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Are Primary Cultures Realistic Models of Prostate Cancer?

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Abstract Primary cultures fill a unique niche among the repertoire of in vitro model systems available to investigate the biology of the normal and malignant human prostate. This review summarizes some of the properties of primary cultures, with special emphasis on two questions: are primary cultures from adenocarcinomas really comprised of cancer rather than normal cells, and do primary cultures faithfully retain characteristics of cells of origin? J. Cell. Biochem. 91: 185–195, 2004. © 2003 Wiley-Liss, Inc.

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During the past 20 years, many of the technical challenges of growing primary cultures of human prostatic epithelial cells have been overcome [Peehl and Sellers, 2002]. Primary cultures derived from nonmalignant tissues in particular are widely used as models of normal prostatic biology and benign prostatic hyperplasia (BPH). The recent commercial availability of normal prostatic epithelial cells has dramatically increased the number and scope of studies carried out with these cells. Primary cultures derived from malignant tissues of the prostate, on the other hand, are less widely available and have had more limited use. Numerous phenotypic differences distinguish primary cultures of normal prostatic epithelial cells from established prostate cancer cell lines. These differences are often assumed to reflect features of normal versus malignant biology, but these may in some cases instead be due to different culture conditions and/or differences between short-term cultures and immortal cell lines. Distinctive features of primary cultures derived from cancers compared to primary cultures from normal tissues, in contrast, have been much more difficult to identify. Because primary cultures derived from normal and malignant tissues share many properties in common, questions have arisen regarding the cells of origin of primary cultures derived from cancers. Since there is currently no mechanism to isolate pure populations of cancer cells from tissues, it is possible that small populations of contaminating nonmalignant cells in the tissues of origin are the cells that populate the primary cultures instead of, or in addition to, cancer cells. No definitive marker has yet been identified to prove the malignant or nonmalignant nature of prostate cells in primary cultures. This article will review the properties of primary cultures of epithelial cells from normal or BPH tissues and tumors and consider the evidence suggesting that cultures derived from adenocarcinomas are indeed in part or in whole populated by malignant cells. The ability of primary cultures to realistically model the biological properties of cells in prostate tissues will also be discussed. Additional steps that need to be taken to fully develop primary cultures as realistic models of prostate cancer will be presented.

AN HISTORICAL PERSPECTIVE OF PRIMARY CULTURE OF PROSTATIC EPITHELIAL CELLS

My lab was among the first to optimize methods to routinely and reproducibly establish primary cultures of human prostatic epithelial cells from normal tissues, BPH, and adenocarcinomas of the human prostate [Peehl, 1985]. Tissues from which cultures were derived were

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usually obtained from radical or open prostatectomy specimens, but primary cultures were also established from needle biopsies [Peehl et al., 1991b]. When tissues were obtained from prostatectomy specimens, very small pieces ($\sim$0.1 cm^3) were grossly dissected by an expert pathologist for culture. The rationale for using small samples was that they were more likely than large samples to be composed of tissue of only one histological type, either normal or BPH or cancer. The edges of the cut tissues in the prostatectomy specimens were inked, then the specimens were fixed, serially sectioned at 3 mm intervals, and embedded in blocks [Schmid and McNeal, 1992]. After thin sections from each block were cut and stained, the pathologist could locate the inked regions and verify that the tissues removed for culture were from areas that were either entirely without cancer (normal or BPH), or were predominantly (>90%) cancer. In the case of needle biopsies, an additional biopsy was taken 2 mm from and parallel to the biopsy used for culture, and histologically characterized after fixing and sectioning as for the prostatectomy specimens. In the absence of any technique to directly isolate cancer versus normal or BPH cells for culture, it was felt that starting with tissues as histologically pure as possible was the only feasible approach to establishing primary cultures representative of each histological type.

