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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.

Keywords

Prostate cancer; cancer-associated fibroblasts; xenograft

Introduction

A role for stroma in cancer progression has been postulated for many years. In the first half of the Nineteenth Century Johannes Müller 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.

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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 60s 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 available and will focus on *in vivo* models currently being applied to address this problem, with a focus on prostate cancer.

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, Sameni et al, 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, Huang et al, 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 T β R11 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, Kedingner 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 subcutaneous 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 subcutaneous 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, Tuxhorn et al, 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.

Carcinoma-associated fibroblasts

Fibroblasts are a poorly defined group of stromal cells that produce extracellular matrix components and growth factors. In general fibroblasts express vimentin and may express other structural proteins that define sub-populations of the cells. It has been known for at least 30 years that there are many sub-populations of cells that broadly fit a definition of fibroblasts, however detailed descriptions of such cells and their markers and interactions are still lacking (Schor et al, 1985, Schor et al, 1988, Schor et al, 1991). While the role of some fibroblasts, for example myofibroblasts in wound healing responses, are relatively well understood (Tomasek et al, 2002), the details of other groups of superficially similar cells are poorly comprehended.

Fibroblast-epithelial interactions in tumorigenesis have been studied in human cancers including breast, colon, and prostate. In the human prostate the stroma is predominantly composed of smooth muscle cells that abut sub-adjacent to the epithelial basement membrane, with few fibroblasts, myofibroblasts or collagen fibers. Masson's Trichrome staining reveals normal prostate stroma to exhibit predominantly red-staining smooth muscle cells with occasionally blue-staining collagen fibers and fibroblasts. A stromal response, of variable intensity, occurs as a component of prostate carcinogenesis. This tumor stroma microenvironment is similar to wound repair granulation, a so called "reactive stroma". Tumor stroma includes activated fibroblasts and myofibroblasts often interspersed with expression of extracellular matrix (ECM) components, prominently including various collagens, evident in Masson's trichrome stained sections (Tuxhorn et al, 2001), as well as endothelial cells, immune cells, inflammatory cells. Phenotypic changes in stromal cells, such as expression of procollagen 1 can be seen adjacent to foci of prostatic intraepithelial neoplasia (PIN), a prostate cancer precursor suggesting that, at least in some patients, this is an early response to changes in stromal-epithelial interactions in carcinogenesis (Tuxhorn et al, 2002c).

Quantitation of prostate tumor stromal changes have demonstrated that this is a useful measure of clinical outcomes, providing additional prognostic information over Gleason grade (McKenney et al, 2016). Seminal work on prostate cancer stromal grading showed that the two groups of tumors that are most aggressive are, 1) those with almost no stromal involvement, these seem to be independent of their microenvironment from early in their pathogenesis; and, 2) tumors that exhibit an abundant stromogenic response, where the stroma appears to be a major contributor to tumor growth and invasion (Ayala et al, 2003). Reactive stroma grading (RSG) is an independent predictive factor for PCa biochemical recurrence and PCa specific death (Ayala et al, 2011, McKenney et al, 2016, Saeter et al, 2015, 2016, Yanagisawa et al, 2007).

Role of CAF in regulating tumorigenesis *in vivo*

Fibroblasts can enhance tumor take rate and growth in experimental systems (Gleave et al, 1991). We examined the effects of CAF on human prostate epithelial cells in a tissue recombination assay. This work demonstrated, for the first time, that fibroblasts derived from human prostate cancer were able to induce malignant transformation in genetically initiated but non-malignant BPH1 human prostatic epithelial cells, but not in normal epithelial cells (Olumi et al, 1999). These data, were subsequently confirmed by others (Barclay et al, 2005), demonstrating that prostate CAF induced highly disorganized and invasive tumors, whereas BPH-derived fibroblasts induced well disorganized and non-invasive tumor. Normal peripheral zone-derived fibroblasts did not generate visible tumors. These differential phenotypes found among those models suggested that CAFs might be defined as ‘isolated from within the tumors of cancer patients’ versus from uninvolved tissue in the same organ. Subsequent work over nearly two decades has demonstrated that there are patient-specific variations in the degree of the tumor-inducing response of CAF, and that not all fibroblasts derived from tumors are able to induce malignant transformation in the BPH1 reporter line. However, we have never seen a case where fibroblasts from non-malignant tissue elicited this effect. For this reason we have added a functional component to the definition of CAF used in our own work, this being the ability to induce tumors in the reporter line *in vivo*. All human prostate CAF used in our own experiments are validated in this way, however this standard is not universally applied and the term is often used simply to describe fibroblastic cells derived from a tumor.

In tissue recombination assays normal epithelial cells (with no genetic lesions predisposing towards malignant transformation) adopt a squamous phenotype in response to human prostate CAF. There is no evidence of invasion in these tissues, and the cells are apparently benign, but adopt a phenotype that can be considered to be similar to a generic wound healing response. This may indicate that the epithelial cells are responding to an abnormal environment by adopting a protective response that falls short of, or perhaps inhibits, malignant transformation.

Genetic and genomic analysis of cancer-associated fibroblasts

Molecular features of normal versus tumor stroma are revealing insights into the ability of CAF to elicit changes to epithelium. It is known that artificially-induced stromal mutations can elicit tumorigenic responses in epithelial cells (Barcellos-Hoff and Ravani, 2000). Using the techniques available in the late 1990s we never saw evidence of gene amplifications or deletions in human CAF (Olumi et al, 1999). Some contemporaneous studies suggested the presence of such mutations in human prostate cancer (Macintosh et al, 1998), however these have not been repeated and may represent artifactual findings due to limitations of the technology available at the time to cleanly isolate cells. Experiments in mouse models demonstrated the potential of stromal mutations to affect tumor evolution (Hill et al, 2005a, b) however while interesting this may not be relevant to human disease. More recently, detailed genetic analysis of CAF by comparative genomic hybridization, DNA sequencing, and microsatellite methods, found no convincing evidence of clonal somatic copy-number alterations in the stromal components of human tumors, leading to a general acceptance that

clonal somatic gene changes are not a major route to the formation of tumor stroma associated with human carcinomas (Bianchi-Frias et al, 2016, Campbell et al, 2009, Qiu et al, 2008).

Traditional histopathologic grading of tumors uses the Gleason grading system to examine the patterns of growth and invasion of tumor epithelium. Reactive stromal grading (RSG) is an independent system based upon stromal phenotypes to divide tumors into one of four reactive stromal grades (0–3) (Yanagisawa et al, 2007, 2008). RSG provides a predictive factor for biochemical recurrence and disease-specific death in prostate cancer patients (Saeter et al, 2015, 2016) and can add significant predictive value to Gleason grading (McKenney et al, 2016). In contrast to clonal somatic gene studies, many differences have been found in gene expression between CAF and normal fibroblasts. Dakhova et al. characterized reactive stroma grade 3 in PCa relative to paired benign prostatic stroma using the gene expression array analysis on laser-captured RNAs (Dakhova et al, 2009). Seven genes (*FOXA1*, *TGFBR2*, *DVL1*, *ERBB2*, *GSK3B*, *SMA4F*, and *GLI2*) were found to be up-regulated in tumor stroma, and two (*CAVI* and *TPM1*) were down-regulated. Gene ontology analysis of grade 3 reactive stroma revealed a significant increase in the level of expression for genes responsible for neurogenesis, axonogenesis, and the DNA damage/repair pathways, as well as evidence of increases in stem cells. Other studies have identified additional genes for paracrine-acting factors including the chemokines, *CCL5* and *CXCL13*, and growth factors, *IGF-1* and *FGF-2*, as being upregulated in tumor stromal cells, compared to adjacent epithelium, supporting the critical role that stromal cells can play in promoting growth and recruiting immune/inflammatory cells (Gregg et al, 2010).

