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Review

Three-Dimensional Cultures of Prostatic Cells: Tissue Models for the Development of Novel Anti-Cancer Therapies

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This review addresses the application of three-dimensional cultures of prostatic cells to the development of novel anti-cancer therapies. A variety of therapeutic agents to combat prostate cancer are currently under development. These include cytotoxins, differentiation agents and, more recently, genetically modified tumor vaccines. Three-dimensional cultures of prostatic cells are increasingly used in preclinical research in the design of new therapies and in the development of delivery strategies for these treatments. These tissue-like structures more realistically model the structural architecture and differentiated function of the human prostate than a cellular monolayer. In doing so, three-dimensional cultures produce an *in vivo*-like response to therapeutic agents. Advances in tissue engineering have improved the variety, fidelity and quantity of these prostate models. To date, they have been applied to estimate the dose of new drug therapies, evaluate drug penetration into solid tumors, assess the effectiveness of drug combinations, and develop tumor vaccines.

KEY WORDS: prostate cancer; spheroid; gel culture; drug therapy; gene therapy.

INTRODUCTION

Prostate cancer is the second most frequent cause of cancer-related death in the United States. As the male population ages, the number of men that develop this disease is increasing yearly and is currently over 300,000 (1). The rate at which prostate cancer grows and metastasizes is highly variable from patient to patient (2). Progression of this disease is associated with genetic changes within the tumor (3) and interactions between the tumor and host environment (4), but many of the specific molecular mechanisms remain undefined. When the cancer has metastasized, treatment options are limited. The primary therapy is androgen deprivation (5). While it initially arrests tumor growth, remission is often temporary as the tumor progresses from androgen-dependent to androgen-independent growth (6). Once prostate cancer has become hormone-refractory, there is no known cure.

Tissue models which mimic the structural architecture and differentiated function of the intact human prostate are essential to the design of novel anti-cancer therapies and development of more effective delivery strategies for these treatments. With *in vivo* prostate models, cancer can occur spontaneously in laboratory animals or as a result of induction, transplantation or transgenic manipulation (7). *In vitro* models provide a well defined environment for cancer studies in contrast to the complex host environment of an *in vivo* model. For years, monolayer cell culture and organ culture have been popular *in vitro* models for prostate carcinogenesis. The advantage of organ culture is that the microenvironment of the tumor remains intact. It is well known that cell-cell interactions, cell-matrix interactions and interstitial fluid within this three-dimensional microenvironment affect differentiated cell function (8). Organ culture, however, is limited by availability of the prostatic tissue and its viability in culture. The converse is true of monolayer cell culture. It is applicable to a variety of primary and immortal cell lines, but the three-dimensional microenvironment of intact tissue is lost.

Three-dimensional cell culture combines the beneficial features of the two established *in vitro* models. With this method, three-dimensional prostatic tissue is regenerated from cell culture. The regenerated tissue more closely mimics intact prostatic tissue than a monolayer cell culture (9). With recent advances in tissue engineering, three-dimensional cultures are now more differentiated and can be produced in larger quantities as a result of greater control over culture composition, new bioreactors for cell cultivation and greater choice in the method of inducing three-dimensional growth. Increasingly, researchers are turning to this technology to produce prostatic tissue models of high fidelity. It is timely, therefore, to review existing methods for three-dimensional cultivation of prostatic cells and discuss the application of these *in vitro* models to the design and delivery of novel anti-cancer therapies.

THREE-DIMENSIONAL CULTURES OF PROSTATIC CELLS

The development of human tissue is dependent on the (i) co-location of dissimilar cell types that will synergistically

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interact through cell-cell linkages and the exchange of growth factors and other biological effectors, (ii) synthesis of extracellular matrix to provide a three-dimensional scaffold for mechanical stability and to regulate cell function, and (iii) formation of a rich interstitial fluid containing biological effectors required for tissue development and maturation (8). In three-dimensional cell culture, multilayer cell growth generates an environment in which the spatial orientation of cell-cell and cell-matrix interactions and the composition of interstitial fluid are akin to those *in vivo*. By satisfying these three requirements for tissue development, three-dimensional cell cultures produce tissue models of high fidelity.

