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Molecular Characterization of Prostatic Small-Cell Neuroendocrine Carcinoma

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OBJECTIVES. A subset of prostate carcinomas is composed predominantly, even exclusively, of neuroendocrine (NE) cells. In this report, we sought to characterize the gene expression profile of a prostate small cell NE carcinoma by assessing the diversity and abundance of transcripts in the LuCaP 49 prostate small cell carcinoma xenograft.
METHODS. We constructed a cDNA library (PRCA3) from the LuCap 49 prostate small cell xenograft. Single pass DNA sequencing of randomly selected cDNA clones followed by sequence assembly and annotation produced a library of Expressed Sequence Tags (ESTs) representing the LuCaP 49 transcriptome. Comparative sequence analysis with ESTs derived from prostate adenocarcinoma libraries was performed using statistical algorithms designed to identify differentially expressed sequences. Putative NE cell-specific genes were further examined by Northern analysis.
RESULTS. Sequence assembly and analysis identified 1,447 distinct genes expressed in the LuCaP 49 cDNA library. These include cDNAs encoding the NE markers secretogranin (SCG2), CD24, and ENO2. Northern analysis revealed that three additional genes, ASCL1, INA, and SV2B are expressed in LuCaP 49 but not in various prostate cancer cell lines or xenografts. Fifteen genes were identified with a statistical probability ($P > 0.9$) of being up-regulated in LuCaP 49 small cell carcinoma relative to prostate adenocarcinoma (two primary prostate adenocarcinomas and the LNCaP prostate adenocarcinoma cell line).
CONCLUSIONS. Prostate small cell carcinoma expresses a diverse repertoire of genes that reflect characteristics of their NE cell of origin. ASCL1, INA, and SV2B are potential molecular markers for small cell NE tumors and NE cells of the prostate. This small cell NE carcinoma gene expression profile may yield insights into the development, progression, and treatment of subtypes of prostate cancer. *Prostate 55: 55–64, 2003.* © 2003 Wiley-Liss, Inc.

KEY WORDS: **xenograft; cDNA; expressed sequence tag; digital expression; database**

INTRODUCTION

The prostate epithelium is composed of three primary cell types: basal cells, luminal secretory cells, and neuroendocrine (NE) cells. NE cells display hybrid epithelial/neural/endocrine characteristics and have variably prominent dendritic processes [1,2]. Based on ultrastructural studies, two subtypes have been identified, an open subtype with apical processes extending to the glandular lumen and a closed subtype [1,2]. Ultrastructural studies, biochemical analyses, and histochemical staining provide evidence for functionally

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diverse subtypes of NE cells within the prostate [3,4]. These cells secrete a wide range of peptides known to stimulate cell growth (and perhaps cell secretion) in an autocrine and paracrine fashion. However, the role of NE cells in both normal prostate development and in prostate carcinogenesis is poorly understood [5,6].

NE differentiation in prostatic malignancy has been grouped into three categories: (1) focal differentiation of cells with NE features in a conventional adenocarcinoma, (2) carcinoid tumor of the prostate, and (3) small cell undifferentiated NE carcinoma of the prostate [7]. Rare NE cells are found in virtually all prostate adenocarcinomas. Carcinoid tumors are usually foci within a conventional adenocarcinoma; pure prostate carcinoids are extremely rare. Small cell undifferentiated carcinoma is rare, representing only 1–2% of all prostate malignancies. Although small cell undifferentiated carcinoma is most often seen as a component of a conventional adenocarcinoma, the pure small cell tumor has an aggressive course [8,9].