Several studies have also characterized gene expression in CAFs compared with normal prostate fibroblasts (NAFs). Gene ontology analysis of the transcriptome of laser-capture microdissected stroma from matched normal and tumor foci showed that CAFs-enriched transcripts were associated with prostate morphogenesis and CAF-depleted transcripts were associated with cell cycle. Gene microarrays identified more than 100 genes differentially expressed between CAF and NAF (Joesting et al, 2005, Madar et al, 2009, Orr et al, 2012, Pascal et al, 2009, Richardson et al, 2007).

Epigenetic changes to CAF can permanently alter the expression of certain genes. It has been observed that there is a loss of TGF β receptor type II (TBR2) in stroma associated with human prostate cancer (Li et al, 2008). Experimental models have demonstrated that this can be a result of promoter hypermethylation that occurs in response to tumor cell expressed interleukin 6 (Banerjee et al, 2014), demonstrating that a cancer epithelia-derived paracrine-acting factor can mediate the loss of TGF- β signaling in the adjacent fibroblasts by silencing the TBR2 expression.

Origin of prostate cancer-associated fibroblasts

The precise origin of CAFs is still unclear (Figure 1), perhaps reflecting the diversity of cell populations and a still imprecise definition of what the CAF phenotype represents. Previous studies have suggested that CAFs could be derived from resident fibroblasts, fibroblasts precursors, or different cell types such as bone marrow-derived mesenchymal stem cells

(MSCs), preadipocytes, or smooth muscle cells (Shimoda et al, 2010). A number of hypothetical mechanisms by which the CAF phenotype evolves include standard connective tissue acute phase and stress responses, fibroblast senescence, reciprocal interactions with the cancer cells, differentiation of precursor cells, and infiltration of MSC (Haviv et al, 2009). A more thorough treatment of the subject of fibroblasts and their roles and heterogeneity in cancer can be found in recent reviews (Kalluri and Zeisberg, 2006, Öhlund et al, 2014).

Several studies have demonstrated that undefined factors secreted from tumors may recruit MSCs into solid tumor. TGF β could induce tumor invasion and differentiation of MSC into myofibroblasts. TGF β stimulates prostatic stromal cells to express a number of genes related to myofibroblastic differentiation and promotes reactive stroma formation and carcinoma growth (Verona et al, 2007). These results suggest that MSCs could be a candidate for the origin of a sub-population of cells within CAF. Another possible origin of prostate CAF is transdifferentiation of resident fibroblasts, potentially in response to factors secreted by tumor cells. Normal fibroblasts co-cultured with cancer cells can become activated and exhibit biochemical characteristics of CAF in a heterogeneous manner (Ishii et al, 2011). This supports the idea that heterogeneous induction of CAF-like differentiation might be dependent on characteristics of adjacent cancer cells. We previously showed in a tissue recombination model that the up-regulation of the cell cycle regulator cyclin D1 (CD1) in normal prostatic fibroblasts mimics aspects of the phenotype of malignant conversion seen in CAFs (He et al, 2007). Moreover, during tumor progression, preexisting stromal fibroblasts within the tumor increasingly acquire two autocrine signaling loops involving TGF β and SDF-1 that mediate transdifferentiation into a myofibroblast phenotype (Kojima et al, 2010). The reactive stromal response probably also reflects the genomic background of the specific patient, suggesting that genomic studies could be undertaken to assess whether there are predisposing variants that favor the formation of an exuberant stromagenic response adjacent to tumors.

***In vivo* modeling of stromal mediation of cancer progression**

In the tumor microenvironment, various chemokines and cytokines act as messengers in reciprocal tumor-stroma interactions. There are numerous signaling pathways acting between CAF and normal and tumor epithelial cells, the immune/inflammatory cells and the various other stromal components (blood vessels, nerves, fat, muscle, etc.) that could act to regulate carcinogenesis. CAF secrete elevated levels of a number of factors, including TGF β , EGF, FGF, HGF, IGF-1, interleukin (IL) 6, and CXCL12 (SDF-1) that might reasonably be expected to act in a paracrine fashion with the potential to promote tumorigenesis (Bhowmick et al, 2004a, Orimo et al, 2005, Osuala et al, 2015).

The two *in vivo* approaches that have been most widely used to address the functional significance of modulating paracrine signaling pathways in human prostate cancer have been the differential reactive stroma (DRS) prostate cancer xenograft model and tissue recombination (summarized in Table 1). The DRS model was developed to investigate the role of reactive stroma in prostate cancer progression. Using this model, Rowley's group showed that human TGF β 1 signaling mediated by prostate stromal cells can promote

angiogenesis and growth of LNCaP human prostate carcinoma cell tumors (Tuxhorn et al, 2002a). The group applied molecular approaches to allow the overexpression of specific proteins in given cell types, for example regulating the expression of WFDC1/ps20 in fibroblasts and showing resultant changes in angiogenesis (McAlhany et al, 2003). Angiogenesis is essential for tumor growth and metastasis. Although anti-vascular therapy has not yet reached its early promise, it might still be a potential treatment choice for specific targets, if appropriate drugs can be developed. We have demonstrated the potential of 'protective' prostate fibroblasts to supply blood and prevent acute tumor hypoxia (with associated evolution of more aggressive tumor phenotypes) after androgen deprivation therapy (Sasaki et al, 2016) suggesting that such approaches might be applied in a manner that at first sight seems counterintuitive.

The Rowley group also modified the DRS model using TBR2 null or dominant-negative Smad3 stromal cells which demonstrated a significant reduction in mass and microvessel density relative to controls and also suggested a role for TGF β stimulated stromal FGF-2 expression as a potential mediator of tumor growth (Yang et al, 2008). Links between TGF β signaling and other growth factors also emerged from studies examining connective tissue growth factor (CTGF). CTGF expression is elevated in tumor-promoting prostate stromal cells and TGF β 1 stimulated CTGF mRNA expression. Expression of CTGF in tumor-reactive stroma in the DRS model induced significant increases in microvessel density and xenograft tumor growth, suggesting that CTGF is a downstream mediator of TGF β 1 action in cancer-associated reactive stroma (Yang et al, 2005).

Tissue recombinants composed of adult human prostate epithelium derived from benign prostatic tissues and embryonic rat urogenital sinus mesenchyme (UGM), the natural inducer of prostatic growth and differentiation, demonstrated the importance of stromal-epithelial interactions in determining the development of prostate epithelium *in vivo* (Hayward et al, 1998, Jiang et al, 2010). These studies also established the ability of paracrine signals involved in prostatic development to be interpreted across human/rodent species barriers.

As described above, we demonstrated the ability of CAF to induce malignant transformation in human prostatic epithelial cells *in vivo* (Olumi et al, 1999). UGM has also been shown, in a rodent model, to be able to "normalize" malignant prostatic epithelium, resulting in enforced differentiation and loss of malignant potential (Hayashi and Cunha, 1991, Hayashi et al, 1990, Wong et al, 1992). To investigate the balance of paracrine mediated tumor induction vs. differentiation, UGM and CAF were mixed in five different ratios and recombined with initiated but non-tumorigenic BPH-1 cells *in vivo* (Franco et al, 2011). Although both rat UGM and CAF increased the size of grafts, BPH1 cells were differentiated with a benign phenotype when combined with 100% UGM, and became progressively less organized and more invasive when combined with increasing proportions of CAF. The gradual progression towards cancer with increasing proportions of CAF that is suggested by these data probably reflect the complex interplay between the various cells involved and suggests that there is not a single switch driving this mechanism but rather that the overall balance of the paracrine signaling environment is critical in determining the overall fate of the grafts.

Tissue recombination approaches have been used to examine specific stromal signaling pathways in tumorigenesis. These models, like the DRS approach, allow investigators to overexpress or suppress the expression of specific genes in a given tissue or in a sub-set of cells in a tissue to explore experimental endpoints.