Over the past decade, the number of prostate models based on three-dimensional cell culture has been steadily growing (Table I). This is partially the result of new culturing technology emerging from the field of tissue engineering which has further improved model fidelity. Prior to this influx of technology, three-dimensional prostate cell models were produced by spontaneous cell aggregation (10) and liquid overlay on agar or basement membrane (11) to form tissue-like spheroids. The introduction of spinner flasks enabled researchers to generate prostatic spheroids in greater quantities (12). More recently, spheroids grown in rotating wall-vessels were more differentiated and larger that those cultured in the more turbulent spinner flasks (13). In addition, prefabricated scaffolds from the tissue engineering field are increasingly used as a three-dimensional matrix to promote multilayer growth of cells derived from prostatic tissue (9).

Spontaneous Cell Aggregation

A limited number of prostatic cell lines will spontaneously aggregate during routine cultivation to form tissue-like spheroids. One of these, 1-LN, is a subline of human prostatic PC 3 cells (10). Another, U4F mesenchymal cells, were derived from fetal rat urogenital sinus organ culture (14). The mean diameter of 1-LN spheroids approached 1 mm by day 16 of cultivation. U4F spheroids were of a similar size, 1 to 3 mm in diameter, 40 days after inoculation and were stable for several months in culture. Typical of large spheroids, 1-LN and U4F cultures exhibited a gradient of viability as a function of radial distance from the spheroid surface to its core (10,14). Proliferating cells were confined mostly to the periphery. Because of mass transfer limitations, the supply of nutrients diminishes and toxic metabolic wastes accumulate at progressively greater depths within the spheroid. At the core, there was mass necrosis with quiescent, but viable, cells scattered within the central region. This gradient in spheroid models reproduces the heterogeneity in cell viability observed in micrometastases prior to vascularization and in intervascular microregions of larger tumors (15).

Spheroid cultures of 1-LN and U4F cells were functionally distinct from their monolayer counterparts. For 1-LN cells, spheroids exhibited differentiated function in that they actively secreted prostatic acid phosphatase (PAP), but secretion was insignificant in monolayers; moreover, there was a positional dependence of protein expression in the spheroids for prostate-restricted antigen p40 (10). Expression was comparable from cells on the spheroid surface and in monolayers, yet it was enhanced by 2-fold in the deeper intermediate region of the spheroids. Urogenital sinus derived growth inhibitory factor was secreted by U4F spheroids but not by monolayers of this cell line (14).

Liquid-Overlay Culture

For tumor cell lines that will not spontaneously aggregate, Yuhas *et al.* developed a method to induce spheroid formation in which tumor cells are grown in static liquid medium over an agar base (16). Agarose (11) and reconstituted basement membrane (17) have been substituted for agar as a substratum for spheroid formation. For prostatic NbMC 2 cells, spheroid formation in liquid-overlay culture is a biphasic process (17). Initially, the cells migrate on the substratum towards each other and aggregate into micro-spheroids. Increase in spheroid size is a result of cell growth. In addition to NbMC 2 cells, liquid-overlay cultures have been used to generate prostatic spheroid models of PC 3, DU 145 and LnCaP cells (11) as well as primary cultures from human benign prostatic hyperplasia (18) and secondary cultures from human prostatic tumors (19).

Spheroids generated in liquid-overlay culture maintain the cellular composition and differentiation of intact tissue. Consider composition. Secondary cultures from human prostatic tumors slowly died after 4 weeks when cultivated as monolayers (19). In contrast, the cultures grew on agarose to form spheroids nearly 350 μm in diameter and could be maintained for 3 months. The percentages of epithelial cells, vimentin-positive fibroblasts and aneuploid cells in the spheroids were similar to values in the excised tissue from which the secondary cultures were derived. Primary cultures from human benign prostatic hyperplasia retained differentiated morphology and function when grown as spheroids (18). This three-dimensional cell culture acquired several features of differentiated epithelium: abundant secretory vesicles, microvilli and desmosomes with associated cytoskeletal elements. These features were far less apparent in monolayer culture. The spheroids also displayed enhanced differentiated function in their increased secretion of

Table I. Summary of Three-Dimensional Cultures of Prostatic Cells

Type	Method	Cell Lines/Cultures	References
Spheroid	Spontaneous aggregation	1-LN, U4F	(10,14)
Spheroid	Liquid-overlay	LnCaP, DU 145, PC 3, NbMC 2, primary prostatic culture	(11,17–19)
Spheroid	Spinner flask	DU 145	(12,13,20,21)
Spheroid	Rotating-wall vessel	LnCaP, DU 145, PC 3	(13,20,24,26,27)
Scaffold-based culture	Collagen matrix	LnCaP, PC 3, minced prostatic tissue, primary cultures of prostatic epithelium	(9,28,29,33–48)

PAP by 5-fold and prostate-specific antigen (PSA) by 8-fold relative to the monolayer.

