Tumor and Stem Cell Biology

Novel Oncogene–Induced Metastatic Prostate Cancer Cell Lines Define Human Prostate Cancer Progression Signatures

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Abstract

Herein, murine prostate cancer cell lines, generated via selective transduction with a single oncogene (c-Myc, Ha-Ras, and v-Src), showed oncogene-specific prostate cancer molecular signatures that were recapitulated in human prostate cancer and developed lung metastasis in immune-competent mice. Interrogation of two independent retrospective cohorts of patient samples using the oncogene signature showed an ability to distinguish tumor from normal prostate with a predictive value for prostate cancer of 98% to 99%. In a blinded study, the signature algorithm showed independent stratification of reduced recurrence-free survival by Kaplan–Meier analysis. The generation of new oncogene-specific prostate cancer cell lines that recapitulate human prostate cancer gene expression, which metastasize in immune-competent mice, are a valuable new resource for testing targeted therapy, whereas the molecular signatures identified herein provides further value over current gene signature markers of prediction and outcome. Cancer Res; 73(2); 978–89. ©2012 AACR.

Introduction

Prostate cancer remains the most common nondermatologic cancer in the United States and the second leading cause of cancer-related death among men with approximately 190,000 new cases diagnosed and approximately 27,000 deaths annually. The adoption of screening with serum prostate-specific antigen (PSA) has led both to the earlier detection of prostate cancer and to the improved understanding of the biologic heterogeneity of the disease. Risk stratification following local therapy has used histopathologic criterion, Gleason scale, tumor grade, the degree of tumor differentiation, and serum PSA. The diverse clinical behavior of prostate cancer with some patients dying within 2 years of diagnosis and others living for 20 years with localized disease emphasizes the need for improved prognostic tests.

The molecular mechanisms contributing to prostate cancer recurrence and therapy resistance are poorly understood. Androgen ablation therapy results in a 60% to 80% initial response rate (1), however, approximately 30% of men who receive radical prostatectomy relapse, attributed to micrometastatic disease. Clinical classification nomograms are used that incorporate individual pre- and postoperative parameters. These clinically efficient models have limited value in predicting the diverse outcomes of patients with similar clinical, histologic, and biochemical parameters. A global pathway analysis view of the prostate cancer genome identified the most commonly altered pathways as the androgen receptor (AR), retinoblastoma (Rb), phosphoinositide 3-kinase (PI3K), and Ras/Raf signaling (2). The use of gene expression analysis has identified a subset of gene clusters that correlated in retrospective analysis with therapy outcomes (3, 4). Biomarkers derived from gene expression profiling studies that use the same dataset for signature discovery and validation (3, 5, 6) typically however, showed limited use when used to interrogate independent datasets (7).

Molecular analysis of human prostate cancer has shown increased c-Myc expression and/or copy number in up to 30% of prostate tumors. Murine prostate tumors induced by prostate-targeted c-Myc give rise to tumors with molecular features of human prostate cancer (8). c-Myc that is overexpressed in human prostate cancer is sufficient to induce prostatic intraepithelial neoplasia (PIN) in transgenic mice (8). In contrast, Ras mutations are relatively uncommon, however, induction of Ras effector pathways has been revealed by microarray and genomic analysis (2). Upregulation of Ras-mediated signaling cascades may also reflect the overexpression of autocrine and paracrine factors [EGF receptor (EGFR), TGF-α; ref. 9]. Although there are conflicting results over whether ErbB2 is overexpressed or amplified in prostate cancer, a population of patients with prostate cancer showed a gene expression pathway of activated ErbB2 signaling (10). Activation of the Src pathway is also found in primary prostate cancer associated with a truncated version of c-kit (11), and a subset of human prostate cancers expresses a Src signature (10).