Morphology

In our hands, the "take" rate of primary cultures from normal or BPH versus malignant tissues was similar and >90% in all cases [Peehl, 1992]. As we began to compare properties of the different types of cultures, our first observation of note was that cultures from normal or BPH tissues were similar in appearance to those from cancers [Peehl and Stamey, 1986b]. While established prostate cancer cell lines such as LNCaP, PC-3, or DU 145 typically express features associated with "transformed" or malignant cells in vitro, such as rounded morphology, limited intercellular adhesions, loose attachment to the substratum, and multilayering, primary cultures from normal, BPH, and cancer are composed of monolayers of cuboidal cells. However, the overall morphology is highly dependent on culture conditions, as exemplified by growth of primary cultures in medium containing epidermal growth factor (EGF) versus keratinocyte growth factor (KGF). In medium with EGF, primary cultures are very migratory and form colonies comprised of widely separated cells, whereas in medium with KGF, cells form tight, cohesive islands [Peehl et al., 1996b]. Because established cancer cell lines are generally grown in different medium from that used for primary cultures, it is difficult to directly compare morphologies. In any case, no consistent morphological differences have been noted between primary cultures derived from normal or BPH tissues versus adenocarcinomas when grown in monolayer culture, and primary cultures from adenocarcinomas do not morphologically resemble established cancer cell lines.

Cytokeratins

When we examined primary cultures for expression of cytokeratins to demonstrate the epithelial nature of the cells, we observed that all of the cells from normal or BPH tissues expressed keratin 5 [Brawer et al., 1986], a marker of basal cells of the benign prostatic epithelium [Brawer et al., 1985]. A subset of cells in primary cultures from normal or BPH tissues also expressed keratins 8 and 18 [Peehl et al., 1994a], markers of differentiated secretory cells of the benign prostatic epithelium [Brawer et al., 1985]. In subsequent years, as specific cell populations in the prostatic epithelium have been characterized in more detail, it has become evident that the characteristics of normal and BPH primary cultures are most like that of "transit amplifying cells" in the prostate. These cells are typified by proliferation and simultaneous expression of markers of both basal and secretory cells, and are believed to be progenitor cells in the process of differentiating [Bonkhoff et al., 1994]. Analyses of additional markers of basal and secretory cells have shown that, indeed, primary cultures are populated by cells that have characteristics of both types of cells [van Leenders et al., 2000; Liu and Peehl, 2001; Goossens et al., 2002; Tran et al., 2002; Garraway et al., 2003].

Invasive prostate cancer is known to have a complete absence of basal cells (at least, basal cells as they exist in normal epithelia) [Brawer et al., 1985]. Therefore, it might be expected that primary cultures of cancer cells would not express markers of basal cells, i.e., keratin 5. However, in my lab, primary cultures derived from cancers express keratin 5 identically to cultures derived from normal or BPH tissues,

that is, keratin 5 is present in every cell [Peehl et al., 1994a]. This might a priori be taken as evidence that primary cultures from cancers are instead populated by normal cells, except for the fact that keratin expression is known to be plastic and has been widely shown to be modified by culture [Knapp and Franke, 1989]. For example, Cussenot et al. [1994] found that when normal prostatic epithelial cells were grown in medium with low calcium, the cells expressed keratins 8 and 18 but not basal cell keratin 14. Another relevant example of plasticity is the expression of keratins by the SV_{40}-immortalized human prostatic epithelial cell line, BPH-1. In culture, these cells only express the secretory cell keratins 8 and 18. Yet when combined with urogenital sinus mesenchyme and grown under the renal capsule of mice, BPH-1 cells form tumors after hormonal stimulation and express basal cell keratin as well as keratins 8 and 18 [Hayward et al., 2001]. It may be proposed that keratin 5 expression is induced in primary cultures of cancer cells upon attachment to the culture substrate or in response to some other element of the in vitro environment. It is interesting to note that prostate cancer-derived cells were reported to behave differently from normal prostate cells in collagen gels containing stromal cells, but the cancer cells still expressed basal cell keratins [Hall et al., 2002]. Expression of cytokeratins 8, 18, and 19 is also similar in normal and cancer-derived primary cultures [Peehl et al., 1996a].

Senescence

Cultures derived from primary adenocarcinomas of the prostate rarely become immortal. Generally, cells derived from normal and BPH tissues as well as from cancers undergo an average of 30 population doublings (five to six passages) before becoming senescent [Peehl et al., 1991b; Iype et al., 1998; Sandhu et al., 2000; Schwarze et al., 2001]. The few immortal lines that supposedly arose from cultures of primary adenocarcinomas are now believed to be contaminants of previously established cell lines [van Bokhoven et al., 2001]. The majority, if not all, of the bonafide cancer cell lines originated from prostatic metastases.