In the case of NbMC 2 cells, spheroid growth on Matrigel in liquid-overlay culture resulted in the appearance of morphologically transformed variants (17). These fusiform cells appeared as outgrowth from the spheroids after 1 to 3 weeks of cultivation and, over time, migrated away from the spheroids and invaded the Matrigel matrix. NbMC 2 cells exhibit an epithelial morphology. In contrast, the fusiform cells were more elongated. Sublines of fusiform cells were characterized by a significantly greater invasion potential, saturation density and random motility than the NbMC 2 parent cell line. Relative motility, for instance, was a factor of 3 times faster for the fusiform subline FB2 than for NbMC 2 cells in a phagokinetic track assay. The altered phenotypic properties of the fusiform cells were stable; they could be inherited by daughter cells and were evident in the absence of Matrigel. Outgrowth of fusiform cells could not be induced on laminin, type I or IV collagen, or fibronectin substratum. These findings indicate that specific interactions between NbMC 2 spheroids and Matrigel promoted the development of this distinct and more aggressive cell type.

Spheroid Culture in Spinner Flask

Spinner flasks are stirred-tank bioreactors which have long been used by bioengineers to cultivate animal cells as a suspension culture in liquid medium. Impeller mixing maintains the cells in suspension. Greater quantities of spheroids can be cultivated in suspension than is possible with liquid-overlay cultures. In addition, fluid movement in spinner flasks may aid in mass transport of nutrients to and wastes from spheroids relative to the static environment in liquid-overlay cultures. The latter is better for investigations of individual spheroids.

DU 145 human prostate carcinoma cells have been cultured as spheroids in spinner flasks by ourselves and others (12,13,20,21). In our laboratory, we observed that the spheroids were slower growing in terms of growth rate and cell-cycle phase distribution relative to a control culture of bi-/trilayers of DU 145 cells grown on a two-dimensional substratum (13). Also, the spheroid culture had stronger immunostaining for select cytoskeletal proteins, epidermal growth factor receptor, transforming growth factor β and collagen IV; however, it had comparable staining results to that of the bi-/trilayer for epidermal growth factor, transforming growth factor β type II receptor and laminin (13,20). Based on these findings, we explored an alternate method to generate spheroids in suspension culture. As described below, we were able to magnify differences between spheroid and bi-/trilayer cultures by growing the spheroids in a rotating-wall vessel instead of a spinner flask.

Spheroid Culture in Rotating-Wall Vessel

The rotating-wall vessel was developed by NASA in 1990 to grow animal cells in an environment which simulates microgravity in a bench-top bioreactor here on earth (8). In actual microgravity, suspension cultures of animal cells float in liquid medium mixed by only minimal hydrodynamic forces. To simulate this environment, the rotating-wall vessel has three key design features: end-over-end mixing, bubble-free aeration and solid-body rotation. The rotating-wall vessel rotates end-over-end around a horizontal axis (Fig. 1). This motion suspends