The current studies were conducted to address several key needs. First, the development of new therapies for prostate cancer remains a significant challenge. Second, the identification of biomarkers to distinguish tumor from normal prostate is desired. Third, the ability to accurately characterize the molecular profile of prostate cancer could lead to improved understanding of the biologic heterogeneity of the disease. Finally, the ability to stratify patients based on these molecular characteristics could inform treatment decision making.
cancer requires model systems that closely resemble human disease. Unfortunately, to date there have been several limitations with currently available cell lines. Although important transplantation experiments have been conducted using human prostate cancer cell lines in immune-deficient animals, the immune system plays an important role in prostate cancer onset and progression (12, 13) making it imperative to develop prostate cancer cell lines that can be studied in immune competent animals. Second, genome-wide expression studies of prostate cancer cell lines showed only a small number of genes with concordant expression in malignant prostate tissue (14). Third, prostate cancer in humans is associated with bone and lung metastasis, however, only a single cell line, a product of Myc and Ras transduction has been developed that develops bone metastasis in immune-competent mice (15). Fourth, although the transgenic mouse had been effectively deployed as a model to study the molecular basis of human cancers, the prostate cancer mouse models have long latency and often unpredictable metastasis (16).

The current studies aimed to generate oncogene-specific cell lines that could be grown in immune-competent mice, determine whether the lines recapitulate human prostate cancer, and validate the signature in distinct populations of patients as a prognostic test. Herein, oncogene transduction of murine prostate epithelium induced prostate cellular transformation with invasive and metastatic properties. Oncogene-specific molecular signatures were defined that distinguished benign from malignant human prostate and predicted outcome of patients with human prostate cancer. The generation of oncogene-specific prostate cancer cell lines that recapitulate molecular signatures of human prostate cancer will be of value in understanding molecular mechanisms and identification of new therapies for this disease.

Materials and Methods

Mice, cell culture, chemicals, and reagents

The experimental procedures with transgenic mice were approved by the ethics committee of Thomas Jefferson University (Philadelphia, PA). Mice were in the FVB strain. Mouse prostate epithelial cell cultures were isolated from prostate glands of 12-week-old male mice and maintained as previously described (17) and analyzed after 25 passages with at least 3 lines of each genotype. Transduction of cells by the retroviral expression vectors encoding a single distinct oncogene (c-Myc, Ha-Ras (V-12), v-Src, and NeuT, an activating mutant of ErbB2). The cellular morphology of the prostate epithelial cells was altered over the 4-week period (Fig. 1A). Nontransformed PECs were characterized for each oncogene (Fig. 1E). Nontransformed PECs were characterized for each oncogene (Fig. 1E). Nontransformed PECs were characterized for each oncogene (Fig. 1E). Nontransformed PECs were characterized for each oncogene (Fig. 1E). Nontransformed PECs were characterized for each oncogene (Fig. 1E).

Cellular growth assays

Cells were seeded in 24-well-plates at a concentration of $1 \times 10^5$ cells per well, with each sample in triplicate for 7 days. Transformed cells were grown in Dulbecco’s Modified Eagle’s Medium with 10% FBS, whereas control prostate epithelial cell (PEC) cells were cultured in prostate epithelial primary culture medium. Cells were harvested, suspended in 100 μL PBS, and an equal volume of 0.4% Trypan blue added after 5 minutes. Cells were counted using a Countess Automated Cell Counter (C10227, Invitrogen).

Colony formation in soft agar

Cells (3 × 10^3/mL) were seeded into 0.3% soft agar Sigma) in a suspension dish (Nalgene Nunc International). Colonies were stained by 0.04% crystal violet acetate and counted under a vertical microscope after 2 weeks of incubation.

Tumor formation assay and detection of lung metastasis

A total of 1 × 10^6 cells suspended in a 100 μL volume were injected subcutaneously into 7 to 8 week-old FVB male mice. The cell suspension was mixed with a 20% by volume BD Matrigel (BD Biosciences), resulting in a final cell concentration of 10^5 cells/mL. Tumor growth was measured by Vernier calipers twice a week. Tumor samples were harvested after 30 days (except NeuT-induced tumors, which were harvested after 16 days). Thirty mice were examined. At autopsy the presence of lung metastasis was examined by visualization and palpation. All lungs were subsequently analyzed for metastasis by histologic sections of hematoxylin and eosin–stained specimens. The prostate cancer lung metastases were confirmed by a trained pathologist to show similar histology to the parental line, with migration of cells through the basement membrane. Ten sections were examined per lung at 100-μm separation. No formal analyses of bone metastasis or lymph node infiltration were conducted in these studies.

