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Are Times a' Changin' in Carcinogenesis?

For the most part, the introductory sentences in many research articles and reviews attempting to explain carcinogenesis do not deviate much from the following narrative ". . . It is generally acknowledged that gene mutations are at the core of the carcinogenic process. . . ". Multiple references usually follow this assertion, aimed at buttressing the role of mutations as the direct cause of so-called sporadic cancers, *i.e.* those responsible for about 95% of human cancers. A bibliographical search of these suggested references perpetuate the introductory statement with equally axiomatic assurances. This routine has been exercised for over four decades now, and we suspect that comparable statements will still be printed long after the publication of this current commentary. However, it seems as though new ideas that challenge the main underlying premise of the somatic mutation theory (SMT) are emerging. For the most part, they have asked the following questions: Which are these mutations? How consistently has their appearance been responsible for cancers? When do they take place, and how are they related to the conversion of previously normal tissues to cancerous ones? Unequivocal answers to these sensible inquiries have not been readily forthcoming (1). In fact, increasing numbers of skeptics are voicing their doubts about the value of the SMT (2–6).

Two main forces fuel this revisionist process: the first and more obvious is that, despite the aggressive effort by laboratories around the world to vindicate the SMT and its ancient and modern variations (aneuploidy, oncogene/repressor gene balance, *etc.*), the bill of goods promised after the dawn of the molecular biology revolution has not been delivered (1, 7). The second force is the increasing accumulation of data emphasizing the role of tissue interactions in carcinogenesis (8–10). This almost imperceptible switch of targets from a cell- to a tissue-based etiopathogenesis does not fit easily into the expectations anticipated by the SMT.

Little by little, for the last two decades, pragmatic tissue-based models have been incorporated into the mix of options to explain both carcinogenesis and metastasis. At times tacitly (11), at other times overtly (12), the preeminence of the oncogene/repressor gene theme (the latest incarnation of the SMT) in explaining carcinogenesis has been challenged on a variety of accounts (2). However, most of these tissue-related concepts were introduced using the narrative of a course correction to the prevalent SMT rather than an overt rejection. In addition to mutations, the final outcome in the cancer phenotype had to accommodate the role of stroma-epithelial tissue interactions. An increasing number of researchers are currently favoring this hybrid theory of carcinogenesis that incorporates elements of the original SMT ("mutations are the cause of cancer") and a role of stroma/epithelial interactions on this process ("context counts") (13). The latter has also been dubbed the epigenetic theory of carcinogenesis, implying that tissue-based phenomena are due to epigenetic gene expression modifications.

In the last few years, theoretical alternatives to the SMT have been postulated, suggesting that carcinogenesis should be considered a problem akin to normal histogenesis and tissue repair, involving the three-dimensional organization of tissues (10, 14). Equally important to this novel approach has been the notion that *proliferation* is the default state of all cells (15, 16). This notion is diametrically opposed to the premise adopted by those who favor the SMT that *quiescence* is the default state of cells in multicellular organisms. This alternative theory incorporating tissues as the target of carcinogens and proliferation as the default state of all cells has coalesced under the name of tissue organization field theory of carcinogenesis and metastasis (TOFT).

Now, in this issue, Barclay *et al.* (17) provide compelling evidence in favor of the TOFT. According to the experimental protocol, it is largely the stroma component of the stroma/epithelial couple, which is responsible for the neoplastic phenotype. Objectively, tissue recombinants involving human prostate cancer-derived fibroblasts and a normal human prostate epithelium cell line derived from a benign prostate hypertrophy (BPH-1) specimen develop as invasive carcinomas when grafted under the kidney capsule of immunocompromised, nude mice. In contrast, recombination of normal stroma or benign prostate hyperplasia stroma with the same epithelial cells does not generate a cancer phenotype. This strengthens the argument that carcinogenesis as well as metastasis is generated by influences of a "modified" stroma over epithelial cells regardless of whether they showed a normal or a cancer phenotype as defined by observations gathered through a light microscope. Also, observations by Barclay *et al.* suggest that the cancer phenotype is reversible provided that the intervening stroma belonged to "normal" or benign prostate hypertrophy sources.

