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Review article

Interaction of prostate carcinoma-associated fibroblasts with human epithelial cell lines *in vivo*

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ABSTRACT

Stromal-epithelial interactions play a crucial and poorly understood role in carcinogenesis and tumor progression. Mesenchymal-epithelial interactions have a long history of research in relation to the development of organs. Models designed to study development are often also applicable to studies of benign and malignant disease. Tumor stroma is a complex mixture of cells that includes a fibroblastic component often referred to as cancer-associated fibroblasts (CAF), desmoplasia or "reactive" stroma. Here we discuss the history of, and approaches to, understanding these interactions with particular reference to prostate cancer and to *in vivo* modeling using human cells and tissues. A series of studies have revealed a complex mixture of signaling molecules acting both within the stromal tissue and between the stromal and epithelial tissues. We are starting to understand the interactions of some of these pathways, however the work is still ongoing. This area of research provide a basis for new medical approaches aimed at stabilizing early stage cancers rendering them chronic rather than acute problems. Such work is especially relevant to slow growing tumors found in older patients, a class that would include many prostate cancers.

1. Introduction

A role for stroma in cancer progression has been postulated for many years. In the first half of the Nineteenth Century Johannes Mu¨ller demonstrated that cancer is made up of cells. His student, Rudolph Virchow, famously proposed that chronic inflammation was the cause of cancer, establishing a possible role for non-epithelial cells in carcinoma pathogenesis. Towards the end of that century Stephen Paget, in his "seed and soil" hypothesis, proposed that metastatic sites represented areas with fertile soil for specific tumor epithelial cells to grow, suggesting, but not explicitly stating, a role for stroma in cancer metastasis.

The role for stromal cells in the study of prostate development forms a basis for studies of prostatic carcinogenesis and tumor progression. The prostate, like many organs, contains cells derived from two of the embryonic germ layers, an epithelium of endodermal origin with surrounding stromal cells that are mesodermal derivatives. The prostate is derived from the urogenital sinus (UGS), which itself is formed from the subdivision of the embryonic cloaca by the urorectal septum.

Ideas relating to a role for mesenchyme in defining the process of organogenesis go back to the 1950s and 60 s with contributions from a number of sources, notably including studies by Grobstein (Golosow and Grobstein, 1962; Grobstein, 1953). Early studies, demonstrated that tissue interactions between urogenital epithelium and urogenital mesenchyme are crucial to prostatic organogenesis (Cunha, 1972). Work was pursued in a number of other organs including the gastro-intestinal tract and its derivatives as well as lungs mammary gland, hair follicles and teeth, to explore the importance of mesenchyme in mediating epithelial growth and differentiation (Dauca et al., 1990; Haffen et al., 1982; Kollar, 1970; Kollar and Baird, 1970; Kollar and Fisher, 1980). In the prostate, the application of tissue recombination technology, using androgen receptor-deficient (tfm) mouse tissues, demonstrated that paracrine interactions between tissues mediate many of the effects of androgens in the prostate relating to proliferation and differentiation, but not to adult epithelial function (Cunha and Chung, 1981; Donjacour and Cunha, 1993). Subsequent work demonstrated that these paracrine interactions are bi-directional, *i.e.* that there is a "conversation" between epithelial and stromal tissues, rather than a stromally-driven monologue (Cunha et al., 1996; Hayward et al., 1997).

A large number of approaches have been developed to examine the role of stromal-epithelial interactions in cancer. Each of these has inherent strengths and weaknesses, and a number of studies proceeding in parallel is often needed to thoroughly investigate a given hypothesis. Here we will briefly summarize the approaches currently

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available and will focus on *in vivo* models currently being applied to address this problem, with a focus on prostate cancer.

2. Technical approaches to examine cellular interactions

A number of approaches have been devised to study cellular interactions. *In vitro* methods include 2- and 3-dimensional tissue culture and the application of microfluidic devices. *In vivo* approaches include transgenic mice, xenografting of tissues and organs and various forms of tissue recombination. All of these have strengths and limitations, and have developed and improved over the years.

The limitations of cell culture technology in the 1970s and 80s meant that there were few practical options to examine interactions between tissues *in vitro* spurring the development of *in vivo* methods at that time. This situation has since changed, with the development of effective three-dimensional tissue culture and co-culture approaches and advances in imaging technology to allow the development of tumor avatars that can be used to test specific paradigms (Chambers et al., 2014; Gao et al., 2014; Ji et al., 2016; Osuala et al., 2015; Sameni et al., 2012, 2017; Windus et al., 2012). Such approaches also provide a valuable method of preclinical testing of potential therapeutics (Aggarwal et al., 2015; Sameni et al., 2016). The combination of old hanging drop approaches with newer microfluidics technology has also provided powerful new *in vitro* tools (Casavant et al., 2013).

Ex vivo culture techniques using a variety of approaches have developed over a number of decades, starting with the work of Isla Lasnitzki in the 1960s and 70s (Lasnitzki, 1963, 1974, 1976). These approaches allow the cells in the pieces of tissue under examination to interact without systemic effects. Effective organ culture techniques developed in the 1990s provided good assays of prostate development (Foster and Cunha, 1999; Huang et al., 2005, 2009; Jarred et al., 2000; Tsuji et al., 1994). More recently a variety of approaches have been developed to culture intact pieces of human prostate tumor *in vivo* (reviewed in (Centenera et al., 2013)). Such approaches allow for the testing of specific drug modalities to assess efficacy (Centenera et al., 2012).