This can be illustrated by studies examining the interplay of TGF β and SDF-1/CXCL12 signaling. Specific responses to TGF β are complex and context dependent, they include regulating cell proliferation, differentiation, extracellular matrix (ECM) production, apoptosis, and epithelial to mesenchymal transformation (EMT) (Bierie and Moses, 2006). CAF express elevated levels of TGF β 1 compared with normal fibroblasts. Non-tumorigenic BPH1 cells are growth inhibited by TGF β in culture. In contrast, the derivative tumorigenic BPH1^{CAFTD} sublines are not growth inhibited but instead undergo EMT in response to TGF β (Ao et al, 2006). In a tissue recombination model, however, expression of a dominant negative TBR2 in epithelial cells was able to ablate the tumor-promoting effects of CAF. This finding was mirrored using a blocking antibody to TGF β (Ao et al, 2007). This was a counterintuitive result, in that the removal of a growth inhibitory factor resulted in the loss of tumor-inducing activity.

CAF also express elevated levels of stromal cell derived factor-1 (SDF-1/CXCL12) (Ao et al, 2007, Orimo et al, 2005). To further evaluate the signaling mechanisms by which CAF induce tumorigenesis in BPH1 cells, we examined the expression of CXCR4, the cognate receptor for SDF-1. CXCR4 is expressed at low levels in benign prostate tissue and BPH1 cells. However, CXCR4 levels increase during tumor progression and in BPH1 cells treated by CAF-conditioned medium or with TGF β treatment. TGF β -regulated CXCR4 activated Akt in epithelial cells. Our data showed interactions of TGF β and SDF-1/CXCR4 pathways in tumorigenesis and tumor progression whereby CAF-expressed TGF β 1 induces the expression of CXCR4 in the epithelial cells allowing them to respond to SDF-1 (Ao et al, 2007). The TGF β /SDF1 axis has been a feature of tumor stroma in other systems too. In a mouse recombination model we were able to identify a more complex set of interactions additionally involving stromal heterogeneity (discussed below) and additional signaling molecules including IL-1 β and Wnt3a that were predicted by mathematical modeling summarized in Figure 2 (Kiskowski et al, 2011). In addition a human mammary fibroblast model was used to demonstrate the importance of TGF β and SDF-1 in the generation of the CAF phenotype (Kojima et al, 2010).

Androgen action in prostate cancer-associated fibroblasts

In general, androgen action mediated by 5 α -dihydrotestosterone is required for the survival and function of prostate epithelial cells. It has long been known that prostate development is dependent upon the action of androgens in the mesenchyme, while adult epithelial function utilizes epithelial AR (Cunha and Chung, 1981, Donjacour and Cunha, 1993, Sugimura et al, 1986). In the adult prostate, AR is expressed not only by epithelial cells but also stromal cells to greater or lesser degrees. AR signaling in stromal cells is important in prostate cancer, yet the mechanisms by which stromal AR contributes to disease development and progression remain unclear (Leach et al, 2015). Several clinical studies demonstrated that decreased stromal AR expression in tumor is associated with recurrence and progression

after radical prostatectomy (Ricciardelli et al, 2005, Wikstrom et al, 2009). In contrast, *in vivo* models of hormone-induced carcinogenesis suggested that loss of stromal AR prevented carcinogenesis, malignant transformation and metastasis (Ricke et al, 2012). It is possible that stromal AR is pro-tumorigenic in early stage disease, but prevents metastasis of evolving prostate cancer cells by altering the composition and permissiveness of the ECM (Leach et al, 2015). However, it is also possible that the artificially elevated levels of hormones present in models of hormonal carcinogenesis activate pathways that are not commonly seen in normal disease progression in patients and thus mask the potential protective effects of androgens. Recent mouse model studies suggest a possible role for stromal androgen receptor in maintaining stem cell marker expression in prostate cancer epithelium and suggest that AR signaling in CAF may negatively regulate cancer growth (Liao et al, 2017).

The actions of androgens and the interactions of the AR with specific DNA response elements and transcription factors to elicit gene expression has been predominantly studied in epithelial cells (DeGraff et al, 2014, Gao et al, 2005, Grabowska et al, 2016, Yu et al, 2005). While this work has provided some detailed insights into androgen action, notably mechanistic data on the expression of specific genes, it has not provided insight into whether such mechanisms are generalizable between tissues. Recent studies using CHIPseq validated by RNAseq show that the profile of genes regulated by androgens in UGM and human prostate CAF bears little resemblance to those seen in epithelium (as might be expected), and also that the consensus binding sites of AR to DNA in stromal cells are not the same as those used in epithelium (Nash et al, 2017). It seems likely that the profile of transcription factors interacting with the AR in stroma will be different from those found in epithelium, likely affecting the choice of specific binding sites, although these may contain members of the same families with substitutions appropriate to specific tissues.

Does cancer-associated fibroblast function reflect a discreet cell type or a collective phenotype derived from cellular interactions?

Prostate cancer epithelium is heterogeneous in terms of the specific mutations occurring and their distribution in tumors (Aihara et al, 1994, Ruijter et al, 1996). We recently showed, using a tissue recombination model of breast cancer, that such epithelial heterogeneity can contribute to tumor growth through paracrine interactions between epithelial populations (Franco et al, 2016). In human prostate tumors the stroma, like the epithelium, is heterogeneous with respect to expression of markers such as the TBR2 (Li et al, 2008).

CAF likely contain cells derived from a number of sources (see figure 1), these might include tissue resident fibroblasts, mesenchymal stem cells, fibrocytes and stellate cells, expressing a range of chemokines and cytokines and with a variety of different effects in promoting tumor growth and metastasis (Öhlund et al, 2014). Experimental mouse models have demonstrated extensive tumor stromal heterogeneity (Sugimoto et al, 2006), which tends to be masked when these cells are placed in culture. At present there are a number of markers that are commonly associated with CAF in various tumors, such as the expression of CD90, smooth muscle α -actin, and elevated TGF β and SDF-1 signaling (Ao et al, 2007,

Kojima et al, 2010, Mishra et al, 2008, Zhao and Peehl, 2009). However, the overall CAF phenotype is perhaps best defined functionally, and it is unclear that any of these markers actually defines CAF as such. Studies on fibroblast heterogeneity suggest that rather than being a specific population of cells, the functional activity of CAF is a product of the interactions of sub-populations of cells.

To test this idea we performed stromal mixing studies using human fibroblasts which expressed TBR2 (responded normally to TGF β), expressed a dominant negative receptor II (these cells could not respond to TGF β), or used a 50/50 mix of the two fibroblast strains. We demonstrated that the mixture of fibroblasts set up a system that resulted in the elevation of a number of growth promoting factors, and in a tissue recombination model we were able to recapitulate some aspects of CAF activity simply by deleting a single receptor function in a subgroup of stromal cells (Franco et al, 2011). Parallel studies using mouse stromal cells yielded results consistent with the human findings (Kiskowski et al, 2011). Of interest, neither the human or the mouse TBR2-deficient fibroblasts would promote tumorigenesis in adjacent epithelium. As previously noted, the TBR2^{flox/flox} FSP-cre mouse dies as a result of squamous cell carcinoma of the forestomach (Bhowmick et al, 2004b). While it was initially assumed that this genotype would have a near total loss of TBR2 in the stromal cells, reanalysis of the samples from that strain reveal that FSP-cre is relatively inefficient and results in the loss of receptor in around 50% of stromal cells, providing an observation that is consistent with the tissue recombination experiment.