Several model systems for studying the role of NE cells in prostate cancer have been described. One approach has been to express genes with oncogenic potential in mice using heterologous promoters [10–15]. Most of these transgenic models used promoters that do not restrict gene expression to the prostate. Employing a more directed approach, Masumori et al. [15] used the rat probasin promoter to drive expression of the SV40 large T antigen specifically in prostate epithelium. With advancing age, low-grade prostatic intraepithelial neoplasia (PIN), high-grade PIN, microinvasion, invasive carcinoma, and poorly or undifferentiated carcinoma with NE differentiation developed in the prostates in sequential order. Alternatively, studies of human prostate cancers implanted into immune deficient mice have also provided insights into the role of NE cells in prostate carcinogenesis. Androgen deprivation of the prohormone convertase-310 human prostate cancer xenograft induces NE differentiation without proliferation, and may serve as a model for the role of NE cells in hormone refractory prostate cancer [16]. Three androgen-insensitive small cell prostate cancer xenografts have been described (UCRU-PR-2, WISH-PC2, and LuCaP 49) that are capable of proliferative growth [17–20]. These xenografts are composed of actively dividing NE-like cells that express a variety of NE-enriched molecular markers, but otherwise, little is known about the genes that they express.

LuCaP 49 is a xenograft that exhibits a rapid growth rate (doubling time 6.5 days) and is composed almost exclusively of cells with a NE/small cell carcinoma phenotype [19]. As such, LuCaP49 provides a rare opportunity to study the repertoire of genes expressed in NE-like cells of the prostate. Here, we report the isolation and characterization of 2,096 Expressed Sequence Tags (ESTs) from a LuCaP 49 cDNA library that identifies 1,447 distinct genes. Together, these sequences represent a partial transcriptome reflecting the diversity and relative abundance of the genes and their cognate transcripts that are expressed in prostate small cell carcinoma. Many of these genes are expressed in other cell types, but several are highly enriched in NE cells. In addition, a statistical analysis of EST frequencies was used to identify genes that are expressed at higher levels in LuCaP 49 than in primary prostate adenocarcinomas and in the LNCaP prostate adenocarcinoma cell line. These expression differences may reflect unique features of small cell prostate cancers that can further the understanding of the role of NE cells in the development of small cell carcinoma and adenocarcinoma of the prostate.

MATERIALS AND METHODS

The establishment and characterization of the LuCaP 49 NE small cell xenograft is described in detail elsewhere [19]. LuCaP 49 was derived from an omental mass removed during surgery. The tumor was isolated from a 71-year-old male originally diagnosed with clinical stage B-II prostate carcinoma 4 years prior to obtaining the tumor. The xenograft was established in Fox Chase CB.17 SCID mice (Charles River Laboratories, Wilmington, MA) and has been serially passaged for 5 years. Histological analysis of sections adjacent to flash-frozen tissue revealed predominantly NE cells interspersed with approximately 5% mouse stromal cells.

PolyA+RNA was isolated from a LuCaP 49 xenograft sample using Trizol reagent (Life Technologies, Carlsbad, CA) and oligo-dT columns (Life Technologies, Carlsbad, CA). A cDNA library designated PRCA3 was constructed in the pSPORT vector according to protocols we have previously described [21]. DNA sequencing and Northern blot analysis was performed by standard methods as described in Clegg et al. [22]. Details of the PRCA3 library construction are also available at http://www.pedb.org. The average insert size of PRCA3 cDNA clones is 1.2 kb.

DNA sequences were stored, clustered, and annotated using the Prostate Expression Database (PEDB) and associated data analysis tools [23,24]. Briefly, vector, *E. coli* and interspersed repeats were masked in ESTs using Cross_Match (bozeman.mbt.washington.edu/phrap.doc/general.html) and RepeatMasker (ftp.genome.edu/RM/RepeatMasker.html). Phrap (P. Green, University of Washington, Washington), which incorporates estimates of sequence quality, was used to cluster the masked sequences and generate a consensus sequence for each assembly. Each distinct cluster was annotated by searching Unigene

[25], GenBank [26], or dbEST [27] using BLASTN (http://blast.wustl.edu). Annotations were assigned using a Perl-based script to select the lowest *P* value where the maximum *P* value was e-20 and the minimum BLAST score was 300. The biological role for each species was assigned using the categories described in Adams et al. [28].