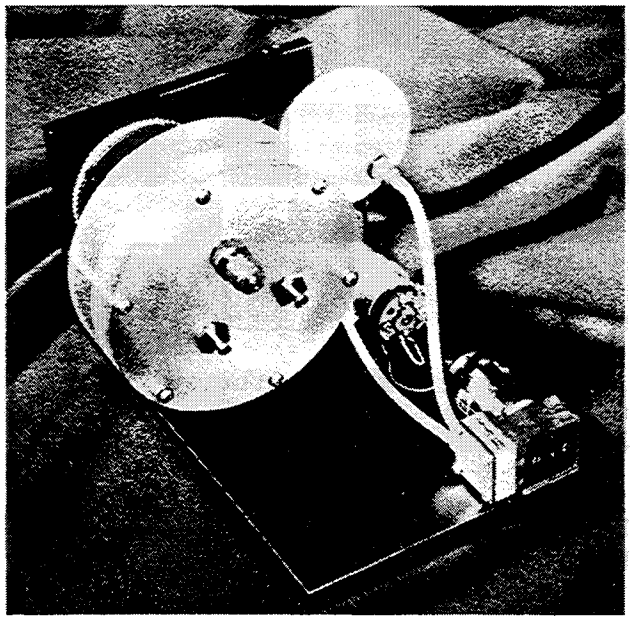

Fig. 1. Photograph of a 50 ml rotating-wall vessel showing the Laxan cultivation chamber, sampling ports, 24-DC motor, air pump and air filter. The medium-filled chamber rotates around a horizontal shaft. Reprinted from Francis *et al.* (60) and used with permission from the Society for *In Vitro* Biology (Columbia, MD).

the cells in the liquid medium as if in microgravity without the aid of an impeller. To minimize fluid turbulence, the vessel is completely filled with medium and aerated by oxygen diffusion across a silicon membrane. Because the medium and vessel wall rotate as a solid body at the same angular rate, shear forces at this interface are greatly reduced. Bubble-free aeration eliminates hydrodynamic forces associated with air-liquid interfaces. Furthermore, end-over-end rotation not only suspends the cells but also eliminates hydrodynamic forces resulting from impeller mixing. In all, the rotating-wall vessel provides a very quiescent environment for animal-cell cultivation with adequate mixing for mass transport.

Since its invention, the rotating-wall vessel has been used to cultivate a variety of normal and neoplastic animal cells (22,23). In this bioreactor, spheroid formation of prostatic cells is characterized by increased differentiation and intercellular adhesion for both non-invasive (LnCaP) and invasive (DU 145 and PC 3) cell lines (24). Relative to monolayer cultures, these spheroids exhibited enhanced expression of cytokeratin 8, as a marker of differentiation, and the cell adhesion molecules CD44 and E-cadherin. Likewise, our laboratory investigated the possibility of increased cell-matrix interactions in the rotating-wall vessel by monitoring the accumulation of extracellular matrix proteins in DU 145 spheroids (20). For collagen IV, as an example, there was upwards of a 4-fold increase in staining intensity for the spheroids relative to bi-/trilayers of DU 145 cells (Fig. 2).

Our laboratory has compared DU 145 spheroids grown in a rotating-wall vessel and spinner flask (13,20). The two reactors differ in the severity of the hydrodynamic forces to which cells are exposed. Rotating-wall vessels are characterized by a maximum shear stress of 0.2 dyne/cm^2; whereas, this value is nearly 0.6 dyne/cm^2 for a spinner flask operating at 60 rpm

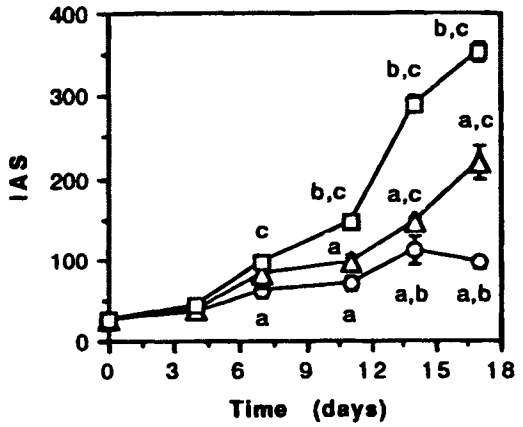

Fig. 2. Staining intensities reported as intensity-times-area scores (IAS) for collagen IV from cultures of DU 145 cells grown as spheroids in a rotating-wall vessel (□) and spinner flask (△). Bi-/trilayers of DU 145 cells served as control cultures (○). Significant differences are labeled as follows: a, $p \leq 0.01$ vs. rotating-wall vessel; b, $p \leq 0.01$ vs. spinner; and c, $p \leq 0.01$ vs. control. Reprinted from O'Connor *et al.* (20) and used with permission from Mary Ann Liebert, Inc. (Larchmont, NY).

(13). The higher fluid turbulence in the spinner flask has been shown to damage fragile animal cells affecting membrane integrity and metabolism, to name a few (25). In a more quiescent environment, cells could possibly redirect metabolic energy from the repair of hydrodynamic damage to other cellular processes. Accordingly, the rotating-wall vessel produces spheroids that are more differentiated and larger than is possible with a spinner flask as is described below.