Tumorigenicity

Primary cultures from cancers do not form tumors when injected subcutaneously into immunocompromised mice [Peehl, 1992]. While this fact may be used as an argument against the malignant nature of these primary cultures, it is also the case that prostate cancer tissues themselves, especially from primary adenocarcinomas, rarely form tumors when transplanted into mice [Stearns et al., 1998].

Clonogenicity

The observations described above suggested that primary cultures from adenocarcinomas might indeed be populated entirely by normal cells. Since the tissues from which our cultures originated were rigorously characterized, we reasoned that any normal cells in the malignant tissues had to be a small minority, since the pathologist had confirmed that the tissues were taken from areas composed of $>90\%$ cancer. Therefore, if only normal cells were growing out from the original mixture of normal and cancer cells, then the colony-forming efficiencies of cells from cancer in primary culture should be much lower than those from normal tissues. We isolated single epithelial cells from normal tissues and cancers by enzymatic digestion, and evaluated the colony-forming efficiencies. Regardless of the tissue of origin, the colony-forming efficiency was about 5% [Peehl et al., 1988]. This suggested that small numbers of contaminating normal cells in the cancer cell populations could not be solely responsible for populating the primary cultures, because if this were the case, the colony-forming efficiencies from cancers should have been much less than from normal tissues. This evidence reaffirmed the premise that primary cultures from adenocarcinomas did indeed contain cancer cells, despite the many similarities to cultures from normal tissues.

PROLIFERATIVE POTENTIAL OF PRIMARY CULTURES AND RESPONSE TO GROWTH-STIMULATORY OR -INHIBITORY FACTORS

We continued our comparative studies of cells from normal tissues and tumors by evaluating growth parameters in culture. Established cancer cell lines are often characterized by a rapid rate of proliferation and by production of autocrine growth factors. In this regard, primary cultures of normal or BPH epithelial cells do not differ much from established prostate cancer cell lines. In optimal growth medium, the doubling time of normal or BPH cells is about

24 h, and these cells also secrete many growth factors, including members of the EGF and fibroblast growth factor families [Torring et al., 1998; Campbell et al., 1999], parathyroid hormone-related protein [Cramer et al., 1996] and interleukin-6 [Giri et al., 2001]. Tang et al. [1998] reported increased survivability of cancer cells compared to normal or BPH cells in the absence of trophic factors, but we have not observed this phenomenon. My lab has also not noted any consistent differences in growth rates between normal, BPH, and cancer-derived primary cultures, although Chopra et al. [1996] reported that primary cultures from cancer grew faster than those from normal tissues. We have also not identified any consistent differences between normal or BPH and cancer-derived primary cultures with regard to responses to growth-stimulatory factors [Peehl and Stamey, 1986a], although we and others have infrequently observed cultures derived from cancers that are unresponsive to stimulatory effects of hydrocortisone or EGF [Peehl et al., 1989; Chopra et al., 1996].

We have also never observed any consistently different response of primary cultures from normal or BPH versus cancer to growth-inhibitory factors. All cultures respond similarly to transforming growth factor-β [Peehl et al., 1989], retinoic acid [Peehl et al., 1993], interferon-γ [Peehl et al., 1994a], 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] [Peehl et al., 1994b], suramin [Peehl et al., 1991a], and agonists of peroxisome proliferator-activated receptor γ [Xu et al., 2003].

Androgen Activity

Androgen receptors are expressed in the epithelium of normal and BPH tissues and in most cancers, especially in untreated tumors [Litvinov et al., 2003]. However, androgen receptors are rarely expressed by primary cultures regardless of the histology of the tissue of origin [Peehl and Stamey, 1986a]. The absence of androgen receptors in normal cells may be explained by incomplete differentiation, since androgen receptors are expressed primarily by secretory prostatic epithelial cells [Leav et al., 1996]. Androgen receptors in cancer cells, however, might be expected to be constitutively expressed and retained in culture. While the reason for absence of androgen receptors in primary cultures is not known, a parallel may be drawn with primary cultures of mammary epithelial cells and estrogen receptors (ERs). Just as primary cultures of prostate cells do not express androgen receptors, breast cells from normal or malignant tissues do not express ERs, in contrast to the situation in tissues [Zajchowski et al., 1988]. Recently, application of novel methodology reportedly maintained expression of ERs in primary cultures of malignant breast cells [Kothari et al., 2003]; this method may have applicability to prostate cultures.