SAGE libraries SAGE_PR317_prostate_tumor and SAGE_PR317_normal_prostate are listed at the NCBI Library Browser web site (www.ncbi.nlm.nih.gov/SAGE/sagelb.cgi); expression tags were downloaded from SAGEmap's anonymous FTP site (ftp://ncbi.nlm.nih.gov/pub/sage/seq/). EST sequences for lung cancer libraries NCI_CGAP_Lu5, NCI_CGAP_Lu6, NCI_CGAP_Lu24, NCI_CGAP_Pr3, and NCI_CGAP_Pr12 are listed at http://cgap.nci.nih.gov/Tissues/LibraryFinder. Pr12 and Pr3 sequences are also stored in the PEDB. Comparisons between sequence tags (ESTs or SAGE tags) were performed using the method of Audic and Claverie [29], which allows statistical analysis of small samples of expression tags.

RESULTS

The prostate small cell cancer xenograft LuCaP 49 was used to construct a cDNA library (PRCA3). Morphological and immunohistochemical data show that LuCaP 49 is nearly identical to the primary tumor from which it was derived, hence it is a good source of material for the study of NE gene expression in the prostate [19]. In brief, both the xenograft and the primary tumor are composed of undifferentiated cells characterized by a high nuclear:cytoplasmic ratio, and nuclei with a fine heterochromatin pattern and inconspicuous nucleoli (Fig. 1). Both the original tumor and the xenograft express the NE markers synaptophysin and neuron specific enolase in similar numbers of cells (40–80% and 80%, respectively); and neither expresses the adenocarcinoma markers PSA and the androgen receptor. One difference between the primary cancer and the xenograft is that fewer xenograft cells express the NE marker Chromogranin A (Fig. 1).

Clones from the PRCA3 library were randomly selected and partially sequenced to generate 2,096 high quality ESTs representing a partial transcriptome of this tissue. ESTs were assembled using the Phrap sequence assembly program to produce 1,577 clusters. Each cluster was used to query the Unigene, non-redundant Genbank, and dbEST databases. Based on shared annotations, the clusters were further consolidated and assigned to 1,447 distinct transcripts. Twenty-four transcripts were of murine origin. These presumably represent tissue contamination from the xenograft host. For classification purposes, the remaining 1,423 are assumed to be of human origin; however, 148 transcripts were not homologous to any known nucleotide or protein sequence recorded in the public databases, and an undetermined number of these could be from murine mRNAs. Alternatively, the unannotated species may represent novel human transcripts.