Statistical analysis

Comparisons between groups were analyzed by 2-sided t test. A difference of $P < 0.05$ was considered to be statistically significant. All analyses were done with SPSS 11.5 software. Data are expressed as mean ± SEM.

Results

Oncogene-transformed prostate cell lines convey contact-independent growth

Primary prostate epithelial cell cultures were established from the ventral prostates of FVB mice. Cells were transduced with retroviral expression vectors encoding a single distinct oncogene (c-Myc, Ha-Ras (V-12), v-Src, and NeuT, an activating mutant of ErbB2). The cellular morphology of the prostate epithelial cells was altered over the 4-week period (Fig. 1A). Individual colonies of oncogene-transduced cells were selected and characterized. Cellular growth assays were conducted by cell counting (Fig. 1B). A substantial growth advantage was observed in each oncogene-transduced cell line compared with primary prostate epithelial cells.

Western blot analysis was conducted to examine the relative expression of each of the oncogenes used to transduce the PEC. The presence of oncogenic c-Myc, Ha-Ras, ErbB2, and v-Src was identified by Western blot analysis (Fig. 1C). The increase in abundance of each oncogene was specific to each cell line (Fig. 1C). The prostate tumor cell lines expressed prostate epithelial cell markers including cytokeratin 5 (CK5) and CK8, and were either AR-positive (NeuT and v-Src) or AR-negative (c-Myc and Ha-Ras; Fig. 1D). Oncogene transfection of fibroblasts or murine epithelial cells conveys contact-independent growth in solid agar. The oncogene-transduced PEC lines were examined for growth in soft agar. Colony size and number were characterized for each oncogene (Fig. 1E). Nontransformed PECs...
failed to grow in soft agar as previously described. Oncogene transduction increased the size and number of colonies (Fig. 1F).

As changes in genome copy number and/or genome structure contribute to tumor progression (20, 21), we conducted genome copy number analysis using array comparative genomic hybridization (CGH; see Materials and Methods). The cell lines, such as human prostate cancer, exhibited genomic gains and losses (Fig. 2A and B). Genome abnormalities were similar within each oncogene-transformed line. The genome copy number abnormality profiles showed distinct levels of genomic complexity. Greater complexity and number of abnormalities was identified in the NeuT lines, followed by the c-Myc lines. Ha-Ras lines showed low-level abnormalities (Fig. 2A). Copy aberration associated with the human prostate cancer derived from 205 primary and metastatic prostate cancer samples (Materials and Methods), were then compared with the genomic aberration of the oncogene-transformed cell lines (Fig. 2C and D). The frequency and copy aberrations associated with the human prostate cancer samples are shown on the right of each schematic. Significant fidelity of conservation between genomic alterations in the prostate cell lines and alterations...
found in human prostate cancer was observed. Analysis of the individual genes within the regions deleted or amplified for each cell line was conducted and tabulated (Supplementary Table S1). Approximately 2,400 genes are included within these regions. A comparison of gene expression and copy number showed the relative frequency of coincident changes (Fig. 2D).

**Lung metastases of prostate cancer cell lines**

Tumor formation studies were conducted in FVB mice. Each of the prostate tumor lines grew subcutaneously in immune-competent mice. Growth was sustained for c-Myc-, Ha-Ras-, and v-Src-transformed PECs (Fig. 3A). The extirpated tumors were hemorrhagic (Fig. 3B) with histologic features of prostate adenocarcinoma (Fig. 3C and Supplementary Fig. S1). Immunostaining of tumors for Von Willebrand factor (VWF) confirmed angiogenesis and showed significantly greater VWF staining in tumors induced by Ha-Ras (Supplementary Fig. S2A). Lung metastases were characterized at autopsy by histopathologic assessment as described in the Materials and Methods (Fig. 4A). The

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**Figure 2.** Copy number aberrations in the 4 oncogene cell lines assessed by array CGH. A, the percentage of the 4 cell lines sharing copy gain or loss regions is shown as a function of genomic position. B, regions of copy gain (red) or loss (blue) for each of the 4 cell lines are shown as a function of genomic position. C, oncogenes are identified with mRNA overexpression (red), DNA amplification (yellow), or both (purple) among the 4 oncogene cell lines, with corresponding amplification in the Memorial Sloan-Kettering Cancer Center (MSKCC) prostate cancer database (listed on right-hand side). D, tumor-suppressor genes are identified with mRNA underexpression only (blue) or both mRNA underexpression and DNA copy loss (orange) among the 4 oncogene cell lines, with corresponding copy loss in the MSKCC prostate cancer database (listed on right-hand side). PCs, prostate cancer.
number of lung metastases derived from subcutaneous injection of the primary PECs was increased in the Ha-Ras, v-Src, and c-Myc sublines (Fig. 4B).