Apoptosis

Compared to established cancer cell lines, prostatic primary cultures seem much more resistant to apoptosis. Agents that induce apoptosis in cell lines often induce growth arrest instead in primary cultures. Several drugs that we identified as capable of inducing apoptosis in primary cultures, brefeldin A and triptolide, induced equivalent apoptosis in cultures from normal and malignant tissues [Wallen et al., 2000; Kiviharju et al., 2002]. Coffey et al. [2001] investigated the ability of the thiol-depleting agent diethyl-maleate (DEM) to sensitize primary cultures to radiation-induced apoptosis and found that DEM sensitized both normal and cancer-derived cultures. Lin et al. [1997] reported that thapsigargin, a calcium ionophore under development as a therapeutic agent for prostate cancer, effectively induced apoptosis in primary cultures of prostate cancer cells, but responses of normal cells were not examined.

DISTINCTIVE PROPERTIES OF PRIMARY CULTURES DERIVED FROM CANCER

As outlined above, primary cultures derived from cancer share many features in common with primary cultures derived from normal tissues or BPH. However, differences have emerged upon further investigation. These phenotypic characteristics often appear to reflect features of the tissues of origin, suggesting that primary cultures are valid models of normal, BPH, and cancer biology.

Chromosomal Aberrations

Prostatic adenocarcinomas typically contain numerous chromosomal abnormalities [Brothman et al., 1999]. In contrast, primary cultures derived from adenocarcinomas are often diploid, especially when analyzed by standard karyotyping methods [Micale et al.,

1992; Konig et al., 1993a; Ketter et al., 1996]. However, clonal chromosomal aberrations are found in a subset of primary cultures of cancer cells [Brothman et al., 1990, 1991; Limon et al., 1990; Arps et al., 1993; Jones et al., 1994; Webb et al., 1996; Chopra et al., 1997; Konig et al., 1998]. When sensitive methods such as fluorescence in situ hybridization (FISH) are employed, up to 80% of primary cultures derived from cancers have been reported to have clonal abnormalities [Brothman et al., 1992; Szucs et al., 1994]. The loss of aneuploid cells in culture has been attributed by one group of investigators to collagenase digestion of the tissue [Konig et al., 1993b], and Zwergel et al. [1998] described specific conditions to serially passage cancer cells so that they do not become diploid. The presence of chromosomal abnormalities in cancer-derived primary cultures but not in cultures from normal or BPH tissues provided the first definitive evidence that primary cultures from adenocarcinomas contained at least some cancer cells.

Integrins

One of the first biological differences reported to distinguish primary cultures of cancer cells from cultures derived from normal or BPH tissues was expression of integrins. As cell surface receptors, integrins have roles in cell migration, proliferation, and regulation of gene transcription. Alterations of integrins in cancer have been associated with tumor growth, invasion, and metastasis. The $\alpha_v\beta_3$ integrin is expressed by primary cultures of prostate cancer cells but not by normal cells [Zheng et al., 1999]. This integrin mediates adhesion and migration on vitronectin, and stimulation of this integrin can result in invasion through the basement membrane. Because of the specificity of expression of $\alpha_v\beta_3$ by cancer cells, it has been proposed that this integrin may provide a cancer-specific target for therapy [Chatterjee et al., 2001]. Primary cultures may, therefore, provide a good model system for conducting preclinical studies focusing on this receptor.

Estrogen Receptors

Two ERs have been cloned. One, ERα, was expressed by all normal and cancer-derived prostatic epithelial primary cultures analyzed by Pasquali et al. [2001]. However, several of the cancer-derived cultures expressed ERα variants, as has been found in established prostate cancer cell lines [Lau et al., 2000]. ERβ, expressed by basal epithelial cells in tissues [Leav et al., 2001], was expressed in primary cultures derived from normal tissues [Lau et al., 2000; Pasquali et al., 2001]. ERβ is for the most part absent in prostate cancer tissue, and ERβ was expressed in only one of six primary cultures of prostate cancer cells [Pasquali et al., 2001]. The biological significance of loss of ERβ from prostatic epithelial cells is indicated by the phenotype of ERβ knock-out mice [Krege et al., 1998]. These mice exhibit hyperplasia with aging, suggesting absence of antiproliferative effects of estrogen or other ligands of ERβ. The role of ERβ in prostate cancer has been extensively discussed by Signoretti and Loda [2001].