Of course, supporters of the SMT may still argue that the fibroblasts in the stroma of cancers may have accumulated mutations that affect the normal functions of these cells and, consequently, normal epithelial cells would be influenced by the mutated stroma cells and behave in a pattern comparable to that of the genuine cancer epithelial cells (5). As mentioned above, for over four decades, the search for those single or multiple somatic mutations in the epithelial cell that would eventually become endowed with the cancer phenotype has remained elusive. The historical record highlights the fact that, regardless of their worthiness, established paradigms are seldom easily replaced. This is due in part to intellectual inertia and, in this particular case, because of powerful methodologies available to study this most stable of molecules, DNA, in which mutations are supposed to take place. Hence,

Abbreviations: SMT, Somatic mutation theory; TOFT, tissue organization field theory of carcinogenesis and metastasis.

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the search for those mutations may resume in this complex population of stroma cells in yet another *ad hoc* alternative before discarding the SMT. Let us hope that this option will not delay the alternative: to address carcinogenesis and metastasis in a way comparable to how developmental biologists have addressed the study of histogenesis and organogenesis.

It would still be fair to ask. . . Will the identification of the stroma as the culprit in carcinogenesis and/or metastatic development make it easier to pinpoint how cancer, in this case, prostate cancer, evolves? Stroma, as is the case with many other collective nouns, represents a good number of cellular types (fibroblasts, endothelial cells, smooth muscle cells, macrophages, mast cells, and a number of cells that traverse the territory through the blood and the lymph) in addition to extracellular matrix components. Moreover, dynamic changes take place among the above-listed cellular components and the matrix they secrete. Thus, further research on the role played by the stroma in carcinogenesis will have to expand further to eventually clarify which and how these cellular and extracellular cellular components interact to generate the cancer phenotype. A realistic assessment of our current understanding of the cancer puzzle would have to acknowledge that we are all at the beginning of a long journey to untangle the complex interactions among these protagonists. This can be interpreted as the glass half-empty/half-full metaphor. The half-full, optimistic counterpart of the metaphor is the identification of carcinogenesis as a problem of tissue organization and the central role of the stroma in this process. This is as if the proverbial lamppost has been successfully moved to the place where the keys were lost. The half-empty counterpart is that the variables to be tested are multiple and they probably interact simultaneously. A productive exploration of such a complex subject will need a steady support of adequate resources, a realistic management of the hype that has surrounded the cancer field, and a humble attitude toward the years spent following false leads. By facing the data that this and other equally significant contributions in this field have made, it is now becoming evident how much more complex the task is than was thought not long ago.

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A System for Studying Epithelial-Stromal Interactions Reveals Distinct Inductive Abilities of Stromal Cells from Benign Prostatic Hyperplasia and Prostate Cancer

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The development of normal and abnormal glandular structures in the prostate is controlled at the endocrine and paracrine levels by reciprocal interactions between epithelium and stroma. To study these processes, it is useful to have an efficient method of tissue acquisition for reproducible isolation of cells from defined histologies. Here we assessed the utility of a standardized system for acquisition and growth of prostatic cells from different regions of the prostate with different pathologies, and we compared the abilities of stromal cells from normal peripheral zone, benign prostatic hyperplasia (BPH-S), and cancer to induce the growth of a human prostatic epithelial cell line (BPH-1) *in vivo*. Using the tissue recombination method, we showed that grafting stromal cells (from any histology) alone or BPH-1 epithelial cells alone produced no visible grafts. Recombining stromal cells from normal peripheral zone with BPH-1 cells also produced no visible grafts (n = 15). Recombining BPH-S with BPH-1 cells generated small, well-organized, and sharply demarcated grafts approximately 3–4 mm in diameter (n = 9), demonstrating a moderate inductive ability of BPH-S. Recombining stromal cells from cancer with BPH-1 cells generated highly disorganized grafts that completely surrounded the host kidney and invaded into adjacent renal tissue, demonstrating induction of an aggressive phenotype. We conclude that acquisition of tissue from toluidine blue dye-stained specimens is an efficient method to generate high-quality epithelial and/or stromal cultures. Stromal cells derived by this method from areas of BPH and cancer induce epithelial cell growth *in vivo*, which mimics the natural history of these diseases. (*Endocrinology* 146: 13–18, 2005)

PRIMARY CULTURE OF prostatic cells from surgical specimens is a valuable *in vitro* model that can offer insight into normal prostatic growth regulation and the changes that occur during disease. However, complex endocrine and/or paracrine interactions between epithelium and stroma that may be important for prostatic disease such as prostate cancer or benign prostatic hyperplasia (BPH) are not easily studied *in vitro*.