**Oncogene-specific molecular signatures in prostate cancer cell lines**

To further characterize the molecular genetic signaling pathways regulated by specific oncogenes in prostate epithelial cells, mRNA was prepared from the oncogene-transformed PEC cell lines. Microarray analysis identified a total of 2,635 of 22,115 genes that were significantly altered in expression (at least 2-fold change) in oncogene overexpressing cell lines when compared with nontransformed prostate epithelial cell control samples (Fig. 5A). The rows of the heatmap represent unique genes and are displayed by their pattern of up- and down-regulation for all 4 of the oncogene-induced cell lines. For example, group 1 contains genes that share concordant differential expression patterns across all 4 cell lines, whereas group 15 contains genes whose differential expression is specific to the v-Src cell lines. A sizable number of up- and downregulated genes were shared among all 4 cell lines (group 1; 251 genes). Genes with up and downregulation specific to Ha-Ras were the most prevalent (group 14; 584 genes), followed by c-Myc-specific genes (group 8; 332 genes), NeuT-specific genes (group 12; 277 genes), and v-Src-specific genes (group 15; 215 genes).

![Figure 3. Prostate epithelial cell lines grow in immune competent mice. A, PEC tumor diameter determined by Vernier caliper measurement is shown as days after inoculation in FVB mice. The diameter mean ± SEM for \( N > 5 \) separate experiments. B, photograph of representative tumor derived from oncogene-induced lines. NeuT-induced tumors were harvested at 15 days after cell injection. C, hematoxylin and eosin staining at low and high magnification (see also Supplementary Fig. S1).](image)
The murine prostate oncogene expression signature in high grade and advanced-stage human prostate cancer

The prostate "oncogene expression signature" (group 1) was defined as genes that were significantly altered in expression level and that were uniquely altered in expression by a specific oncogene compared with primary prostate epithelial cells (Fig. 5A). The oncogene expression signature was compared with gene signatures obtained from other published databases to identify similarities to other well-studied disease phenotypes and cell lines. Gene ontology enrichment analysis for each of the cell lines identified enrichment for pathways involved in tumor progression. The top 40 gene ontology biologic process terms sorted on the basis of their overall significance in the 4 oncogene cell lines are shown in Supplementary Fig. S3 and show enrichment of pathways involved in cellular proliferation, DNA replication, and reduced expression of pathways promoting adhesion and epithelial cell differentiation. Comparisons were conducted against gene signatures representative of differential expression in advanced state versus early-stage prostate cancer, high grade versus low-grade prostate cancer, recurrent versus nonrecurrent prostate cancer (4).

The gene signature heatmaps representing advanced stage/early stage, high grade/low grade, and recurrent/nonrecurrent prostate cancer phenotypes (4; Fig. 5B and C) are shown on the left and the heatmaps on the right represent genes that are differentially expressed in the prostate oncogene expression signature. The heatmaps are labeled with the percentage of genes within the "oncogene expression signature" that are differentially expressed. P values for the statistical significance of the similarity between the genes expressed in the prostate cancer cell lines and the gene signature of the disease phenotype are shown. P values are based on the hypergeometric distribution and represent the probability of these genes being differentially expressed in the disease phenotype if they were selected at random.

A "high grade" prostate cancer gene signature was previously determined from 61 primary prostate tumors (4). Figure 5B shows that 34 genes from the prostate oncogene expression signature were common to the "high grade" gene signature ($P = 2.97 \times 10^{-3}$). For each oncogene-induced prostate cancer cell line, the proportion of significant genes contributing to that cell line is shown. For example, the overlap between the
prostate oncogene expression signature and high-grade disease includes a combination of genes that are significant genes in c-Myc (47%), NeuT (53%), Ha-Ras (71%), and v-Src (62%). Figure 5C depicts the 72 genes that were common between the prostate oncogene signature and the “advanced stage” gene signature ($P = 4.13 \times 10^{-8}$). These results indicate a significant degree of similarity between the prostate oncogene signature and the “advanced stage” gene signature ($P = 4.13 \times 10^{-8}$) and between the oncogene expression signature and the advanced-stage disease phenotype ($P = 4.13 \times 10^{-8}$).