Summary and future directions

The concept of stromal targeting as an approach to treat cancer first came to fame through the anti-angiogenic approaches developed by Dr. Judah Folkman. However stromal cells beyond the vasculature have also been seen as potential targets. Features of CAF, such as the expression of the membrane bound serine protease FAP- α have been used experimentally to target therapies to tumors. As a proof of principle of this approach the bee sting venom melittin was modified so that it could be injected in a harmless form and be locally activated at a tumor site (Lebeau et al, 2009). Further developing this approach Brennen et al. demonstrated that an FAP-activated prodrug can elicit antitumor efficacy based in a xenograft model (Brennen et al, 2012). Recombination models have been used to examine the effects of regulating specific mesenchymally expressed molecules. Dlk1 and SCUBE1, previously identified in a gene screening approach of cancer stroma and identified as potential growth suppressors were examined. CAF expressing these molecules resulted in reduced tumor size, epithelial growth and invasion compared to controls (Orr et al, 2013). These studies provide evidence that targeting CAF, independent of cancer cells, could be developed into a treatment choice for cancers that show a reactive stroma.

Summary

In vivo models that allow for interactions between human epithelial and stromal cells, and for the specific manipulation of gene expression in target cell types provide a powerful tool for understanding the interactions and action of CAF in driving tumorigenesis. They also provide a platform to identify novel therapeutic targets and to test potential therapies.

All models have strengths and weaknesses, the major weakness of systems using human cells *in vivo* is the loss functional immune/inflammatory cells that play a critical role in so many aspects of cancer progression. The benefit is allowing testing of preclinical models *in vivo*. We have focused here on insights derived from models using human cells interacting in mouse models. To develop a clear picture of the tumor environment requires the use of multiple complementary *in vivo* and *in vitro* models.

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References

- Aggarwal N, Santiago AM, Kessel D, Sloane BF. Photodynamic therapy as an effective therapeutic approach in MAME models of inflammatory breast cancer. *Breast cancer research and treatment*. 2015; 154:251–262. [PubMed: 26502410]
- Aihara M, Wheeler TM, Ohori M, Scardino PT. Heterogeneity of prostate cancer in radical prostatectomy specimens. *Urology*. 1994; 43:60–66. [PubMed: 8284886]
- Ao M, Williams K, Bhowmick NA, Hayward SW. Transforming growth factor-beta promotes invasion in tumorigenic but not in nontumorigenic human prostatic epithelial cells. *Cancer research*. 2006; 66:8007–8016. [PubMed: 16912176]
- Ao M, Franco OE, Park D, Raman D, Williams K, Hayward SW. Cross-talk between paracrine-acting cytokine and chemokine pathways promotes malignancy in benign human prostatic epithelium. *Cancer research*. 2007; 67:4244–4253. [PubMed: 17483336]
- Ayala G, Tuxhorn JA, Wheeler TM, Frolov A, Scardino PT, Ohori M, Wheeler M, Spitler J, Rowley DR. Reactive stroma as a predictor of biochemical-free recurrence in prostate cancer. *Clin Cancer Res*. 2003; 9:4792–4801. [PubMed: 14581350]
- Ayala GE, Muezzinoglu B, Hammerich KH, Frolov A, Liu H, Scardino PT, Li R, Sayeeduddin M, Ittmann MM, Kadmon D, Miles BJ, Wheeler TM, Rowley DR. Determining prostate cancer-specific death through quantification of stromogenic carcinoma area in prostatectomy specimens. *The American journal of pathology*. 2011; 178:79–87. [PubMed: 21224046]
- Bandari RP, Jiang Z, Reynolds TS, Bernskoetter NE, Szczodroski AF, Bassuner KJ, Kirkpatrick DL, Rold TL, Sieckman GL, Hoffman TJ, Connors JP, Smith CJ. Synthesis and biological evaluation of copper-64 radiolabeled [DUPA-6-Ahx-(NODAGA)-5-Ava-BBN(7-14)NH₂], a novel bivalent targeting vector having affinity for two distinct biomarkers (GRPr/PSMA) of prostate cancer. *Nuclear medicine and biology*. 2014; 41:355–363. [PubMed: 24508213]
- Banerjee J, Mishra R, Li X, Jackson RS 2nd, Sharma A, Bhowmick NA. A reciprocal role of prostate cancer on stromal DNA damage. *Oncogene*. 2014; 33:4924–4931. [PubMed: 24141771]
- Barcellos-Hoff MH, Ravani SA. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. *Cancer research*. 2000; 60:1254–1260. [PubMed: 10728684]
- Barclay WW, Woodruff RD, Hall MC, Cramer SD. A system for studying epithelial-stromal interactions reveals distinct inductive abilities of stromal cells from benign prostatic hyperplasia and prostate cancer. *Endocrinology*. 2005; 146:13–18. [PubMed: 15471963]
- Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature*. 2004a; 432:332–337. [PubMed: 15549095]
- Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science (New York, N Y)*. 2004b; 303:848–851.
- Bianchi-Frias D, Basom R, Delrow JJ, Coleman IM, Dakhova O, Qu X, Fang M, Franco OE, Ericson NG, Bielas JH, Hayward SW, True L, Morrissey C, Brown L, Bhowmick NA, Rowley D, Ittmann

- M, Nelson PS. Cells Comprising the Prostate Cancer Microenvironment Lack Recurrent Clonal Somatic Genomic Aberrations. *Mol Cancer Res.* 2016; 14:374–384. [PubMed: 26753621]
- Bierie B, Moses HL. Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nature reviews.* 2006; 6:506–520.
- Brennen WN, Rosen DM, Wang H, Isaacs JT, Denmeade SR. Targeting carcinoma-associated fibroblasts within the tumor stroma with a fibroblast activation protein-activated prodrug. *J Natl Cancer Inst.* 2012; 104:1320–1334. [PubMed: 22911669]
- Campbell I, Polyak K, Haviv I. Clonal mutations in the cancer-associated fibroblasts: the case against genetic coevolution. *Cancer research.* 2009; 69:6765–6768. discussion 6769. [PubMed: 19706773]
- Carlucci G, Ananias HJ, Yu Z, Hoving HD, Helfrich W, Dierckx RA, Liu S, de Jong IJ, Elsinga PH. Preclinical evaluation of a novel (1)(1)(1)In-labeled bombesin homodimer for improved imaging of GRPR-positive prostate cancer. *Molecular pharmaceutics.* 2013; 10:1716–1724. [PubMed: 23590837]
- Casavant BP, Berthier E, Theberge AB, Berthier J, Montanez-Sauri SI, Bischel LL, Brakke K, Hedman CJ, Bushman W, Keller NP, Beebe DJ. Suspended microfluidics. *Proceedings of the National Academy of Sciences of the United States of America.* 2013; 110:10111–10116. [PubMed: 23729815]
- Centenera MM, Raj GV, Knudsen KE, Tilley WD, Butler LM. Ex vivo culture of human prostate tissue and drug development. *Nature reviews Urology.* 2013; 10:483–487. [PubMed: 23752995]
- Centenera MM, Gillis JL, Hanson AR, Jindal S, Taylor RA, Risbridger GP, Sutherland PD, Scher HI, Raj GV, Knudsen KE, Yeadon T, Tilley WD, Butler LM. Australian Prostate Cancer B. Evidence for efficacy of new Hsp90 inhibitors revealed by ex vivo culture of human prostate tumors. *Clin Cancer Res.* 2012; 18:3562–3570. [PubMed: 22573351]
- Chambers KF, Mosaad EM, Russell PJ, Clements JA, Doran MR. 3D Cultures of prostate cancer cells cultured in a novel high-throughput culture platform are more resistant to chemotherapeutics compared to cells cultured in monolayer. *PLoS ONE.* 2014; 9:e111029. [PubMed: 25380249]
- Cunha GR. Tissue interactions between epithelium and mesenchyme of urogenital and integumental origin. *Anat Rec.* 1972; 172:529–541. [PubMed: 5011946]
- Cunha GR, Chung LW. Stromal-epithelial interactions--I. Induction of prostatic phenotype in urothelium of testicular feminized (Tfm/y) mice. *J Steroid Biochem.* 1981; 14:1317–1324. [PubMed: 6460136]
- Cunha GR, Hom YH. Role of mesenchymal-epithelial interactions in mammary gland development. *J Mammary Gland Biology and Neoplasia.* 1996; 1:21–35.
- Cunha, GR., Boutin, EL., Turner, T., Donjacour, AA. Role of mesenchyme in the development of the urogenital tract. In: Colborn, T., Clement, C., editors. *Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection.* Princeton Scientific Publishing Co., Inc; Princeton: 1992. p. 85-106.
- Cunha GR, Hayward SW, Dahiya R, Foster BA. Smooth muscle-epithelial interactions in normal and neoplastic prostatic development. *Acta Anat (Basel).* 1996; 155:63–72. [PubMed: 8811117]
- Dakhova O, Ozen M, Creighton CJ, Li R, Ayala G, Rowley D, Ittmann M. Global gene expression analysis of reactive stroma in prostate cancer. *Clin Cancer Res.* 2009; 15:3979–3989. [PubMed: 19509179]
- Dauca M, Bouziges F, Colin S, Kedingier M, Keller MK, Schilt J, Simon-Assmann P, Haffen K. Development of the vertebrate small intestine and mechanisms of cell differentiation. *The International journal of developmental biology.* 1990; 34:205–218. [PubMed: 2203458]
- DeGraff DJ, Grabowska MM, Case TC, Yu X, Herrick MK, Hayward WJ, Strand DW, Cates JM, Hayward SW, Gao N, Walter MA, Buttyan R, Yi Y, Kaestner KH, Matusik RJ. FOXA1 deletion in luminal epithelium causes prostatic hyperplasia and alteration of differentiated phenotype. *Laboratory investigation; a journal of technical methods and pathology.* 2014; 94:726–739. [PubMed: 24840332]
- Del Buono R, Fleming KA, Morey AL, Hall PA, Wright NA. A nude mouse xenograft model of fetal intestine development and differentiation. *Development (Cambridge, England).* 1992; 114:67–73.