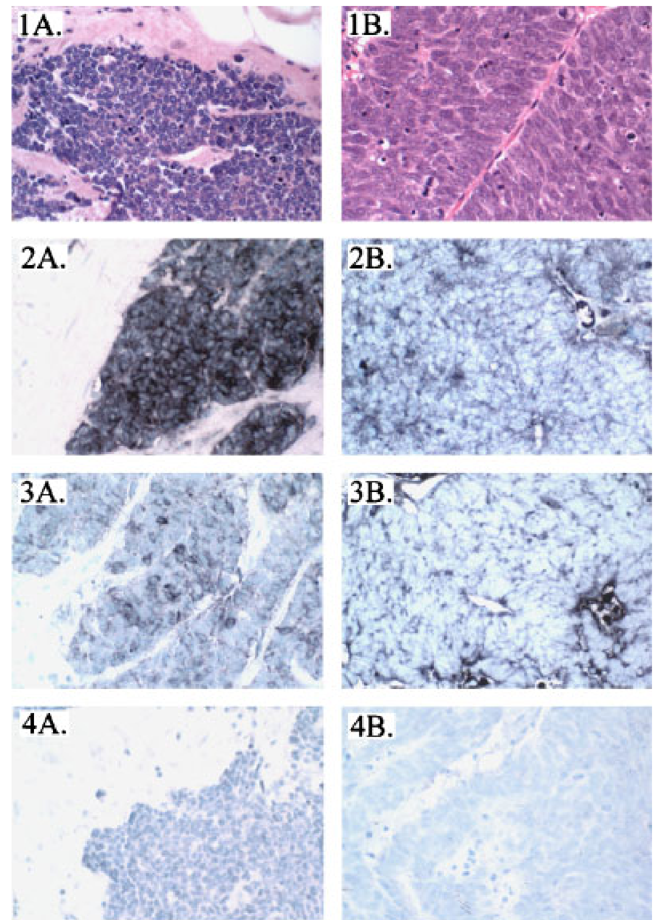

Fig. 1. Phenotypes of a prostate small cell carcinoma (**left panel**) and the LuCaP 49 xenograft that was derived from it (**right panel**). **1A**: Primary tumor. Sheet of undifferentiated carcinoma cells invading omental fat. The tumors have a high nuclear:cytoplasmic ratio, a fine heterochromatin pattern, and inconspicuous nucleoli. **1B**: Xenograft. Aggregate of cohesive, undifferentiated carcinoma cells with histologic features similar to 1A, and frequent mitoses. [1A, 1B: hematoxylin and eosin, original magnification 400×]. **2A**: Tumor. Uniform, intense, synaptophysin immunoreactivity. **2B**: Xenograft. Variably intense, cytoplasmic synaptophysin immunoreactivity. **3A**: Tumor. Focal cytoplasmic expression of chromogranin A. **3B**: Xenograft. Chromogranin A expression in the periphery of the cytoplasm of a minority of tumor cells. **4A,B**: Negative controls with no immunoreactivity. [All immunostains: Ni.DAB black, cytoplasmic reaction product; faint grey, nuclear green counterstain; 400× magnification].

A complete summary of all isolated transcripts representing both known and uncharacterized genes can be found at http://www.pedb.org. Mitochondrial sequences represent 4.3% of all the ESTs, but were counted as a single species. The majority of the non-mitochondrial genes are represented by a single EST

(1141; 80%), which is expected because most tissues contain 15,000–30,000 different transcript types [30], and our study sampled 2,096 ESTs. Only 13 (<1%) of all genes were represented by more than six ESTs. When the genes were assigned functional roles, 3 of the 13 most abundant transcripts were ribosomal, 3 were cytoskeletal, and the remainders were of diverse function. The most frequently sampled transcript encodes translation elongation factor 1 alpha 1, represented by 31 ESTs.

The general distribution of biological roles in the NE PRCA3 sample (Table I) is similar to those observed for both LNCaP prostate adenocarcinoma cells and the normal prostate [21,22]. A high proportion of all transcripts (56%) could not be assigned any functional role. If the unclassified species are excluded, the functional category of gene expression comprised the greatest number of transcripts (38%) and cell signaling the second (20%). The gross similarity to LNCaP cells may reflect the secretory nature of both the epithelial and NE cells.

Potential Markers for NE-Like Cells

A variety of molecular markers have been described for NE cells and small cell tumors of the prostate. Among these are members of the granin family of acidic glycoproteins, which are quantitatively the major components of dense-core secretory granules and which are required for the regulated secretion of prohormones [31,32]. ESTs for secretogranins 1, 2, and 3 (CHGB, secretogranin (SCG2), and SCG3) were detected in the PRCA3/LuCaP 49 library (Table II). ESTs for chromogranin A, a key regulator of dense-core secretory biogenesis [32], were not isolated; however, chromogranin A is histochemically detectable in the LuCaP 49 xenograft (Fig. 1; [19]). An EST was also detected that encodes NE secretory protein 55 (NESP55), a chromogranin-like protein that may function in secretion [33].

TABLE I. Roles of LuCaP 49/PRCA3 Species

Category	ESTs	Species
Gene expression	424 (0.202)[a]	231 (0.381)
Metabolism	162 (0.077)	94 (0.155)
Cell division	66 (0.032)	45 (0.074)
Cell signaling	164 (0.079)	121 (0.200)
Defense	106 (0.051)	66 (0.109)
Cell structure	100 (0.048)	49 (0.081)
Unclassified	949 (0.459)	816 (—)
Mitochondrial	90 (0.043)	1 (—)
Mouse	35 (0.017)	24 (—)
Total	2,096	1,447

[a]Proportion of total.