No significant overlap was identified between the prostate oncogene expression signature and the recurrent/nonrecurrent disease signature identified by Lapointe and colleagues (4). When the prostate oncogene expression signature was compared with the Lapointe data, the Ha-Ras cell line captured the highest level of similarity with high-grade disease (71%), whereas the v-Src cell line showed the highest similarity with advanced-stage disease (67%).

c-Myc–specific gene expression signature in prostate cancer epithelial cells resembles the c-Myc signature in fibroblasts and mammary tumors

A comparison was made between gene expression pathways regulated in the c-Myc cell line with previous studies of gene
expression in transgenic mice expressing the c-Myc oncogene targeted to the prostate under control of the probasin promoter (8) double transgenic mice expressing c-Myc and NKX3.1 (22) and curated gene signatures (23; Supplementary Fig. S4A). These studies showed a significant concordant correlation between c-Myc–repressed and c-Myc–induced genes in the c-Myc PEC line, with the gene expression profiles of the transgenic mice and several curated gene signatures (Supplementary Fig. S4B).

In previous studies, we identified gene expression signatures that were specific to the oncogene used to transform fibroblasts (3T3 cells), which were recapitulated in mammary tumors induced by c-Myc or Ha-Ras (24). The previously defined Ha-Ras- and c-Myc–induced molecular signature was compared with the gene expression signature induced by these oncogenes in the prostate cancer epithelial cells (oncogene superset in Fig. 5A). A comparison was conducted of the Ha-Ras prostate oncogene expression signature against Ha-Ras–transduced fibroblasts (Fig. 6A). Significant overlap was identified between the prostate oncogene expression signature and genes differentially expressed upon Ras oncogene transduction in the mouse fibroblasts (33 genes, hypergeometric \( P = 3.7 \times 10^{-3} \)).

The heatmaps in Fig. 6B depicts the genes shared between c-Myc–transduced fibroblasts (Fig. 6B), c-Myc–induced mammary tumors (Fig. 6C; left-hand heatmaps), and the c-Myc–induced prostate oncogene expression signature (right-hand heatmaps). A significant overlap was identified between the c-Myc oncogene expression signature (Superset) and the genes differentially regulated by c-Myc transduction in mouse fibroblasts (108 genes, hypergeometric \( P = 5.84 \times 10^{-12} \)) or mammary tumors (363 genes; hypergeometric \( P = 7.5916e^{-012} \)). Within the prostate oncogene expression signature, c-Myc cell lines showed the largest proportion of similarity with both the Myc-transduced fibroblasts (92%; Fig. 6B) and the c-Myc–induced mammary tumors (85%; Fig. 6C).

Kaplan–Meier analysis was used to evaluate the difference in recurrence-free survival associated with high expression versus low expression of these genes. Genes that correspond to the 14 most upregulated genes of the c-Myc signature were used to assign the samples as high (upper 25th percentile) or low (lower 75th percentile). Genes that were highly expressed in the c-Myc signature had a significant association with poor outcome when used to interrogate the clinical dataset of Taylor and colleagues (\( P < 0.005; \) Fig. 6D). Comparison was made between the c-Myc signature and a recently described gene prognostic signature (25) by using these signatures to interrogate a clinical dataset (2). The comparison of the c-Myc signature identified herein, compared favorably as a discriminator of outcome (Supplementary Fig. S5).