Gerald R. Cunha (1–6) has developed tissue reconstitution methods for urogenital-derived tissues that allow for evaluating the roles of epithelial-stromal interactions in glandular morphogenesis and tumorigenesis. These landmark experiments over the last several decades have driven our understanding of the complexity of phenomena that contribute to the genetic and epigenetic control of tissue structure and function. Of particular note is the development by Cunha and colleagues (7) of a model of human prostate cancer development that relies on epithelial-stromal interactions. This model system uses the human prostatic epithelial cell line (BPH-1), which was originally derived from benign prostatic tissue after Simian virus 40 large T antigen immortalization of primary cells. These cells represent an initiated prostatic cell. Alone they are not tumorigenic when xenografted sc or under the renal capsule of nude mice. However, when the cells are recombined with tumor-associated prostatic stromal cells and grafted under the renal capsules of nude mice, the BPH-1 cells form invasive tumors. In contrast, normal prostatic stromal cells do not stimulate BPH-1 tumorigenesis. These elegant experiments highlight the intimate contribution of the stromal microenvironment to prostate tumorigenesis. BPH is a nonmalignant disease of the prostate that may be the result of disruption of normal adult epithelial-stromal signaling. The effects of BPH-derived prostatic stromal cells on the growth of BPH-1 grafts have not been previously investigated.

A major limitation to use of the prostate recombination model described above is the availability of stromal cells from histologically distinct regions of the prostate with well-defined pathology. Peehl and colleagues (8, 9) developed the best-characterized culture system for the isolation and maintenance of human prostatic cells. In their method, a small sample of tissue is dissected from the prostatectomy specimen under aseptic conditions. The area of the prostate from which the specimen is removed is inked and fixed for subsequent pathological evaluation.

The advantage of the system described by Peehl *et al.* (10) is the ability to isolate cells from the various regions of the prostate (peripheral zone, central zone, or transition zone) and specific histological phenotypes (normal, BPH, or cancer).

The major disadvantage of the methods developed by Peehl and colleagues is that the initial identification of tumor in the fresh tissue relies on palpation of the prostate to identify nodules. The pathology of the sample is not known until

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Abbreviations: BPH, Benign prostatic hyperplasia; BPH-1, human prostatic epithelial cell line; CA, cancer; PZ, peripheral zone.

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considerable cell culture work has been performed to grow and bank the cells. In the era of prostate-specific antigen screening, palpable nodules of sufficient size are increasingly rare in many clinical practices. In addition, the exact margins of the tumor are not readily visible by gross examination. Fischer *et al.* (11) described a method of tissue acquisition in which cross-sections of the prostate are stained with toluidine blue dye. Toluidine blue dye stains nuclei dark blue and allows for the immediate visual examination of glandular morphology using a dissecting microscope. In that report they described the utility of this system for epithelial cell acquisition. However, the utility of this system for stromal cell acquisition and studying epithelial-stromal interactions has not been explored.

Materials and Methods

Sample acquisition

All prostatectomies were performed by urologists in the urology department at Wake Forest University School of Medicine. All patients gave informed consent for use of surgical specimens for research. Use of surgical specimens for tissue culture was approved by the Institutional Review Board of Wake Forest University School of Medicine. The procedure used for sample acquisition was an adaptation of the procedure described by Fischer *et al.* (11) (Fig. 1A). A 3- to 5-mm cross-section was cut in the horizontal plane of the prostate. The sample was then stained with sterile 0.5% aqueous toluidine blue. Inspection of glandular structure was performed using an SMZ 1500 stereo dissecting scope (Nikon, Tokyo, Japan) connected to a Nikon DXM1200 digital camera. A sterile cork borer (no. 1, 2, or 3, depending on the size of the tumor) was used to remove a plug from the area of interest. Each hole in a prostate cross-section was inked with a different color dye. These areas were incorporated into the routine processing of the prostatectomy for pathological analyses. The plug that was removed for culture was processed by our normal primary culture technique (see below) except that a longitudinal slice was removed for histologic examination. This slice was fixed, embedded in paraffin, and stained with hematoxylin and eosin.