ESTs were identified that encode several other known NE-enriched markers. Enolase 2 gamma (ENO2), the CD24 antigen (CD24), and synaptophysin (SYP) are standard histochemical markers in the prostate. Our analysis identified two other potential markers of prostate NE cells, the achaete-scute complex (Drosophila) homolog-like 1 (ASCL1), and secretagogin (SECRET). ASCL1 encodes a basic-helix-loop-helix transcription factor that is highly expressed in medullary thyroid cancers and lung tumors with NE properties [34,35]. SECRET encodes a calcium binding protein that is enriched in some NE cells and may function in cell proliferation [34].

The origin of small cell cancers and adenocarcinomas of the prostate is an area of current debate and it remains uncertain to what extent gene expression profiles in the two cancer types may overlap [36–39]. To investigate whether the 'NE-enriched' transcripts in PRCA3 are also expressed and abundant in adenocarcinomas or normal prostate, we compared EST frequencies in the PRCA3 library to expression tag frequencies in the PR317 and LNCaP SAGE libraries available at the National Cancer Institute SAGE website. The original PR317 tumor was a primary adenocarcinoma of the prostate while the LNCaP cell line is derived from a metastatic adenocarcinoma. These SAGE libraries comprise 65109 and 22637 sequence tags respectively, and thus represent a deep sampling of the transcriptomes expressed by these cell and tissue types. Using the probability function of Audic and Claverie [29], 8 of the 9 potential NE markers had a high likelihood ($P > 0.9$) of elevated expression in PRCA3/LuCaP 49 relative to the other samples (Table II). Only NESP55 had a P-value less than 0.9. Five of the 9 potential markers (SYP, SCG2, SCG3, CHGB, and ASCL1) were represented exclusively in the PRCA3/LuCaP 49 library sample; and one (SECRET) was represented only in the PRCA3/LuCaP 49 NE and PR317 adenocarcinoma library samples. Surprisingly, CD24 and ENO2 ESTs were found in all of the libraries. Since CD24 and ENO2 are used as markers for prostate NE cells, this finding demonstrates the importance of using statistical methods to evaluate expression profiles instead of relying exclusively on the presence or absence of ESTs.

To confirm the statistical observations of Table II, we examined the expression of three transcripts, SCG2, ASCL1, and SECRET, in a variety of cancer cell lines using Northern analysis (Fig. 2). SCG2 RNA is expressed in the LuCaP 49 small cell carcinoma, but is not detectable in the adenocarcinoma xenograft LuCaP 73; nor is it detectable in whole normal prostate tissue or the cell lines DU145, LNCaP, and PC3. Like SCG2, the ASCL1 gene is expressed exclusively in LuCaP 49. SECRET is expressed in all of the samples, but it is

TABLE II. Neuroendocrine (NE) and Neural Genes Expressed in LuCaP 49

			cDNA library (tags per million)					
		Unigene	Known prostate NE	PRCA3[a] small cell	PR317 normal[b]	Tumor[c]	LNCaP[d] cell line	Lowest probability of differential expression[e]
Neuroendocrine								
ASCL1	Achaete–scute complex (*Drosophila*) homolog-like 1	Hs.1619	–	954	0	0	0	$0.998 < P < 0.999$
CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	Hs.286124	+	2,386	117	307	176	$0.999 < P < 1.000$
ENO2	Enolase 2, gamma	Hs.146580	+	477	50	15	44	$0.95 < P < 0.96$
NESP55	NE secretory protein 55	Hs.113368	–	477	302	230	132	$0.70 < P < 0.80$
CHGB	Secretogranin 1 (chromogranin B)	Hs.2281	+	477	0	0	0	$0.98 < P < 0.99$
SCG2	Secretogranin 2 (chromogranin C)	Hs.75426	+	1,908	0	0	0	$0.999 < P < 1.00$
SCG3	Secretogranin 3	Hs.22215	+	477	0	0	0	$0.98 < P < 0.99$
SECRET	Secretagogin	Hs.116428	–	477	0	61	0	$0.97 < P < 0.98$
SYP	Synaptophysin	Hs.75667	+	954	0	0	0	$0.998 < P < 0.999$
Neural								
INA	Internexin neuronal intermediate filament protein, alpha	Hs.76888	–	477	0	0	0	$0.98 < P < 0.99$
KAL1	Kallmann syndrome 1	Hs.89591	–	477	0	0	0	$0.98 < P < 0.99$
SV2B	Synaptic vesicle protein 2B homolog	Hs.194035	–	477	16	30	0	$0.98 < P < 0.99$
NLGN3	Neuroligin 3	Hs.47320	–	477	65	15	0	$0.96 < P < 0.97$
SYT13	Synaptogamin 13	Hs.12365	–	477	0	0	0	$0.98 < P < 0.99$
TPH	Tryptophan hydroxylase	Hs.144563	–	477	0	0	0	$0.98 < P < 0.99$
RTN3	Reticulon 3	Hs.252831	–	477	49	76	441	$0.40 < P < 0.50$
RTN4	Reticulon 4 (foocen, ASY, NOGO)	Hs.65450	+	477	251	122	44	$0.70 < P < 0.80$
ROBO1	Roundabout	Hs.301198	–	1,908	0	15	0	$0.99 < P < 1.00$