- Dijkgraaf I, Franssen GM, McBride WJ, D'Souza CA, Laverman P, Smith CJ, Goldenberg DM, Oyen WJ, Boerman OC. PET of tumors expressing gastrin-releasing peptide receptor with an 18F-labeled bombesin analog. *J Nucl Med*. 2012; 53:947–952. [PubMed: 22570329]
- Donjacour AA, Cunha GR. Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgen-insensitive mice. *Endocrinology*. 1993; 132:2342–2350. [PubMed: 7684975]
- Foster BA, Cunha GR. Efficacy of various natural and synthetic androgens to induce ductal branching morphogenesis in the developing anterior rat prostate. *Endocrinology*. 1999; 140:318–328. [PubMed: 9886841]
- Franco OE, Tyson DR, Konvinse KC, Udyavar AR, Estrada L, Quaranta V, Crawford SE, Hayward SW. Altered TGF- α /beta signaling drives cooperation between breast cancer cell populations. *FASEB J*. 2016
- Franco OE, Jiang M, Strand DW, Peacock J, Fernandez S, Jackson RS 2nd, Revelo MP, Bhowmick NA, Hayward SW. Altered TGF- β signaling in a subpopulation of human stromal cells promotes prostatic carcinogenesis. *Cancer research*. 2011; 71:1272–1281. [PubMed: 21303979]
- Gao D, Vela I, Sboner A, Iaquinata PJ, Karthaus WR, Gopalan A, Dowling C, Wanjala JN, Undvall EA, Arora VK, Wongvipat J, Kossai M, Ramazanoglu S, Barboza LP, Di W, Cao Z, Zhang QF, Sirota I, Ran L, MacDonald TY, Beltran H, Mosquera JM, Touijer KA, Scardino PT, Laudone VP, Curtis KR, Rathkopf DE, Morris MJ, Danila DC, Slovin SF, Solomon SB, Eastham JA, Chi P, Carver B, Rubin MA, Scher HI, Clevers H, Sawyers CL, Chen Y. Organoid cultures derived from patients with advanced prostate cancer. *Cell*. 2014; 159:176–187. [PubMed: 25201530]
- Gao N, Ishii K, Mirosevich J, Kuwajima S, Oppenheimer SR, Roberts RL, Jiang M, Yu X, Shappell SB, Caprioli RM, Stoffel M, Hayward SW, Matusik RJ. Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. *Development (Cambridge, England)*. 2005; 132:3431–3443.
- Gleave M, Hsieh JT, Gao CA, von Eschenbach A, Chung LW. Acceleration of human prostate cancer growth in vivo by factors produced by prostate and bone fibroblasts. *Cancer research*. 1991; 51:3753–3761. [PubMed: 1712249]
- Golosow N, Grobstein C. Epithelio-mesenchymal interaction in pancreatic morphogenesis. *Develop Biol*. 1962; 1:242–255.
- Grabowska MM, Kelly SM, Reese AL, Cates JM, Case TC, Zhang J, DeGraff DJ, Strand DW, Miller NL, Clark PE, Hayward SW, Gronostajski RM, Anderson PD, Matusik RJ. Nfib Regulates Transcriptional Networks That Control the Development of Prostatic Hyperplasia. *Endocrinology*. 2016; 157:1094–1109. [PubMed: 26677878]
- Gregg JL, Brown KE, Mintz EM, Piontkivska H, Fraizer GC. Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection. *BMC Cancer*. 2010; 10:165. [PubMed: 20426842]
- Grobstein C. Epithelio-mesenchymal specificity in the morphogenesis of mouse submandibular rudiments in vitro. *J Exp Zool*. 1953; 124:383–444.
- Haffen, K., Keding, M., Simon-Assmann, PM., Lacroix, B. Mesenchyme-dependent differentiation of intestinal brush-border enzymes in the gizzard endoderm of the chick embryo. In: Weber, M., Burger, M., editors. *Embryonic Development, Part B: Cellular Aspects*. A. R. Liss; New York: 1982. p. 261-270.
- Haviv I, Polyak K, Qiu W, Hu M, Campbell I. Origin of carcinoma associated fibroblasts. *Cell Cycle*. 2009; 8:589–595. [PubMed: 19182519]
- Hayashi N, Cunha GR. Mesenchyme-induced changes in the neoplastic characteristics of the Dunning prostatic adenocarcinoma. *Cancer research*. 1991; 51:4924–4930. [PubMed: 1893381]
- Hayashi N, Cunha GR, Wong YC. Influence of male genital tract mesenchymes on differentiation of Dunning prostatic adenocarcinoma. *Cancer Res*. 1990; 50:4747–4754. [PubMed: 2369750]
- Hayward SW, Rosen MA, Cunha GR. Stromal-epithelial interactions in the normal and neoplastic prostate. *Br J Urol*. 1997; 79(Suppl 2):18–26. [PubMed: 9126066]
- Hayward SW, Del Buono R, Deshpande N, Hall PA. A functional model of adult human prostate epithelium. The role of androgens and stroma in architectural organisation and the maintenance of

differentiated secretory function. *Journal of cell science*. 1992; 102(Pt 2):361–372. [PubMed: 1400638]

Hayward SW, Haughney PC, Rosen MA, Greulich KM, Weier HU, Dahiya R, Cunha GR. Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. *Differentiation; research in biological diversity*. 1998; 63:131–140. [PubMed: 9697307]

He Y, Franco OE, Jiang M, Williams K, Love HD, Coleman IM, Nelson PS, Hayward SW. Tissue-specific consequences of cyclin D1 overexpression in prostate cancer progression. *Cancer research*. 2007; 67:8188–8197. [PubMed: 17804732]

Hill R, Song Y, Cardiff RD, Van Dyke T. Heterogeneous tumor evolution initiated by loss of pRb function in a preclinical prostate cancer model. *Cancer research*. 2005a; 65:10243–10254. [PubMed: 16288012]