Muscarinic Cholinergic Receptors

Receptors for neurotransmitters, including muscarinic acetylcholine receptors, have been localized in the human prostate. It has been proposed that activation of muscarinic receptors via release of neurotransmitters by the autonomous nervous system may induce mitogenesis in target cells. Treatment of primary cultures of prostatic epithelial cells from BPH and cancer with a stable analog of acetyl choline stimulated proliferation of both types of cells, but cancer cells exhibited a dramatically higher response [Rayford et al., 1997]. This finding supports the concept that muscarinic cholinergic receptors may be upregulated in prostate cancer and play a significant role in tumor growth.

Three-Dimensional Cultures

Hall et al. [2002] found differences in the behavior of normal versus cancer-derived primary cultures in co-cultures with stromal cells in type I collagen gels. Stromal cells were seeded in a collagen matrix, then epithelial cells were inoculated on top of the collagen gel. If the stromal cells were from normal tissue, the normal epithelial cells formed tightly coherent colonies on top of the gel, then migrated into the gel. If the stromal cells were from adenocarcinomas, the normal epithelial cells formed a loosely associated layer of cells on the gel surface. Cancer-derived epithelial cells developed an elongated morphology and invaded the gels containing normal stromal cells. On gels with tumor-derived stromal cells, the cancer-derived epithelial cells grew as a monolayer on the gel surface. Contraction of the collagen

gels depended on the particular mix of normal and cancer-derived stromal and epithelial cells. E-cadherin expression was also strikingly different between normal and cancer-derived epithelial cells in this study, reflecting aberrations in E-cadherin that have been observed in prostate cancer [Kallakury et al., 2001]. It will be of interest to identify the molecular basis of these intriguing phenomena.

Hart et al. [2002] compared cells from BPH versus cancer grown on bone marrow stromal cells. BPH cells only formed round and compact colonies, whereas cancer cells also formed spiky colonies and caused retraction of the stromal cells. BPH and cancer cells expressed matrix metalloproteinase (MMP)-1 and -7, and urokinase plasminogen activator. If cells were grown on plastic, all three enzymes were expressed more intensely in cancer versus BPH cells; on bone marrow stroma, only MMP-1 was higher in cancer than in BPH cells. Neutralizing antibodies against the enzymes resulted in smaller colonies of both BPH and cancer cells. Lang et al. [1998] previously reported that both cancer and BPH cells grew on and invaded bone marrow stroma similarly, so invasive behavior is not confined to cancer-derived cells. My coinvestigators and I also observed that other proteases linked to invasive behavior, including cathepsin D and MMP-2 and -9, were not expressed differently between normal or BPH and cancer-derived primary cultures [Nunn et al., 1997; Wilson et al., 2002].

Hyaluronidase and Angiogenesis

Hyaluronidase is another enzyme that is differentially expressed in normal versus cancer-derived primary cultures. Hyaluronidase degrades hyaluronic acid into small angiogenic fragments and was found to be increased in prostate cancer tissues compared to normal or BPH [Lokeshwar et al., 2001]. When primary cultures were examined, cancer-derived cells were noted to secrete threefold to eightfold more hyaluronidase than cells from normal or BPH tissues [Lokeshwar et al., 2001]. Primary cultures may serve as a useful model to further investigate the role of hyaluronidase-generated fragments of hyaluronic acid in angiogenic activity in cancer.

Doll et al. [2001] measured the secretion by primary cultures of several other factors known to regulate angiogenesis. Normal cells expressed higher levels of the anti-angiogenic factor thrombospondin-1 and lower levels of the angiogenic factors, vascular endothelial growth factor, and fibroblast growth factor-2, compared to cells cultured from BPH or adenocarcinomas. Immunohistochemical staining of tissues revealed a similar pattern of differential expression, showing that the primary cultures maintained characteristic expression of these factors.