The reduced turbulence in the rotating-wall vessel has a profound effect on the physical properties of a culture (13). DU 145 spheroids in the spinner flask were smaller. On day 14 of cultivation, the mean size of spheroids in the rotating-wall vessel ranged between 5 to 6 and 7 to 10 microcarrier beads per spheroid versus between 2 and 3 to 4 beads per spheroid in the spinner flask. As a result, there was more three-dimensional growth in the rotating-wall vessel. The morphology of the spheroids from the rotating-wall vessel was clearly differentiated with extensive, intertwined filopodia between cells and a number of microvilli-like structures on the cell surface. These features were also evident on spheroids from the spinner flask, but they had accumulated to a greater degree in the rotating-wall vessel.

These morphological changes are consistent with immunostaining results from this system. Staining intensity for cytokeratin 8 and 18 is reduced by loss of differentiation within secretory epithelial cells. DU 145 spheroids from the rotating-wall vessel had a higher intensity score for cytokeratin 8 than the spheroids from the spinner flask by a factor of 1.7 and the cellular bi-/trilayer by a factor of 2.8 (13). The intensity scores for cytokeratin 18 follow a similar trend. In addition to these two cytoskeletal proteins, our cultures were immunostained for 8 other biomarkers (13,20). All our data to date suggest that the spheroid culture from the rotating-wall vessel was the most differentiated of the three.

The rotating-wall vessel is conducive to co-culturing of multiple cell types. Spheroids formed by culturing prostate cancer epithelial cells with either prostate or bone stromal cells have demonstrated the usefulness of this bioreactor for investigating stromal-epithelial interactions in the prostate (26,27). For example, dihydrotesterone maintained differentiation of LnCaP cells over a 10-day period when co-cultured with isolated human prostate fibroblasts but not when the cancer cells were cultured alone (26). In this set of experiments, the differentiated state of LnCaP was monitored by PSA expression which was relatively constant on a per cell basis in the co-culture and rapidly diminished after 4 days in the mono-culture.

Scaffold-Based Culture

Three-dimensional cultures of prostatic cells have been grown on prefabricated scaffolds. Cells attach to and migrate along intertwined scaffold fibers. As cells divide, they fill the interstices within the scaffold to form a three-dimensional culture. The majority of scaffolds have been composed of collagen (28,29); however, other materials are available (30). The choice of scaffold can profoundly affect the properties of a culture. Collagen and other extracellular matrix proteins are known regulators of cell function in addition to substrata for cell attachment (31). Moreover, the scaffold may also contain growth factors and other regulatory agents (32). To date, scaffolds have been inoculated with established cell lines (27–29,33), minced tissue (34–48) and primary cells derived from tissue (9,49–52). The inoculum has been embedded within or seeded atop the scaffold.

In contrast to the tightly packed cell aggregates within the spheroid models of prostatic tissue, three-dimensional cultures of primary epithelial cells derived from the rat ventral prostate formed differentiated acinus-like structures within collagen matrix extracted from rat tail tendon (9,49). These structures consisted of a single layer of cells surrounding the lumen. The cells had microvilli on the luminal surface and secretory vacuoles in the cytoplasm facing the lumen. Adjacent cells were joined together with a junctional complex. At the cell borders facing the collagen gel, a basal lamina-like structure formed. Similar features were observed in collagen gels containing epithelial cells derived from mouse anterior and ventral prostate (52).

Co-culture of metastatic PC 3 cells and MG 63 osteoblast-like cells in collagen gel was used to model the ability of metastatic prostate adenocarcinoma to promote new bone formation (28,29). Inoculation of PC 3 cells into collagen gel containing MG 63 cells did produce an osteoblastic-like reaction. Specifically, there was an increase in the number of MG 63 cells and in the density of type I collagen adjacent to the site of inoculation. Furthermore, KLE endometrial cancer cells and cell-free spent medium produced no reaction at the inoculation site, suggesting that the osteoblastic-like response is cancer-cell specific. At the site of PC 3 inoculation, immunostaining for the urokinase-type plasminogen activator was substantially higher than in either neighboring regions occupied by MG 63 cells or at the site of KLE inoculation. Expression of this serine protease correlates well with the number of bone metastases in prostate cancer patients.