Histochemistry and pathology

All histology including fixation, embedding, sectioning, staining, and cytokeratin 903 immunohistochemistry of prostatectomy samples and longitudinal sections of plugs was performed by the pathology department's clinical laboratory using routine procedures. Blocks that incorporated the stained hole for each sample taken were made. In addition, blocks containing the longitudinal slice of plugs were prepared. Samples were classified as cancer if the hole and longitudinal section from which it was derived was surrounded by cancer glands in 90% of the area. Cork-borer samples were classified as mixed if the hole (and longitudinal section) from which it was derived was surrounded by a mixture of cancer and benign glands, in which the proportion of cancer glands was less than 90% of the total. Samples were classified as benign if there were no cancer glands surrounding the hole or in the longitudinal section.

Isolation and maintenance of prostatic cells

Prostatic epithelial cells were isolated using minor modifications of the protocol of Peehl (12, 13) as previously published by us (14, 15). Stromal cells were isolated by methods previously described (16). A schematic of these procedures is depicted in Fig. 1B. Nomenclature for epithelial cell strains is WFU followed by the strain number (in serial order of acquisition) and then the histology of origin (PZ, peripheral zone; BPH; CA, cancer) (*e.g.* WFU9CA). Stromal cell strains have an additional S at the end (*e.g.* WFU9CA-S).

Prostatic tissue recombinants and renal grafting

All use of animals was in accordance with policies and guidelines from the Animal Care and Use Committee of Wake Forest University School of Medicine. BPH-1 cells were obtained from Dr. Cunha. Stromal cells were cultured from fresh prostatic tissue as described above. To prepare tissue recombinants, semiconfluent stromal cells (PZ-S, BPH-S, or CA-S) were mixed with BPH-1 cells at a ratio of 250,000 stromal cells to 100,000 BPH-1 cells in a sterile microcentrifuge tube in 1 ml HEPES-buffered saline. The cell mixture was pelleted and resuspended in 50 μl of rat-tail collagen (pretitrated to pH 7.4) and inoculated in the center of a well of a multiwell plate. After collagen polymerization the button was overlaid with normal growth medium (RPMI 1640 plus 5% fetal bovine serum). Sixteen to 24 h later the tissue recombinant button was grafted under the renal capsule of a nude mouse using published protocols from Dr. Cunha's laboratory (1–6).

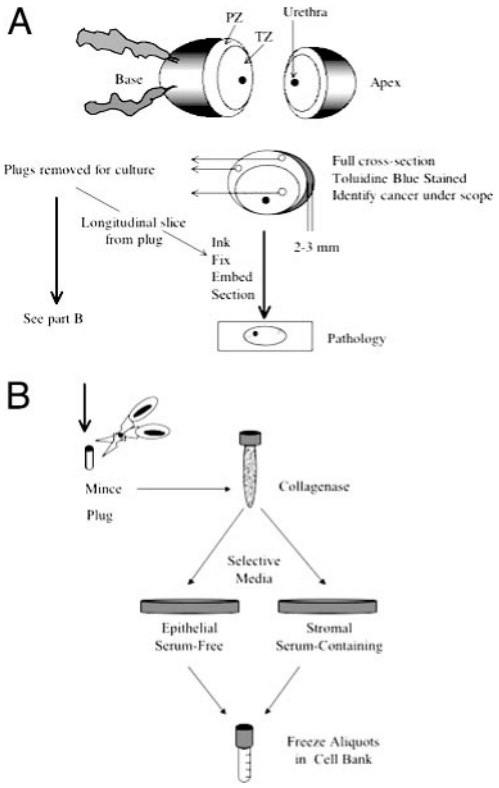

FIG. 1. Acquisition and culture of human prostate specimens. A, Flow chart and schematic of sample acquisition. Posterior view of prostate with attached seminal vesicles showing removal of a complete cross-section. *Open circles* in cross-section indicate hypothetical locations of plug removal after inspection of toluidine staining under a high-power dissecting scope. *Arrows from circles* indicate the flow of plugs for tissue culture. *Arrow below* indicates flow of the remainder of prostate specimen. *Diagonal arrow* indicates the flow of the longitudinal slice for pathological evaluation. TZ, Transition zone. B, Schematic of processing of samples removed for growth of epithelial and/or stromal cells as described above.