Figure 6. c-Myc- and Ha-Ras–specific oncogene signatures in prostate tumors are conserved in other tissues. Heatmaps show genes that are differentially expressed in the oncogene-induced prostate cancer cell lines and in Ha-Ras (A) and c-Myc (B) fibroblasts (3T3 cell line). C, a heatmap shows the intersection of genes that are differentially expressed in the c-Myc prostate cancer cell line and mouse mammary tumor samples. The \( P \) values shown under each prostate cell line heatmap represent the significance of the overlap between the prostate and fibroblast/mammary tumor signatures. WT, wild type D, Kaplan–Meier curves are shown for high (upper 25th percentile) and low (lower 75th percentile) expression populations for the c-Myc overexpression signature used to interrogate the clinical data of ref. 2.
To examine the relationship between genes expressed in the oncogene-transformed cell lines and survival rates from human prostate cancer, a previously published microarray dataset of human prostate tumor samples with known clinical recurrence-free survival time was used (7). Figure 7A provides a heatmap showing the expression profiles of genes in the human prostate cancer samples from the Glinsky’s dataset, which was upregulated in the c-Myc (Fig. 5A, group 8) oncogene-transformed prostate cancer cell lines.

Canonical analysis was conducted using the c-Myc–specific genes that were also found to be differentially expressed between tumor and normal samples in 2 human prostate datasets. The first canonical variable, resulting from this analysis, was used to discriminate tumor from normal samples in each human prostate dataset. A plot of tumor and normal samples along the first canonical variable axis revealed a pronounced separation between tumor and normal samples (Fig. 7B). Receiver operating characteristic (ROC) curves have been used previously to evaluate the diagnostic ability of PSA (26, 27) as well as its ability to identify metastatic disease (28).

Figure 7. The c-Myc–specific expression profile distinguishes tumor from normal tissue. A, hierarchical clustering conducted in the subset genes exclusively deregulated in the c-Myc cell lines separates normal (green), localized tumor (light blue), and tumor metastasis (pink) samples. B, a classifier based on canonical analysis of c-Myc signature distinguishes human tumor (red) from normal tissue (light blue), along the x-axis, in the Lapointe and colleagues dataset. ROC curves for the classifier performance are shown for the Lapointe and colleagues dataset (C) and the Taylor and colleagues MSKCC dataset (D), with AUC values of 0.990 and 0.977, respectively.
A ROC analysis of tumor and normal samples, separated by the first canonical variable, produced area under the curve (AUC) values of 0.977 and 0.990 for the Taylor and colleagues (Fig. 7C) and Lapointe and colleagues (Fig. 7D) datasets, respectively (2, 4).

Discussion

The relative paucity of murine prostate cell lines that can be grown in immune-competent mice to form metastases has limited preclinical studies of prostate cancer therapies in vivo. The immune system plays an important role in the onset and progression of prostate cancer (12). The current studies were conducted to generate murine prostate cancer cell lines that could be studied in immune-competent mice. The prostate cancer cell lines generated herein resembled human prostate cancer histologically, and grew in immune-competent mice in a reproducible manner and formed lung metastasis. The gene expression changes and genomic rearrangements resembled human prostate cancer. By more faithfully recapitulating the tumor host environment, these lines provide a valuable advance for preclinical testing new therapies in vivo.

These studies identified patterns of gene expression within newly engineered prostate cancer cell lines that are observed in human prostate cancer and are associated with poor outcome (Supplementary Fig. S5C). The algorithm distinguished recurrence-free survival (prognostic signature) and with superior characteristics to several published signatures (23, 25) and Gleason scale. Prior studies have shown a paucity of genes with concordant expression in established prostate cancer cell lines and human prostate cancer (29). Herein, the generation of oncogene-specific transformed prostate cancer cell lines allowed a comparison with the molecular signatures of human prostate cancer. Comparison was made between whole tumor mRNA in our studies and whole tumor mRNA in the clinical studies (not laser capture microdissected samples). The prostate oncogene expression signature correlated with a previously defined high-grade prostate cancer and with advanced stage in a second patient population. The c-Myc signature correlated with the signature induced by c-Myc when targeted to the prostate gland in transgenic mice (Supplementary Fig. S4A and S4B) and gene enrichment analysis identified significant overlap with several recent published datasets and transgenic models of prostate cancer (8, 23). The c-Myc signature was used to derive algorithms that distinguished normal from cancerous prostate tissue with approximately 99% predictive value (diagnostic signature).