In another co-culture study, MS and Saos-2 bone fibroblasts were more effective at supporting LnCaP cell growth in collagen gel than MG 63 fibroblasts (27). This result suggests that metastasis of prostate cancer may, in part, depend on the influence of stroma on malignant cell survival. Also, this gel

culture demonstrates that CCD16 lung fibroblasts can support LnCaP growth in addition to prostate and bone stromal cells (27). This is consistent with prostate cancer progression *in vivo* which will metastasize preferentially to the bone and occasionally to the lung.

When prefabricated scaffolds are inoculated with minced tissue, the resulting three-dimensional histoculture is a mixture of multiple cell types which approximates the heterogeneous composition of the parent tissue. Histocultures of human prostatic tissue have been established directly from normal prostate, benign hyperplasia and prostate cancer (35–38,40–47), as well as from xenographs of human tissue passaged in immunodeficient rodents (34,39,48). In one study, greater than 85% of tumor specimens generated proliferating histocultures (37). Once established, these three-dimensional cultures maintained differentiated PAP and PSA expression for at least 8 weeks.

Recently, the total-immersion method of histoculture was developed (38). Unlike conventional histocultures which are supported by collagen sponge gel or similar material, cultures prepared by this new method do not require a support. Instead, minced tissue is placed within 6-well plates and immersed in culture medium. This procedure has been performed with tissue from benign prostate hyperplasia and prostate cancer. Cell proliferation, as measured by ^{3}H-thymidine incorporation per microgram total protein, was 2–5 fold higher in total-immersion histocultures than in sponge-gel histocultures. This increase was attributed to greater diffusion of medium biochemicals into the immersed tissue. The performance of total-immersion histocultures from other tissue sources remains to be evaluated. Care should be taken with this method to avoid monolayer cell growth at the bottom of the culture well.

APPLICATION OF THREE-DIMENSIONAL MODELS TO THE DEVELOPMENT OF PROSTATE CANCER THERAPIES

For *in vitro* testing of a novel tumor therapy to be clinically relevant, the therapeutic agent must elicit an *in vivo*-like response in the tissue model. This can be more readily achieved with three-dimensional cell culture than with a cell monolayer as a result of the structural architecture and transport limitations within the former (53). Clinical relevance can be further enhanced when the three-dimensional models are prepared from human tumors that contain both epithelial and stromal cells (37).

Structural architecture in a tissue model regulates differentiated cell function through changes in cell shape, as well as increased cell-cell and cell-matrix interactions. For instance, expression of E-cadherin, a cell-cell adhesion molecule promotes differentiation and inhibits metastasis of several cancer cell lines (54). Three-dimensional cell growth may induce cell differentiation through enhanced expression of E-cadherin and other cell adhesion molecules. Consider cell shape which regulates cellular function by stimulating signal transduction from the cell membrane through the cytoskeleton and nuclear matrix to the DNA (55). Changes in cell shape that occur in three-dimensional culture may affect gene expression.

These intrinsic changes in cell function profoundly affect the response of a tissue model to external agents. As an example, mammary tumor cells exhibit greater drug resistance to melphalan and 5-fluorouracil as a multicellular aggregate than as a monolayer (56). This resistance was not a result of mass transport effects. Instead, it was an intrinsic property of the cells themselves. To demonstrate this point, the tumor cells were exposed to these therapeutic agents as a monolayer and subsequently grown as a multicellular aggregate. The culture remained more drug resistant than cells grown as a monolayer throughout the experiment.

Mass transport limitations induce cellular heterogeneity within three-dimensional culture that resembles the multiple phenotypes found in solid tumors. Gradients of nutrients, wastes and other biologicals within a tumor apply a selective pressure on tumor cells. Coupled with the instability of the malignant genome, the gradients promote the formation of diverse phenotypes within the tumor (15). New phenotypes with increased metastatic potential can develop. As described in the previous section, three-dimensional cultures are composed of proliferating cells, non-proliferating viable cells and necrotic cells resembling the multiple phenotypes found in intact tumors. Monolayer cultures are not limited by mass transport and are, thus, more homogeneous.