Results and Discussion

Toluidine blue dye tissue acquisition

We adopted a prospective technique for acquiring cell populations with defined pathological origin. Our procedure is an adaptation of the procedure described by Fischer *et al.* (11). Figure 2 shows typical images from a toluidine blue-stained cross-section. The sample is scanned at low magnification (×10) to identify the areas of potential benign tissue (Fig. 2A). These areas are then compared with areas of potential tumor tissue (Fig. 2D). The prostate specimen depicted in Fig. 2 contains a transition zone tumor (Fig. 2D).

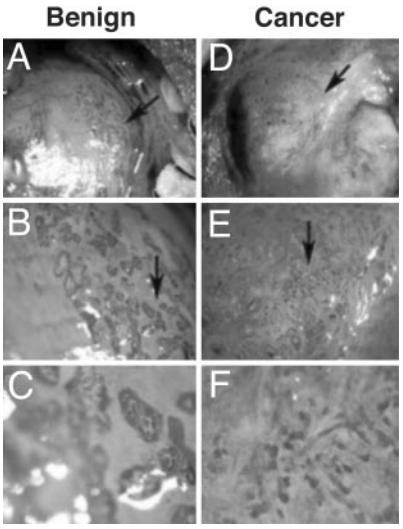

FIG. 2. Toluidine blue-stained cross-sections of fresh prostatectomy specimens. Typical images of toluidine blue-stained prostate cross-section (n = 10 cross-sections). All photomicrographs are from the same cross-section prepared as described in *Materials and Methods* under Sample acquisition. An area of benign-appearing glands is depicted in A–C. An area of apparent cancerous-appearing glands is depicted in D–F. The *arrows* indicate the area used for further magnification. A and D, magnification, ×10; B and E, magnification, ×30; C and F, magnification, ×100. Compare gland size, closeness, and regularity between B and E or between C and F.

This is evident by comparing the size of the adjacent benign glands with putative tumor glands at a given magnification. Usually we use ×30 magnification for this comparison (Fig. 2, B and E). Magnification at ×100 is used for further validation (Fig. 2, C and F). Also shown in Fig. 2D is the presence of a stromal nodule. These are often clearly delineated from surrounding tissue by their paucity of glandular structures and bulging nature. Samples from these nodules can be removed and grown for BPH-derived stromal and epithelial cells.

Once we are confident of the pathology of the area, we obtain a plug using a sterile cork borer as described in *Materials and Methods*. Each hole in a prostate cross-section is inked with a different color dye. Figure 3 shows sections from the pathology blocks containing the inked holes in the prostate sample depicted in Fig. 2. The hole remaining after the plug was removed from the predicted benign sample is depicted in Fig. 3A. The adjacent glandular architecture is indicative of a benign sample. To confirm this, we probed an adjacent section for the expression of basal cytokeratins (Fig. 3, B and C), a marker of benign glandular structure. The results confirm our prospective analysis of the sample that this region contains only benign glands with an intact basal layer. The hole remaining after the plug was removed for the predicted cancer specimen was inked *orange* and is visible in Fig. 3D. The adjacent glandular architecture indicates a cancer sample. There is a complete lack of basal cells in these adjacent glands shown by a lack of basal-specific cytokeratin expression (Fig. 3, E and F) and the structure of the glands as a single cell layer (Fig. 3F).