Hill R, Song Y, Cardiff RD, Van Dyke T. Selective Evolution of Stromal Mesenchyme with p53 Loss in Response to Epithelial Tumorigenesis. *Cell*. 2005b; 123:1001–1011. [PubMed: 16360031]

Huang L, Pu Y, Alam S, Birch L, Prins GS. The role of Fgf10 signaling in branching morphogenesis and gene expression of the rat prostate gland: lobe-specific suppression by neonatal estrogens. *Dev Biol*. 2005; 278:396–414. [PubMed: 15680359]

Huang L, Pu Y, Hu WY, Birch L, Luccio-Camelo D, Yamaguchi T, Prins GS. The role of Wnt5a in prostate gland development. *Dev Biol*. 2009; 328:188–199. [PubMed: 19389372]

Ishii K, Mizokami A, Tsunoda T, Iguchi K, Kato M, Hori Y, Arima K, Namiki M, Sugimura Y. Heterogenous induction of carcinoma-associated fibroblast-like differentiation in normal human prostatic fibroblasts by co-culturing with prostate cancer cells. *J Cell Biochem*. 2011; 112:3604–3611. [PubMed: 21809373]

Ittmann M, Huang J, Radaelli E, Martin P, Signoretti S, Sullivan R, Simons BW, Ward JM, Robinson BD, Chu GC, Loda M, Thomas G, Borowsky A, Cardiff RD. Animal models of human prostate cancer: the consensus report of the New York meeting of the Mouse Models of Human Cancers Consortium Prostate Pathology Committee. *Cancer research*. 2013; 73:2718–2736. [PubMed: 23610450]

Jarred RA, Cancilla B, Prins GS, Thayer KA, Cunha GR, Risbridger GP. Evidence that estrogens directly alter androgen-regulated prostate development. *Endocrinology*. 2000; 141:3471–3477. [PubMed: 10965920]

Ji K, Heyza J, Cavallo-Medved D, Sloane BF. Pathomimetic cancer avatars for live-cell imaging of protease activity. *Biochimie*. 2016; 122:68–76. [PubMed: 26375517]

Jiang M, Strand DW, Fernandez S, He Y, Yi Y, Birbach A, Qiu Q, Schmid J, Tang DG, Hayward SW. Functional remodeling of benign human prostatic tissues in vivo by spontaneously immortalized progenitor and intermediate cells. *Stem Cells*. 2010; 28:344–356. [PubMed: 20020426]

Jin R, Yamashita H, Yu X, Wang J, Franco OE, Wang Y, Hayward SW, Matusik RJ. Inhibition of NF-kappa B signaling restores responsiveness of castrate-resistant prostate cancer cells to anti-androgen treatment by decreasing androgen receptor-variant expression. *Oncogene*. 2015; 34:3700–3710. [PubMed: 25220414]

Joesting MS, Perrin S, Elenbaas B, Fawell SE, Rubin JS, Franco OE, Hayward SW, Cunha GR, Marker PC. Identification of SFRP1 as a candidate mediator of stromal-to-epithelial signaling in prostate cancer. *Cancer research*. 2005; 65:10423–10430. [PubMed: 16288033]

Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nature reviews*. 2006; 6:392–401.

Kedinger M, Duluc I, Fritsch C, Lorentz O, Plateroti M, Freund JN. Intestinal epithelial-mesenchymal cell interactions. *Annals of the New York Academy of Sciences*. 1998; 859:1–17.

Kiskowski MA, Jackson RS 2nd, Banerjee J, Li X, Kang M, Iturregui JM, Franco OE, Hayward SW, Bhowmick NA. Role for stromal heterogeneity in prostate tumorigenesis. *Cancer research*. 2011; 71:3459–3470. [PubMed: 21444670]

Kojima Y, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-Porath I, Onder TT, Wang ZC, Richardson AL, Weinberg RA, Orimo A. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:20009–20014. [PubMed: 21041659]

- Kollar EJ. Induction of hair follicles by embryonic dermal papillae. *J Invest Dermatol.* 1970; 55:374–378. [PubMed: 5489079]
- Kollar EJ, Baird GR. Tissue interactions in embryonic mouse tooth germs. *J Embryol exp Morph.* 1970; 24:173–186. [PubMed: 5487155]
- Kollar EJ, Fisher C. Tooth induction in chick epithelium: Expression of quiescent genes for enamel synthesis. *Science (New York, N Y.)* 1980; 207:993–995.
- Lasnitzki I. Growth pattern of mouse prostate gland in organ culture and its response to sex hormones, vitamin A and 3-methylcholanthrene. *Natl Cancer Inst Monogr.* 1963; 12:381–403. [PubMed: 14073011]
- Lasnitzki, I. The prostate in organ culture. In: Brandes, D., editor. *Male Accessory Sex Organs: Structure and Function.* Academic Press; New York: 1974. p. 348–382.
- Lasnitzki, I. Human benign prostatic hyperplasia in cell and organ culture. Grayback, JT, Wilson, JD., Scherbenski, MJ., editors. 1976. p. 235–248. DHEW Publication no (N111) 76, 1113. 1075
- Leach DA, Need EF, Toivanen R, Trotta AP, Palethorpe HM, Tamblyn DJ, Kopsaftis T, England GM, Smith E, Drew PA, Pinnock CB, Lee P, Holst J, Risbridger GP, Chopra S, DeFranco DB, Taylor RA, Buchanan G. Stromal androgen receptor regulates the composition of the microenvironment to influence prostate cancer outcome. *Oncotarget.* 2015; 6:16135–16150. [PubMed: 25965833]
- Lebeau AM, Brennen WN, Aggarwal S, Denmeade SR. Targeting the cancer stroma with a fibroblast activation protein-activated promelittin protoxin. *Mol Cancer Ther.* 2009
- Li X, Placencio V, Iturregui JM, Uwamariya C, Sharif-Afshar AR, Koyama T, Hayward SW, Bhowmick NA. Prostate tumor progression is mediated by a paracrine TGF-beta/Wnt3a signaling axis. *Oncogene.* 2008; 27:7118–7130. [PubMed: 18724388]
- Liao CP, Chen LY, Luethy A, Kim Y, Kani K, MacLeod AR, Gross ME. Androgen receptor in cancer-associated fibroblasts influences stemness in cancer cells. *Endocrine-Related Cancer.* 2017; 24:157–170. [PubMed: 28264911]
- Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancers reveals genotypic heterogeneity. *Cancer research.* 1998; 58:23–28. [PubMed: 9426051]
- Madar S, Brosh R, Buganim Y, Ezra O, Goldstein I, Solomon H, Kogan I, Goldfinger N, Klocker H, Rotter V. Modulated expression of WFDC1 during carcinogenesis and cellular senescence. *Carcinogenesis.* 2009; 30:20–27. [PubMed: 18842679]
- McAlhany SJ, Ressler SJ, Larsen M, Tuxhorn JA, Yang F, Dang TD, Rowley DR. Promotion of angiogenesis by ps20 in the differential reactive stroma prostate cancer xenograft model. *Cancer research.* 2003; 63:5859–5865. [PubMed: 14522910]
- McKenney JK, Wei W, Hawley S, Auman H, Newcomb LF, Boyer HD, Fazli L, Simko J, Hurtado-Coll A, Troyer DA, Tretiakova MS, Vakar-Lopez F, Carroll PR, Cooperberg MR, Gleave ME, Lance RS, Lin DW, Nelson PS, Thompson IM, True LD, Feng Z, Brooks JD. Histologic Grading of Prostatic Adenocarcinoma Can Be Further Optimized: Analysis of the Relative Prognostic Strength of Individual Architectural Patterns in 1275 Patients From the Canary Retrospective Cohort. *The American journal of surgical pathology.* 2016; 40:1439–1456. [PubMed: 27635949]
- Mishra PJ, Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW, Banerjee D. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer research.* 2008; 68:4331–4339. [PubMed: 18519693]
- Nash C, Boufaied N, Mills IG, Franco OE, Hayward SW, Thomson AA. Genome-wide analysis of AR binding and comparison with transcript expression in primary human fetal prostate fibroblasts and cancer associated fibroblasts. *Molecular and cellular endocrinology.* 2017
- Öhlund D, Elyada E, Tuveson D. Fibroblast heterogeneity in the cancer wound. *Journal of Experimental Medicine.* 2014; 211:1503–1523. [PubMed: 25071162]
- Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer research.* 1999; 59:5002–5011. [PubMed: 10519415]
- Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal Fibroblasts Present in Invasive Human Breast Carcinomas Promote Tumor Growth and Angiogenesis through Elevated SDF-1/CXCL12 Secretion. *Cell.* 2005; 121:335–348. [PubMed: 15882617]