Transgenic mice have provided significant insights into prostate cancer biology, but until recently have been limited in the ability to target to the stroma, limiting their ability to provide insights into stromal-epithelial interactions (Swonger et al., 2016). Most of the mouse models of prostate cancer have applied one of the three versions of the probasin promoter, or the NKX3.1 promoter to drive gene expression (Ittmann et al., 2013; Shappell et al., 2004). Both of these promoters target epithelial cells, and until recently there have been few transgenics that target the stroma. Those that do, using for example the FSP1 and smooth muscle α-actin promoters target populations of stromal cells that are widespread throughout the body and often mask prostate-specific phenotypes. For example the Fsp1-cre TßRII flox mouse exhibits widespread changes including squamous cell carcinoma of the fore-stomach, and dies before any significant prostatic phenotype is obvious (Bhowmick et al., 2004b). Tissue rescue, in which prostatic tissues from these animals is grown for extended periods in immunocompromised or syngeneic hosts is needed to demonstrate a prostate cancer phenotype resulting from the loss of stromal Transforming growth factor receptor II (TBR2) (Li et al., 2008). The recent development of the SRD5A2-cre mouse by the McMahon laboratory at USC, provides a model in which cre-recombinase expression is restricted, in males, to specific stromal cells in organs of the urogenital tract (mouse generated for the GUDMAP consortium, see mouse strains page at: https://www-gudmap3.gudmap.org This new tool should help to start to unravel some of the many questions that until now have not been amenable to investigation by transgenic approaches.

A number of *in vivo* approaches have been applied to provide models of development and carcinogenesis. All *in vivo* systems have advantages and drawbacks. They can give relevant data on the species investigated, however, this is not necessarily applicable to humans. Another problem common to all *in vivo* model systems is that it can be difficult to separate the local influences of individual cell groups within an organ from the systemic effects of the whole animal.

The tissue recombination model was initially developed to interrogate interactions occurring in development in a number of organs including teeth, intestine, breast and prostate (Cunha and Hom, 1996; Cunha et al., 1992; Kedinger et al., 1998; Kollar and Baird, 1970; Kollar and Fisher, 1980). Mesenchymal and epithelial tissues were separated and recombined in various ways to explore developmental signaling. Initial studies used tissues derived from inbred mouse strains that could be grafted back into syngeneic hosts. The development of immunocompromized host strains such as the athymic nude, severe combined immunodeficient (SCID) and V(D)J recombination gene deficient (RAG) mice allowed these approaches to be pursued across species boundaries including the use of human cells. Since these mice do not have a cell mediated immune system they cannot recognize non-self tissues and therefore provide a convenient environment in which to grow foreign tissues. The advantages of xenografts are that grafted tissue can be exposed to a complex *in vivo* environment whilst still being discernible from the host tissue by species or cell type-specific markers. Early studies tested engraftment sites with extensive vascular beds, these included the iris. However, the renal capsule has proved useful implantation site because of both its capacity to quickly vascularize any tissue transplanted into the region and the good level of tolerance shown by host animals. Renal capsule implants have been used to pursue a range of studies in both cancer and developmental biology. A comparison of orthotopic, renal capsule and sub-cutaneous grafting demonstrated the high take rate and technical simplicity of the renal capsule, this can be balanced against the lower take rate of the more simple to perform sub-cutaneous grafts and the high take rate but high degree of difficulty of prostatic orthotopic grafts (Wang et al., 2005a).

An alternative approach, also utilizing immunodeficient mice has been the subcutaneous implantation of collagen gels containing epithelial cells. This method has been used to develop models of human and rat gut and human prostate (Del Buono et al., 1992; Hayward et al., 1992). The Rowley group at Baylor College of Medicine developed an approach known as differential reactive stroma (DRS) grafting. DRS recombines LNCaP prostate carcinoma cell line with engineered prostate stromal cells as an approach to model the effects of specific stromal changes on tumor growth (Tuxhorn et al., 2002a, 2002b; Yang et al., 2005).

One of the major limitations of immunocompromised mouse models is the absence of a functional immune system. While these mice all retain elements of the inflammatory response and varying immune cell components (such as B cells in athymic nude mice), the close interactions between elements of the immune and inflammatory networks mean that in addition to the loss of immune function, inflammatory responses are also not completely normal. This is a cost of using an *in vivo* model and must be weighed against the understanding coming from performing experiments in a more complex living environment *versus* more reductionist *in vitro* approaches.

Xenografting of cell lines, mostly to the subcutaneous site, have been used extensively to study the effects of drugs and other potentially therapeutic and diagnostic agents *in vivo* (Bandari et al., 2014; Carlucci et al., 2013; Dijkgraaf et al., 2012; Jin et al., 2015). Such models are probably an improvement over 2D culture of cell lines but lack paracrine interactions with either normal or cancer stroma and suffer the limitations of lack of immune/inflammatory input described for other forms of grafting.

Xenografting of benign and malignant tissue from patients has a long history and retains normal local paracrine interactions. Efficiency varies with graft site but can be very high (Wang et al., 2005a). Such models can also show metastatic spread (Wang et al., 2005b). As techniques have developed the use of patient derived xenograft (PDX) models has become more popular and these are now available from a number of commercial sources.