The removed plug is processed by our normal primary culture technique except that a longitudinal slice is removed

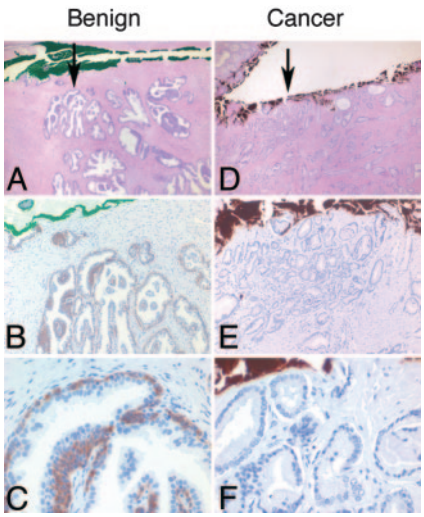

FIG. 3. Histopathology of prostate surrounding plugs removed for culture. Photomicrographs of areas of benign (A–C) and cancer (D–F) prostatic tissue obtained from the same prostate cross-section depicted in Fig. 2 and adjacent to plugs used for culture. Samples for culture were acquired by the toluidine blue dye method, the hole was inked (note *green* and *orange ink* in holes), and the adjacent tissue was fixed and embedded in paraffin by routine histological techniques. Sections from the blocks were prepared and stained with hematoxylin and eosin (A and D, magnification, ×40). Adjacent sections were incubated with an antibody against basal-specific high-molecular-weight cytokeratins (Cytokeratin 903) and peroxidase stained (B and E, magnification, ×100; C and F, magnification, ×400). *Arrows* in A and D indicate areas used for higher-magnification photomicrographs.

for histology before dissociation of the tissue. The growth of the derived cultures does not appear to be compromised by the toluidine staining technique (data not shown).

The longitudinal slice removed from the plug is fixed, embedded in paraffin, and stained with hematoxylin and eosin. Figure 4 shows the sections from the longitudinal slices from the plugs removed from the samples depicted in Fig. 2. The results demonstrate our ability to prospectively predict

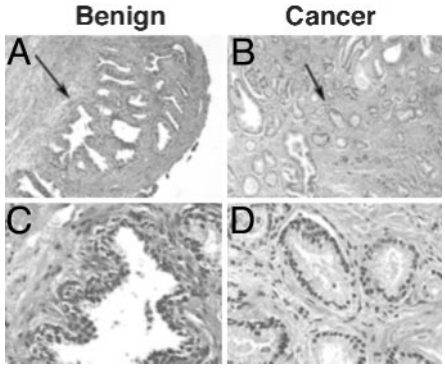

FIG. 4. Histopathology of longitudinal section of plugs removed for culture. Photomicrographs of a longitudinal slice from a plug of benign (A and D) and cancer (B and C) prostatic tissue obtained from the same prostate cross-section depicted in Figs. 2 and 3. A longitudinal slice from the acquired plug was removed, and the tissue was fixed and embedded in paraffin by routine histological techniques. Sections from the blocks were prepared and stained with hematoxylin and eosin (A and B, magnification, ×100; C and D, magnification, ×400). *Arrows* in A and B indicate areas used for higher-magnification photomicrographs.

the location of benign or cancer glands in fresh specimens (Fig. 4, A and B).

We sampled 10 prostatectomy specimens between July 2002 and December 2002 using the toluidine blue staining method to observe glandular architecture. Of 27 samples collected from these specimens, 14 samples appeared to be cancer and 13 appeared to be benign judging from the toluidine blue dye-staining pattern. Twelve of 14 putative cancer samples (86%) were confirmed cancer by pathologic examination of adjacent tissue and the longitudinal plug. The remaining two putative cancer samples were determined to be of mixed origin. All 13 putative benign samples (100%) were confirmed benign samples by pathologic examination. Of the confirmed benign and cancer-derived samples, eight sets are matched to the same prostate. This system allows for efficient acquisition of patient-matched normal and diseased prostatic tissue.

BPH-S and CA-S induce prostate epithelial growth in vivo

To validate our histological findings in a functional assay and test the utility of this acquisition for studying epithelial-stromal interactions, we xenografted prostate tissue recombinants under the renal capsules of nude mice. Figure 5A shows a schematic of the protocol. Using this protocol, we established 15 grafts from PZ-S/BPH-1 recombinants, nine grafts from BPH-S/BPH-1 recombinants, and seven grafts from CA-S/BPH-1 recombinants. Each animal was grafted with two or three PZ-S/BPH-1 recombinant buttons on the left kidney, and the contralateral kidney was grafted with two grafts of either BPH-S/BPH-1 or CA-S/BPH-1 recombinants. We also established two grafts each of PZ-S, BPH-S, CA-S, or BPH-1 cells alone as controls. No growth of any of these control grafts was observed (data not shown). Figure 5B shows representative results from grafts with prostate tissue recombinants. All PZ-S/BPH-1 recombinants produced grafts that contained little or no growth (1 mm or less). The *top left* and *right* images in Fig. 5B are typical of results from BPH-1 recombinants with the two different PZ-S strains (WFU236PZ-S and WFU234PZ-S). Note the flattened opaque collagen disks visible at the site of the grafts. These results are consistent with other observations that show that stromal cells from the normal peripheral zone have little or no inductive ability.