- Orr B, Riddick AC, Stewart GD, Anderson RA, Franco OE, Hayward SW, Thomson AA. Identification of stromally expressed molecules in the prostate by tag-profiling of cancer-associated fibroblasts, normal fibroblasts and fetal prostate. *Oncogene*. 2012; 31:1130–1142. [PubMed: 21804603]
- Orr B, Grace OC, Brown P, Riddick AC, Stewart GD, Franco OE, Hayward SW, Thomson AA. Reduction of pro-tumorigenic activity of human prostate cancer-associated fibroblasts using Dlk1 or SCUBE1. *Dis Model Mech*. 2013; 6:530–536. [PubMed: 23136397]
- Osuala KO, Sameni M, Shah S, Aggarwal N, Simonait ML, Franco OE, Hong Y, Hayward SW, Behbod F, Mattingly RR, Sloane BF. Il-6 signaling between ductal carcinoma in situ cells and carcinoma-associated fibroblasts mediates tumor cell growth and migration. *BMC Cancer*. 2015; 15:584. [PubMed: 26268945]
- Pascal LE, Goo YA, Vencio RZ, Page LS, Chambers AA, Liebeskind ES, Takayama TK, True LD, Liu AY. Gene expression down-regulation in CD90+ prostate tumor-associated stromal cells involves potential organ-specific genes. *BMC Cancer*. 2009; 9:317. [PubMed: 19737398]
- Qiu W, Hu M, Sridhar A, Opeskin K, Fox S, Shipitsin M, Trivett M, Thompson ER, Ramakrishna M, Gorringer KL, Polyak K, Haviv I, Campbell IG. No evidence of clonal somatic genetic alterations in cancer-associated fibroblasts from human breast and ovarian carcinomas. *Nature genetics*. 2008; 40:650–655. [PubMed: 18408720]
- Ricciardelli C, Choong CS, Buchanan G, Vivekanandan S, Neufing P, Stahl J, Marshall VR, Horsfall DJ, Tilley WD. Androgen receptor levels in prostate cancer epithelial and peritumoral stromal cells identify non-organ confined disease. *Prostate*. 2005; 63:19–28. [PubMed: 15378523]
- Richardson AM, Woodson K, Wang Y, Rodriguez-Canales J, Erickson HS, Tangrea MA, Novakovic K, Gonzalez S, Velasco A, Kawasaki ES, Emmert-Buck MR, Chuaqui RF, Player A. Global expression analysis of prostate cancer-associated stroma and epithelia. *Diagn Mol Pathol*. 2007; 16:189–197. [PubMed: 18043281]
- Ricke EA, Williams K, Lee YF, Couto S, Wang Y, Hayward SW, Cunha GR, Ricke WA. Androgen hormone action in prostatic carcinogenesis: stromal androgen receptors mediate prostate cancer progression, malignant transformation and metastasis. *Carcinogenesis*. 2012; 33:1391–1398. [PubMed: 22535887]
- Ruijter ET, van de Kaa CA, Schalken JA, Debruyne FM, Ruiter DJ. Histological grade heterogeneity in multifocal prostate cancer. Biological and clinical implications. *J Pathol*. 1996; 180:295–299. [PubMed: 8958808]
- Saeter T, Vlatkovic L, Waaler G, Servoll E, Nesland JM, Axcrona K, Axcrona U. The prognostic value of reactive stroma on prostate needle biopsy: a population-based study. *Prostate*. 2015; 75:662–671. [PubMed: 25620661]
- Saeter T, Vlatkovic L, Waaler G, Servoll E, Nesland JM, Axcrona K, Axcrona U. Combining lymphovascular invasion with reactive stromal grade predicts prostate cancer mortality. *Prostate*. 2016; 76:1088–1094. [PubMed: 27271973]
- Sameni M, Anbalagan A, Olive MB, Moin K, Mattingly RR, Sloane BF. MAME models for 4D live-cell imaging of tumor: microenvironment interactions that impact malignant progression. *J Vis Exp*. 2012
- Sameni M, Tovar EA, Essenburg CJ, Chalasani A, Linklater ES, Borgman A, Cherba DM, Anbalagan A, Winn ME, Graveel CR, Sloane BF. Cabozantinib (XL184) Inhibits Growth and Invasion of Preclinical TNBC Models. *Clin Cancer Res*. 2016; 22:923–934. [PubMed: 26432786]
- Sameni M, Cavallo-Medved D, Franco OE, Chalasani A, Ji K, Aggarwal N, Anbalagan A, Chen X, Mattingly RR, Hayward SW, Sloane BF. Pathomimetic avatars reveal divergent roles of microenvironment in invasive transition of ductal carcinoma in situ. *Breast Cancer Res*. 2017; 19:56. [PubMed: 28506312]
- Sasaki T, Ishii K, Iwamoto Y, Kato M, Miki M, Kanda H, Arima K, Shiraishi T, Sugimura Y. Fibroblasts prolong serum prostate-specific antigen decline after androgen deprivation therapy in prostate cancer. *Lab Invest*. 2016; 96:338–349. [PubMed: 26641067]
- Schor SL, Schor AM, Rushton G, Smith L. Adult, foetal and transformed fibroblasts display different migratory phenotypes on collagen gels: evidence for an isoformic transition during foetal development. *J Cell Sci*. 1985; 73:221–234. [PubMed: 4019593]