In three-dimensional cultures, transport limitations generate an *in vivo*-like response to external agents by restricting accessibility and inducing cellular heterogeneity. For chemicals that have poor diffusion in tissue like adriamycin, internal cells within a three-dimensional culture are less accessible to these agents than those at the culture periphery (57). The previous paragraph mentioned that transport effects also produce cellular heterogeneity in three-dimensional culture whereby internal cells can have a higher metastatic potential. Both mechanisms can make internal cells more resistant to anti-cancer drugs. In this respect, transport limitations are frequently responsible for failure of tumor regression treatments in patients.

Clinical relevance of *in vitro* therapy testing also is affected by the composition of the tissue model. Interactions between epithelial and stromal cells (fibroblasts and smooth muscle cells) regulate normal and neoplastic development of the prostate (58). Given the importance of these interactions, prostate models which reproduce the composition of the prostate are likely to elicit an *in vivo*-like response during testing. As described in the previous section, three-dimensional cultures can support the co-cultivation of multiple cell types. This has been demonstrated for prostate models with liquid-overlay cultures, spheroid cultures in rotating-wall vessels and scaffold-based cultures (19,26,27,39). For example, histocultures of CWR22, CWR22R and CWR91 xenographs contained human epithelial cells derived from prostate cancer and normal mouse stroma cells (39). The antiproliferative and cytotoxic response of these tissue models to two drugs, doxorubicin and paclitaxel, was qualitatively similar to that of histocultures from patient tumors.

Another aspect of model composition that affects clinical relevance is tissue source. Models prepared from human tissue are arguably more relevant than those of animal origin because of known differences in prostate structure and function from these sources (59). There are additional differences in cancer cell behavior between patients and even within a given tumor (15,59). This inter- and intratumoral heterogeneity can be accounted for by testing a new therapy with several models. Consider histocultures of CWR22, CWR22R and CWR91 which were described in the previous paragraph. In these three

models, expression of PSA, Pgp, p53 and Bcl-2 are representative of their expression in 100, 85, 90 and 60%, respectively, of histocultures from patient tumors (39).

Drug Therapy Development with Three-Dimensional Models

A variety of three-dimensional prostate models have been used to evaluate drug activity (Table II). For 1-LN cells, spheroids are more resistant to the cytotoxic effects of bleomycin, cisplatin and doxorubicin than monolayers (10). The difference between the two cultures in the response to these drugs can be quite dramatic. For example, spheroids exhibited a 16-fold decrease in chemosensitivity to cisplatin.

Breul *et al.* demonstrated that the response of a prostate model to a given drug is highly dependent on the cell line/tissue from which it was derived (11). As in the case of 1-LN cells, there was reduced chemosensitivity to 5-fluorouracil in DU 145 and LnCaP spheroids relative to monolayers of the same cell lines; however, the inverse is true of PC 3 spheroids. Only 35 μM of 5-fluorouracil was required for a 50% growth inhibition of PC 3 cells in spheroid culture as compared with 200 μM in monolayer culture. As another example, histocultures from primary rat prostate tumors are more sensitive to the antiproliferative activity of taxol than paired histocultures derived from lymph node metastases from the same animal (46).

In a second phase of their research, Breul *et al.* used spheroid cultures to assess the effectiveness of a drug combination (11). They investigated the combined effects on 5-fluorouracil and folinic acid on cell proliferation. For PC 3, DU 145 and LnCaP cells, folinic acid enhanced the chemosensitivity of monolayer cultures to 5-fluorouracil. In the presence of folinic acid, the effective dose of 5-fluorouracil could be reduced 4- to 5-fold in monolayer culture. In spheroid cultures of PC 3, DU 145 and LnCaP cells, the effects of folinic acid on the toxicity of 5-fluorouracil were less pronounced perhaps due to mass transport limitations (11). This may be why some patients do not benefit from the combination of these two drugs during treatment.

Estramustine is not subject to the conventional penetration barriers within three-dimensional culture (12). If a drug were mass transport limited, it would accumulate in the outer layers of the cultures. In contrast, estramustine was preferentially bound to the degenerative cells in the core of DU 145 spheroids. As much as 4 times more drug bound to the degenerative cells than to viable cells at the periphery. This preferential binding may be exploited to kill cells within this central region that would escape treatment otherwise. Morphometric evaluation of the cell packing density within DU 145 spheroids revealed that exposure to estramustine increased the relative volumetric percentage of extracellular space especially within the deeper regions of the spheroids. Based on these findings, pretreatment of a prostatic tumor with estramustine may facilitate penetration of a second drug by enlarging extracellular space.