Metabolism of Vitamin D

The active metabolite of vitamin D, $1,25(OH)_2D_3$, inhibits the growth of primary cultures of normal and cancer cells equivalently [Peehl et al., 1994b; Chen et al., 2000]. Circulating $1,25(OH)_2D_3$ is manufactured in the kidney from the precursor, 25-hydroxyvitamin D_3 [$25(OH)D_3$], by the enzymatic activity of vitamin D 1α-hydroxylase. Extrarenal production of $1,25(OH)_2D_3$ also takes place in other organs, including the prostate, and primary cultures of normal prostatic epithelial cells synthesize $1,25(OH)_2D_3$ [Schwartz et al., 1998]. Accordingly, the growth of normal prostatic epithelial cells is inhibited by $25(OH)D_3$ through conversion to the active metabolite, $1,25(OH)_2D_3$, by action of 1α-hydroxylase [Barreto et al., 2000; Hsu et al., 2001]. Some investigators reported that $25(OH)D_3$ inhibited cultured prostatic cancer as well as normal cells [Barreto et al., 2000; Chen et al., 2000], but my collaborators and I had different results [Hsu et al., 2001]. We confirmed the previous finding of high levels of activity of 1α-hydroxylase in normal cells, but found that activity in the majority of primary cultures from cancer (13 of 15) was significantly decreased by as much as tenfold. This was similar to the low levels of activity reported in established prostate cancer cell lines [Schwartz et al., 1998]. Our finding was confirmed in primary cultures studied by Whitlatch et al. [2002]. The low activity of 1α-hydroxylase in cancer cells made them insensitive to growth suppressive effects of $25(OH)D_3$ due to lack of conversion to the active metabolite [Hsu et al., 2001]. Reduced activity of 1α-hydroxylase in prostate cancer may endow the malignant cells with an intrinsic growth advantage as they escape the tumor suppressor activity of locally produced $1,25(OH)_2D_3$.

Metabolism of Retinoids

Retinoids, like vitamin D, have many anticancer activities and generally inhibit growth

and increase differentiation and/or apoptosis of prostatic epithelial cells. The metabolism of vitamin A (retinol) to retinyl esters is carried out primarily by the enzyme lecithin:retinol acyltransferase (LRAT). This enzyme is reduced in many types of cancer cell lines, and it is reduced in prostate cancer compared to normal prostatic epithelium [Guo et al., 2002]. Retinol is stored as retinyl esters, which provide a pool of precursors for conversion to the active metabolite, retinoic acid. The lower concentration of retinoic acid in prostate cancer compared to normal prostate tissue [Pasquali et al., 1996] may be related to lack of LRAT in cancer. In this manner, prostate cancer cells may escape tumor suppressor activities of retinoic acid by losing LRAT activity, similar to escape from inhibitory activity of vitamin D through loss of 1α-hydroxylase. Studying retinol metabolism in primary cultures of normal prostatic epithelial cells, Lewis and Hochadel [1999] found normal retinol esterification. My colleagues and I confirmed activity of LRAT in normal cells, but found that primary cultures from cancers metabolized only trace amounts of retinol to retinyl esters and were deficient in LRAT [Guo et al., 2002].

Androgen Metabolism

Type 3 3α-hydroxysteroid dehydrogenase (aldo-keto reductase 1C2 or AKR1C2) is highly expressed in the human prostate. AKRs can convert potent sex hormones (androgens, estrogens, and progestins) into inactive metabolites, or vice versa. In a study to determine whether AKR1C2 activity could be a source of 5α-dihydrotestosterone (the ligand that activates the androgen receptor in prostatic epithelial cells) in the prostate, my collaborators and I compared levels of AKR1C2 mRNA transcripts in primary cultures derived from normal tissues versus cancer [Rizner et al., 2003]. Epithelial cells from normal tissues had low levels of AKR1C2 transcripts, with only 3 of 12 cultures showing significant RNA expression. In contrast, 14 of 14 cancer-derived cultures showed high levels of AKR1C2 RNA. This study also showed that AKR1C2 acts as a reductase and eliminates 5α-dihydrotestosterone in prostatic epithelial cells, which would reduce activity of the androgen receptor. However, in a recent study, we found that primary cultures of cancer cells have higher levels of 17β-hydroxysteroid dehydrogenase type 10 (17β-HSD10) and synthesize more 5α-dihydrotestosterone from 5α-androstane-3α,17β-diol than normal cells [He et al., 2003]. The functional and biological significance of higher expression of AKR1C2 and 17β-HSD10 in prostate cancer cells remains to be investigated.