- Schor SL, Schor AM, Grey AM, Rushton G. Foetal and cancer patient fibroblasts produce an autocrine migration-stimulating factor not made by normal adult cells. *J Cell Sci.* 1988; 90:391–399. [PubMed: 3253289]
- Schor, SL., Grey, AM., Picardo, M., Schor, AM., Howell, A., Ellis, I., Rushton, G. Heterogeneity amongst fibroblasts in the production of migration stimulating factor (MSF): Implications for cancer pathogenesis. In: Goldberg, ID., editor. *Cell motility factors*. Berkhäuser Verlag; Basel: 1991. p. 127-146.
- Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R, Ward JM, Cardiff RD. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer research.* 2004; 64:2270–2305. [PubMed: 15026373]
- Shimoda M, Mellody KT, Orimo A. Carcinoma-associated fibroblasts are a rate-limiting determinant for tumour progression. *Semin Cell Dev Biol.* 2010; 21:19–25. [PubMed: 19857592]
- Sugimoto H, Mundel TM, Kieran MW, Kalluri R. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer biology & therapy.* 2006; 5:1640–1646. [PubMed: 17106243]
- Sugimura Y, Cunha GR, Bigsby RM. Androgenic induction of deoxyribonucleic acid synthesis in prostatic glands induced in the urothelium of testicular feminized (Tfm/y) mice. *Prostate.* 1986; 9:217–225. [PubMed: 2946028]
- Swonger JM, Liu JS, Ivey MJ, Tallquist MD. Genetic tools for identifying and manipulating fibroblasts in the mouse. *Differentiation; research in biological diversity.* 2016; 92:66–83. [PubMed: 27342817]
- Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nature reviews Molecular cell biology.* 2002; 3:349–363. [PubMed: 11988769]
- Tsuji M, Shima H, Boutin E, Young P, Cunha GR. Effect of mesenchymal glandular inductors on the growth and cytodifferentiation of neonatal mouse seminal vesicle epithelium. *J Androl.* 1994; 15:565–574. [PubMed: 7721659]
- Tuxhorn JA, Ayala GE, Rowley DR. Reactive stroma in prostate cancer progression. *The Journal of urology.* 2001; 166:2472–2483. [PubMed: 11696814]
- Tuxhorn JA, McAlhany SJ, Yang F, Dang TD, Rowley DR. Inhibition of transforming growth factor-beta activity decreases angiogenesis in a human prostate cancer-reactive stroma xenograft model. *Cancer research.* 2002a; 62:6021–6025. [PubMed: 12414622]
- Tuxhorn JA, McAlhany SJ, Dang TD, Ayala GE, Rowley DR. Stromal cells promote angiogenesis and growth of human prostate tumors in a differential reactive stroma (DRS) xenograft model. *Cancer research.* 2002b; 62:3298–3307. [PubMed: 12036948]
- Tuxhorn JA, Ayala GE, Smith MJ, Smith VC, Dang TD, Rowley DR. Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res.* 2002c; 8:2912–2923. [PubMed: 12231536]
- Verona EV, Elkahlon AG, Yang J, Bandyopadhyay A, Yeh IT, Sun LZ. Transforming growth factor-beta signaling in prostate stromal cells supports prostate carcinoma growth by up-regulating stromal genes related to tissue remodeling. *Cancer Res.* 2007; 67:5737–5746. [PubMed: 17575140]
- Wang Y, Revelo MP, Sudilovsky D, Cao M, Chen WG, Goetz L, Xue H, Sadar M, Shappell SB, Cunha GR, Hayward SW. Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. *Prostate.* 2005a; 64:149–159. [PubMed: 15678503]
- Wang Y, Xue H, Cutz JC, Bayani J, Mawji NR, Chen WG, Goetz LJ, Hayward SW, Sadar MD, Gilks CB, Gout PW, Squire JA, Cunha GR, Wang YZ. An orthotopic metastatic prostate cancer model in SCID mice via grafting of a transplantable human prostate tumor line. *Laboratory investigation; a journal of technical methods and pathology.* 2005b; 85:1392–1404. [PubMed: 16155594]
- Wikstrom P, Marusic J, Stattin P, Bergh A. Low stroma androgen receptor level in normal and tumor prostate tissue is related to poor outcome in prostate cancer patients. *Prostate.* 2009; 69:799–809. [PubMed: 19189305]

- Windus LC, Kiss DL, Glover T, Avery VM. In vivo biomarker expression patterns are preserved in 3D cultures of Prostate Cancer. *Experimental cell research*. 2012; 318:2507–2519. [PubMed: 22841689]
- Wong YC, Cunha GR, Hayashi N. Effects of mesenchyme of embryonic urogenital sinus and neonatal seminal vesicle on the cytodifferentiation of the Dunning tumor: Ultrastructural study. *Acta Anat*. 1992; 143:139–150. [PubMed: 1598820]
- Yanagisawa N, Li R, Rowley D, Liu H, Kadmon D, Miles BJ, Wheeler TM, Ayala GE. Stromogenic prostatic carcinoma pattern (carcinomas with reactive stromal grade 3) in needle biopsies predicts biochemical recurrence-free survival in patients after radical prostatectomy. *Human pathology*. 2007; 38:1611–1620. [PubMed: 17868773]
- Yanagisawa N, Li R, Rowley D, Liu H, Kadmon D, Miles BJ, Wheeler TM, Ayala GE. Reprint of: Stromogenic prostatic carcinoma pattern (carcinomas with reactive stromal grade 3) in needle biopsies predicts biochemical recurrence-free survival in patients after radical prostatectomy. *Human pathology*. 2008; 39:282–291. [PubMed: 18206496]
- Yang F, Strand DW, Rowley DR. Fibroblast growth factor-2 mediates transforming growth factor-beta action in prostate cancer reactive stroma. *Oncogene*. 2008; 27:450–459. [PubMed: 17637743]
- Yang F, Tuxhorn JA, Ressler SJ, McAlhany SJ, Dang TD, Rowley DR. Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. *Cancer research*. 2005; 65:8887–8895. [PubMed: 16204060]
- Yu X, Gupta A, Wang Y, Suzuki K, Mirosevich J, Orgebin-Crist MC, Matusik RJ. Foxa1 and foxa2 interact with the androgen receptor to regulate prostate and epididymal genes differentially. *Annals of the New York Academy of Sciences*. 2005; 1061:77–93. [PubMed: 16467259]
- Zhao H, Peehl DM. Tumor-promoting phenotype of CD90hi prostate cancer-associated fibroblasts. *Prostate*. 2009; 69:991–1000. [PubMed: 19267366]

Figure 1. Possible origins of carcinoma associated fibroblasts

CAF are heterogeneous and may arise from a number of sources, or combinations of sources. These include phenotypic alterations of resident fibroblasts, mesenchymal transformation of endothelium, migration of pericytes, and the incorporation of stromal cells from the bone marrow. Specific therapies aimed at treating cancer such as the use of cytotoxic chemotherapy and irradiation could also induce a secretory senescent phenotype in fibroblastic cells contributing to a pro-proliferative microenvironment. The role of epithelial to mesenchymal transformation of tumor epithelium to contribute to CAF has been considered but has not been found in the tumors that have been examined closely. The complex mixture of signaling molecules acting both within the tumor stroma and on the tumor epithelium can contribute to migration, invasion and metastasis of the tumor.

Figure 2. Heterogeneity between carcinoma-associated fibroblasts helps to drive cooperation between signaling pathways to enhance tumorigenesis

Consensus figure showing some of the paracrine interactions occurring within the tumor stroma and between the stromal and epithelial cells. In this example, stromal heterogeneity due to loss of TGF receptor 2 activity in a subpopulation of the CAF leads to local increases in TGFβ and SDF1 expression. IL-1β activity is restricted to the stromal cells in this model, while Wnt3a and TGFβ can act directly on the epithelium. One of the consequences of TGFβ action on the epithelium is the expression of CXCR4 (the receptor for SDF1) allowing the SDF1 to affect epithelial cell proliferation. Based upon data from (Ao et al, 2007, Banerjee et al, 2014, Kiskowski et al, 2011).

Table 1

Comparison of the characteristics of the differential reactive stroma (DRS) and tissue recombination models.

Xenografts	Differential Reactive Stroma (DRS)	Tissue Recombination
Purpose	Reactive stroma/tumor interactions in cancer progression	Stromal-epithelia interactions in development, benign and malignant disease
Cells and tissues	Epithelium: Human prostate cancer cells (LNCaP) Stroma: Human prostate stromal cell lines	Epithelium: A range of human or rodent tissues or cultured cells Stroma: A range of human or rodent tissues or cultured cells
Graft preparation	Cells suspended in Matrigel	Tissues can be directly recombined before grafting, cells suspended type I collagen
Graft Sites	Subcutaneous	Orthotopic Sub-renal capsule
Advantages	High take rate, tumor homogeneity, and early angiogenesis Technically simple procedure Genetic manipulation of stromal and/or epithelial cells can be performed before grafting	High take rate at renal capsule site most rapid site for angiogenesis Orthotopic grafting appropriate for metastasis studies Genetic manipulation of stromal and/or epithelial cells can be performed before grafting
Disadvantages	Data must be carefully validated, not necessarily applicable to human patients Difficult to separate the local influences of individual cell groups from the systemic effects Needs immunocompromised host Lack of metastasis	Data must be carefully validated, not necessarily applicable to human patients Difficult to separate the local influences of individual cell groups from the systemic effects Needs immunocompromised host for any human cell-based studies Needs significant training to acquire competence at prostatic orthotopic and renal capsule graft sites