Clinical relevance of *in vitro* prostate models has been demonstrated with histocultures. After a 4-day exposure to clinically achievable drug concentrations, only 23% of histocultures from 13 patient tumors were sensitive to the antiproliferative effects of suramin, 31% to doxorubicin and 15% to 5-fluorouracil (37). The percentages approximate the historical clinical response rates for these drugs. The response of histocultures from CWR22, CWR22R and CWR91 xenographs of prostate tumors to doxorubicin and paclitaxel is also clinically relevant (39). In histocultures from both xenographs and patient tumors, doxorubicin produced complete antiproliferation and cytotoxicity; whereas, paclitaxel produced incomplete effects.

In addition to the drugs mentioned above, three-dimensional cultures of prostatic cells have been used to evaluate the activity of androstene derivatives (42), cytochalasin E (48), diethylstilbestrol (49), geldanamycin (48), genistein (40), pregnane/pregnene derivatives (42) and thiacetazone (48).

Gene Therapy Development with Three-Dimensional Models

Because gene therapy is in its infancy, three-dimensional cell models have had only limited application in this field. Recently, they have been applied to produce a vaccine against prostatic tumors (34). This research was performed with histocultures derived from CWR22 xenografts of human prostate cancer. The three-dimensional cultures served as an *ex vivo* model for transfection with one of two cytokine genes coding

Table II. Summary of Drug Therapies Evaluated with Three-Dimensional Cultures of Prostatic Cells

Drug	Description	Culture Type	References
Adriamycin (doxorubicin)	Anti-tumor antibiotic	Spheroid, scaffold-based	(10,37,39,41,47)
Androstene derivatives	Inhibitors of androgen synthesis	Scaffold-based	(42)
Bleomycin	Anti-tumor antibiotic	Spheroid	(10)
Cisplatin	Platinum-based chemotherapy	Spheroid	(10)
Cytochalasin E	Actin-disrupting agent	Scaffold-based	(48)
Diethylstilbestrol	Synthetic estrogen	Scaffold-based	(50)
Estramustine	Microtubule-disrupting agent	Spheroid	(12)
Fluorodeoxyuridine/5-fluorouracil	Inhibitors of DNA synthesis	Spheroid, scaffold-based	(11,37)
Geldanamycin	Anti-tumor antibiotic inhibiting Hsp90	Scaffold-based	(48)
Genistein	Soybean isoflavonoid	Scaffold-based	(38,40)
Hydroxyflutamide	Antiandrogen	Scaffold-based	(38)
Ketoconazole	Inhibitor of androgen synthesis	Scaffold-based	(42)
Paclitaxel (taxol)	Inhibitor of microtubule disassembly	Scaffold-based	(39,46)
Pregnane/pregnene derivatives	Inhibitors of androgen synthesis	Scaffold-based	(42)
Suramin	Growth factor inhibitor	Scaffold-based	(37,48)
Thiacetazone	Antitubercular agent	Scaffold-based	(48)

for interleukin-2 or granulocyte-macrophage colony-stimulating factor. An Accell gene gun introduced these genes into the histoculture. With this method, up to 10% of the cells were transfected. The cytokine genes were incorporated into both CRW22 cells within the deeper regions of the histoculture and those on the culture surface. Gene expression was sustained in the transfected cells throughout the 10-day experiment.

CONCLUSIONS

Over the past decade, there has been much progress in the treatment of prostate cancer to prolong survival and improve quality of life. Despite these achievements, there is no known cure for hormone-refractory prostate cancer. Models that closely mimic the prostate are essential to the development of new treatments. With advances in tissue engineering, three-dimensional cell cultures produce tissue models of high fidelity. This review summarizes existing three-dimensional cultures of prostatic cells and highlights their benefits for *in vitro* testing of novel tumor therapies. Three-dimensional cultures provide a realistic portrayal of the *in vivo* response to an experimental treatment. As such, greater use of these models may expedite the discovery of a cure for this deadly disease.

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