combination of these three key factors – expense, long tumour latency and nonhuman origin – has represented the major hurdle for models of prostate cancer that, to date, can only be collectively overcome through the use of *in vitro* models. *In vitro* models, however, come with the caveat that some inherent advantages of *in vivo* systems, such as being able to follow the natural progression of PCa and/or metastasis, are lost.

Traditionally, the vast majority of *in vitro* models of prostate cancer have almost exclusively consisted of immortalised cancer cell lines in monoculture [13,20]. While these cell lines and model systems have significantly advanced our understanding of the mechanisms of PCa, they remain poorly representative of human disease *in vivo* due to the highly abnormal culture environment and inherent inability to incorporate parameters of disease development and progression, such as invasion, and multicellular interaction.

Appropriate *in vitro* experimental models suitable for the analysis of cell growth and interaction, homeostasis, EMT, invasion and metastasis, are becoming increasingly important for basic research and the development of new therapeutics. However, the need for rapid, high-throughput screening in drug development and testing has resulted in most contemporary platforms remaining based on two-dimensional (2D) monoculture cell assays. Using these systems, and animal models, anticancer drugs screened for PCa can show significant promise in the laboratory, but ultimately have little or no impact or benefit on the survival of patients [21]. A key factor underlying this discrepancy is that 2D *in vitro* models do not faithfully recreate the complex multi-cellular, 3D tumour microenvironment seen *in vivo* in humans [22,23]. It is the combined lack of these attributes/features that is the underlying reason for the limited predictive power of 2D systems in terms of clinical efficacy when used for drug testing and discovery [22].

While progress has being made in this area with 3D models such as spheroids providing a more accurate biological readout, the current model systems still only represent a highly limited reconstruction of the native prostatic heterogeneity and complex *in vivo* architecture (Fig. 1). Ultimately, the development of more robust and effective *in vitro* PCa models that accurately mimic the *in vivo* tumour niche microenvironment is of vital importance for drug discovery, drug testing, and to advance our knowledge of PCa biology.

2. The Prostate Tumour Microenvironment

The prostate and PCa are both highly heterogeneous tissues. In addition to the luminal epithelial and tumour cells that have been the typical and traditional basis of *in vitro* models, the prostate is also comprised of basal cells and a small number of neuroendocrine cells in the epithelium, which itself is surrounded by stroma tissue that also plays a major role in cancer cell growth, survival, invasion and metastatic progression [24]. The prostatic stroma is primarily composed of smooth muscle and extracellular matrix, but also consists of nerves, lymphatics and the blood vessels of the organ. Other cell types present include stromal cells (fibroblasts and myofibroblasts), endothelial cells, pericytes and inflammatory cells (including resident mast cells); collectively these form the prostate microenvironment (Fig. 1). It is the combined effect and interaction of these components that define prostate tumourigenesis, progression, invasion and the potential to respond to various therapeutics.

Prostate fibroblasts form particularly important stromal components that have a well-established role in driving tumourigenesis. Very early studies showed morphological changes identified by pathology in prostate carcinoma associated fibroblasts (CAFs) compared to normal prostatic fibroblasts (NPFs), while recent work has unequivocally shown that CAFs can induce transformation and tumourigenesis in benign epithelia, whereas NPFs do not [25–28]. Inflammation also has a well-documented role in the development and progression of many

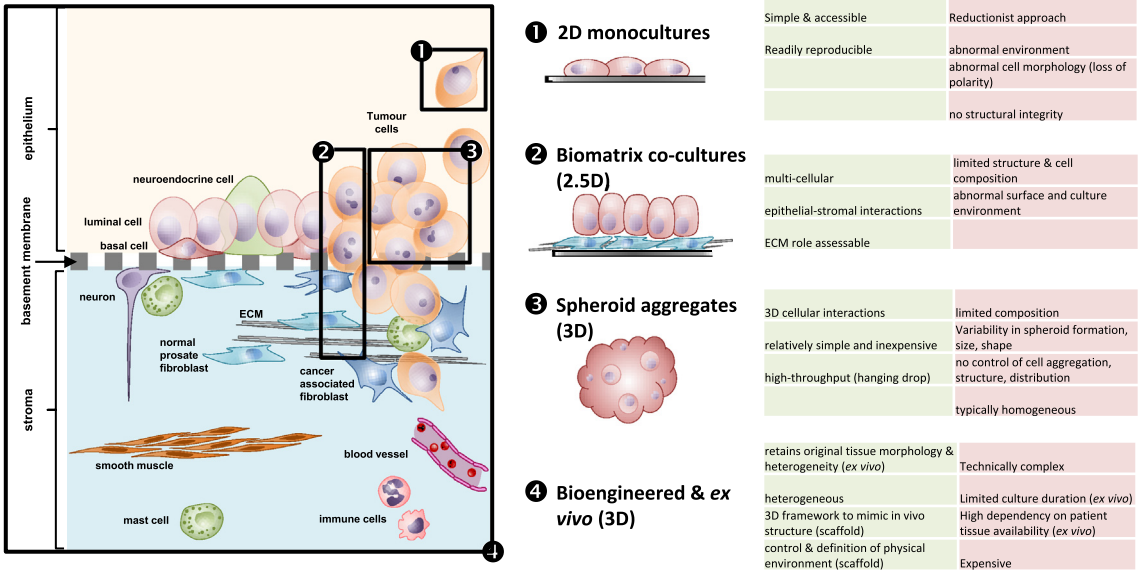

Fig. 1. Prostate architecture and relevance of *in vitro* model systems. The prostate and prostate cancer are highly heterogeneous tissues, consisting of multiple compartments and cell types within which cell–cell and cell–matrix interactions define cell behaviour and response to therapy. Current *in vitro* models, ranging from simple 2D monoculture to complex bioengineered 3D systems, harbour intrinsic advantages and limitations and vary significantly in their recapitulation of the *in vivo* tissue architecture, biological relevance, and drug response.

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