Herein, transduction of primary prostate epithelial cells of FVB mice by a single oncogene was sufficient for transformation. Oncogene genomic analysis identified loss of tumor suppressor regions and amplification of additional oncogenic regions that resemble genomic changes in human prostate cancer (Fig. 2). The cell lines reflect both AR-response and AR-unresponse prostate cancers (Supplementary Fig. S6) although each line expresses plasma Kallikrein 1B (PK1B; Supplementary Fig. S2B). Kallikrein gene expression is regulated by basal and androgen-independent factors, thus PK1B in the tumors may not necessarily be correlated directly with AR expression (Fig. 1D). The tumors expressed CK8, whereas the c-Myc and v-Src lines expressed CK5 by Western blot analysis (Fig. 1D) and by immunofluorescence in the primary cell lines (Supplementary Fig. S7A) as well as in the lung metastasis (Supplementary Fig. S7B). CK5 is considered a marker of basal cell type origin of prostate cancer and is more common in metastatic prostate cancer and after androgen deprivation (30). In previous studies, prostate epithelial cells have been transformed with DNA tumor viruses and murine prostate cancer cell lines have been derived from oncomice (31). Human telomerase reverse transcriptase (hTERT) together with SV40T/t immortalizes human PEC and together with either Ha-Ras or c-Myc results in cells that grow in tissue culture. However, unlike the lines developed herein, these PEC lines were unable to grow tumors after subcutaneous implantation or form metastasis in vivo (32).

The transition of normal somatic epithelial cells into an immortalized metastatic cell requires dysregulation of distinct cellular processes including cell survival motility, cell proliferation, altered metabolism, and angiogenesis. The transformation of human and murine cells has distinct requirements (33, 34). Human fibroblasts can be transformed by 2 oncogenes (35). Primary human cells can be immortalized by the introduction of the catalytic subunit of hTERT and SV40 large-T to inactivate the p53 and p53 pathways (36). Immortal human cells are converted into transformed cells with tumorigenic growth by the further induction of an oncogenic Ha-Ras allele and the SV40 early region oncprotein small t antigen (35, 37, 38). The requirements for transformation are cell type specific (34). Human mammary epithelial cells immortalized by SV40 and hTERT undergo tumorigenic conversion with oncogenic Ras (36) or by the use of a mutant p53, a CDK4 mutant and cyclin D1 overexpression (39). SV40 Large T antigen contributes to transformation through inactivation of p53 and pRb, as attested by siRNA analysis. Cautionary caveats to the use of these cell lines include the potential importance of the local prostate microenvironment in progression of prostate cancer, the capacity of the FVB murine strain background to enhance tumor progression (40) and the tendency of human prostate cancer metastasis to target bone rather than lung (41). The mechanisms governing the spontaneous regression of the NeuT lines remains speculative at this time.

The current studies defined oncogene-specific signatures and then mapped these signatures to human prostate cancer. The oncogenes were chosen because of evidence that these oncogenes and/or tumor pathways are activated in human prostate cancer. The c-Myc signature was conserved in prostate, breast, and fibroblasts. The c-Myc gene copy number is increased in up to 30% of cases at the preneoplastic stage in patients with PIN (42, 43). The v-Src, ErbB2, Ha-Ras oncogenes have also been implicated in human prostate cancer, and a truncated version of c-kit identified in primary prostate tumors correlates with activation of the Src pathway (44). Although the role of ErbB2 in human prostate cancer is controversial, a subset of patients with prostate cancer expresses ErbB2 and its pathway, and activated ErbB2 is sufficient to induce prostate cancer in transgenic mice (45, 46). In vivo, the activating Ras mutation (Ha-Ras V12) induced low-grade PIN in mice when directed to prostate epithelial cells under the control of the minimal probasin promoter (47).
The current studies identified oncogene-specific signatures in murine prostate epithelia that were recapitulated within human prostate cancer samples. Oncogene-specific signatures have been proposed to exist within human prostate cancer (10). In human breast cancer, the identification of tumor subsets with patterns of transcriptional and biologic activity has allowed a deeper understanding of the disease. It has been anticipated that the identification of these patterns of gene function in otherwise heterogeneous biologic material may enable the identification of targets for therapies, and to thereby improve management of prostate cancer. The development herein of oncogene-specific prostate cancer cell lines may be of value in developing targeted therapies for prostate cancer.

References


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