In contrast, all BPH-1 recombinants with BPH-S cells produced 3- to 4-mm-diameter grafts that were sharply circumscribed (the *lower left image* of Fig. 5B shows a representative graft). Both BPH-S strains (WFU225BPH-S and WFU228BPH-S) showed a similar inductive ability. Histological examination of these grafts revealed densely packed, well-organized tubular epithelium with minimal stroma, sharply demarcated from surrounding renal tissue (Fig. 6, A and B). To our knowledge, the use of BPH-S in this recombination system has not been previously tested.

McNeal (17) suggested that BPH is a disease of the prostatic stroma. His observations have led to the hypothesis that BPH is a result of the reawakening of the embryonic inductive abilities of the prostatic stroma. The inductive abilities of the BPH stroma are thought to be the normal embryonic signals that induce normal epithelial structures. Our results

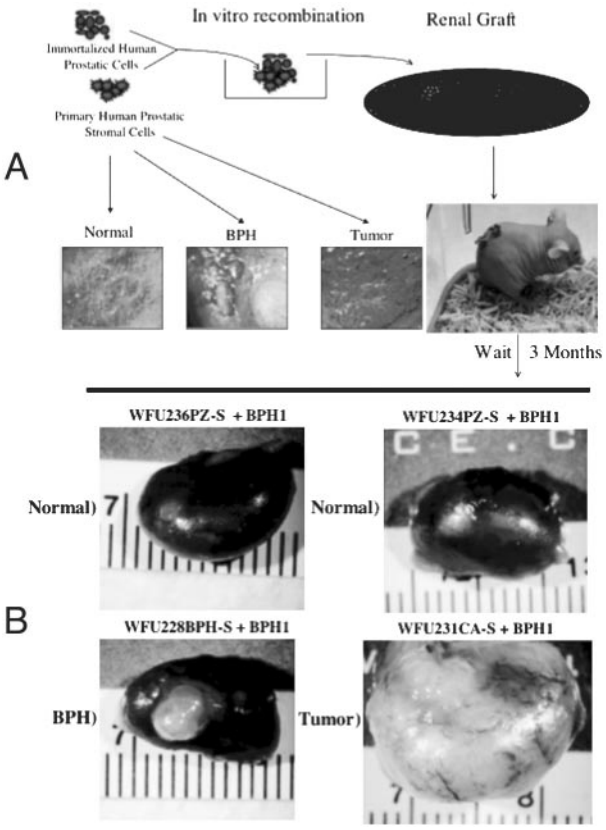

FIG. 5. BPH-S and CA-S cells induce prostate epithelial growth. A, Schematic of prostate tissue recombination protocol. Isolated prostatic stromal cells of defined histological origin (representative toluidine-stained sections are depicted) are combined with BPH-1 cells *in vitro* in a collagen gel matrix. The recombinant button is grafted under the renal capsule of a nude mouse and allowed to grow for 3 months. B, Representative photomicrographs of dissected kidneys grafted with prostate tissue recombinants. The stromal strain used for each graft is indicated above the corresponding photomicrograph.

are suggestive of this. BPH-1 cells are a transformed cell line that cannot differentiate into ductal structures with differentiated lumenal and basal cells. However, the sharp demarcation between the graft and kidney as well as the well-ordered appearance of the solid tubes reflects a relatively benign structure. Some *in vitro* data that show disruptions of the IGF signaling axis in BPH-S support McNeal's hypothesis (18, 19). However, our studies are the first evidence to support a functional inductive capacity of BPH-S in prostatic tissue recombinants. The simplified method of acquiring prostatic stromal cells described here, combined with the prostate tissue recombination model, should facilitate dissecting the signaling pathways leading to BPH.