15-Lipoxygenase-2 (15-LOX-2)

Lipid signaling molecules have been implicated in prostatic carcinogenesis. One of the major pathways that metabolizes arachidonic acid, derived from linoleic acid, the major polyunsaturated fatty acid in the human diet, involves 15-LOX-2. This lipoxygenase, which shows tissue-restricted expression in only a few organs including the prostate, produces 15(S)-hydroxyeicosatetraenoic acid [15(S)-HETE] from arachidonic acid. While 15-LOX-2 is expressed in normal prostatic epithelium, its expression is markedly decreased in prostate cancer [Shappell et al., 1999; Jack et al., 2000]. Tang et al. [2002] verified loss of 15-LOX-2 in prostate cancer tissues, then showed that 15-LOX-2 was expressed in primary cultures of normal prostatic epithelial cells. The growth of these cells was also inhibited by 15(S)-HETE, the product of 15-LOX-2 activity. Although only one primary culture from an adenocarcinoma was investigated, these cells had reduced expression of 15-LOX-2 compared to matched normal cells cultured from the same donor, and the cancer cells were more sensitive to inhibition of growth by 15(S)-HETE. These results suggest that 15-LOX-2 is a tumor suppressor, and that prostate cancer cells lose expression of 15-LOX-2 to escape the growth inhibitory activity of 15(S)-HETE, the metabolic product of 15-LOX-2.

Inducible Nitric Oxide Synthase (iNOS)

Nitric oxide is an important signaling molecule in many biological processes and iNOS is a major contributor to production of nitric oxide. A role for iNOS in tumor survival and progression has been suggested. Wang et al. [2003] demonstrated increased expression of iNOS at both mRNA and protein expression levels in prostate cancer compared to normal prostatic epithelium, and found that iNOS expression was higher in six of six cancer-derived primary cultures compared to matched normal cells from the same donor. Overexpression of iNOS may contribute to angiogenesis, tumor growth, and tumor-related immunosuppression.

CONCLUSIONS

The contribution of primary cultures to our knowledge of prostate biology has perhaps been underappreciated. This review illustrates widespread use of primary cultures that is expanding all the time. The features of primary cultures derived from normal or BPH tissues suggest that these cultures are models of transit amplifying cells of the prostatic epithelium. One goal that remains unfulfilled is to achieve complete differentiation of primary cultures to serve as an in vitro model of secretory prostatic epithelium.

While the similarities between primary cultures derived from normal tissues versus adenocarcinomas suggested that the latter were perhaps comprised of normal rather than cancer cells, mounting evidence shows that this is not the case. The many emerging cancer-related traits expressed by primary cultures from adenocarcinomas emphasize the value of this model system for prostate cancer research. However, existing data should be regarded with caution since many of the putative cancer-specific properties of primary cultures have been reported by individual labs and have not been confirmed. Similarly, lack of differential behavior of normal and cancer-derived cultures may also not be accurate if the tissues of origin were not well characterized. A cancer cell-specific marker that can be used to definitely isolate cancer cells from tissues or allow selective growth of cancer cells in culture remains to be identified.

It is noteworthy that many of the factors that are differentially expressed in primary cultures of normal versus cancer cells are enzymes. Furthermore, differential expression in culture appears to mimic differential expression in normal and malignant tissues when comparisons have been feasible. The products of many of the enzymes that are differentially lost or diminished in prostate cancer cells have tumor suppressor activities. Decreased activity of vitamin D 1α-hydroxylase, LRAT, and 15-LOX-2 would contribute to escape from local tumor suppressor activities of $1,25(OH)_2D_3$, retinoids, and 15(S)-HETE. On the other hand, the products of enzymes that are differentially increased in prostate cancer cells, such as hyaluronidase, 17β-HSD10 and iNOS, could contribute to angiogenesis, increased production of 5α-dihydrotestosterone and other tumor-promoting activities in prostate cancer. The ability to establish primary cultures from early stage cancers and from multiple donors provides the opportunity to investigate the pathological events that occur early and frequently in the development of prostate cancer.

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