Stunningly, BPH-1 recombinants with CA-S cells produced much more robust growth than the other recombinants tested. The *lower right image* in Fig. 5B shows an extreme example of this aggressive growth induced by the CA-S cell strain WFU231CA-S (note that all images in Fig. 5B are of the same magnification). A second CA-S strain (WFU233CA-S) also produced aggressive-looking grafts, but these grafts did not completely encapsulate the kidney (data not shown). Histological assessment of the graft reveals a moderately differentiated, highly vascularized tumor (Fig. 6, C and D)

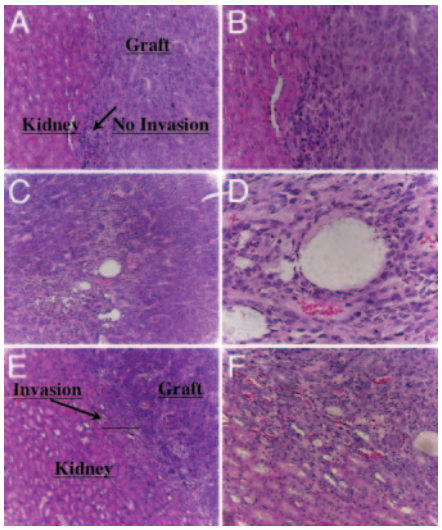

FIG. 6. Histology of BHP-S/BPH-1 and CA-S/BPH-1 recombinants. Kidneys were dissected from animals, fixed in 10% formalin, and embedded in paraffin, and 5-μM sections through the grafts were made. Digital images show hematoxylin-eosin stained sections. A (magnification, ×10) and B (magnification, ×20), Graft-kidney interface of a BPH-S/BPH-1 recombinant showing sharp demarcation between the interfaces. Note the organization of the graft into well-ordered solid tubular structures. C (magnification, ×10) and D (magnification, ×40), CA-S/BPH-1 grafts showing a more disorganized structure with high vascularity. E (magnification, ×10) and F (magnification, ×20), Graft-kidney interface of a CA-S/BPH-1 recombinant showing infiltration of the graft and associated neovasculature into the adjacent renal tissue.

with invasion into surrounding renal tissue (Fig. 6, E and F). Our data confirm the findings reported by Olumi *et al.* (7) that demonstrate the ability of CA-S to produce aggressive prostatic tumors when recombined with BPH-1 cells. It also appears that the phenotype from the CA-S induction is more aggressive that that from the BPH-S induction. The CA-S induces more ragged margins with invasion into the kidney, whereas the BPH-S induces more sharply circumscribed margins with no evidence of invasion. These differences in the phenotypes between these two recombinant models suggest fundamental differences in the nature of the inducers. Further investigation of these models will be facilitated by the systems outlined here.

Development of adenocarcinoma is usually thought of in the context of the somatic mutation theory in which the epithelium is the primary target of mutagenesis (20). The results reported here support a growing body of work from a number of different epithelial tumor systems demonstrating that the tumor microenvironment and specifically, epithelial-mesenchymal interactions are critically important for tumorigenesis (reviewed by Ingber in Ref. 21). Additionally, recent evidence from rodent mammary carcinogenesis demonstrates that the stroma is a direct target of carcinogenic insult that may supersede genetic insult to the epithelium (22). These studies argue that more focused attention should be directed toward the nature of cancer-associated stroma. The system reported here will provide a means to study the specific roles of the stroma and its interaction with epithelium in tumorigenesis.

In summary, we have validated a method of determining prospectively the glandular architecture (malignant or benign) of fresh prostatic samples collected for use in tissue culture. The system reduces the waste of collecting and culturing specimens with undesirable attributes (mixed benign and malignant origin). We can acquire defined cancer samples from a majority of prostatectomy specimens. This will increase the availability of primary cancer strains for prostate cancer research. Stromal nodules can also be easily identified, which should contribute to the availability of BPH-derived stromal lines. Using this system, we have demonstrated fundamental differences in the inductive capabilities of stromal cells derived from normal or diseased prostatic tissue. The model systems described here should facilitate the investigation of epithelial stromal interactions in the etiology of prostatic diseases.

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