[a]PRCA3 cDNA library from small cell xenograft LuCaP49; 2,069 ESTs.
[b]PR317 normal prostate tissue 59419 SAGE tags.
[c]PR317 adenocarcinoma 65109 SAGE tags.
[d]LNCaP cell line 22637 SAGE tags.
[e]PRCA3 vs. PR317 and LNCaP; method of Audic and Claverie [29].

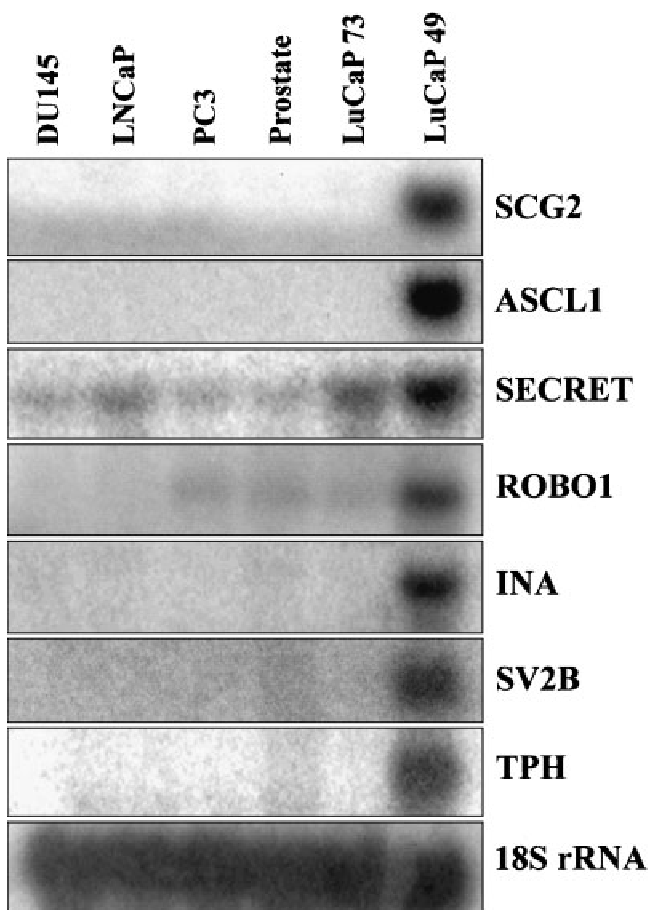

Fig. 2. LuCaP 49-enriched transcripts. Northern blots of total RNA from cell lines (DU145, LNCaP, PC3), an adenocarcinoma xenograft (LuCaP 73) [20], and LuCaP 49. SCG2, secretogranin 2; ASCL1, achaete-scute complex-like 1 (*Drosophila*); SECRET, secretagogin; ROBO1, roundabout, axon guidance receptor, homolog 1 (*Drosophila*); INA, internexin neuronal intermediate filament protein alpha; SV2B, synaptic vesicle protein 2B homolog; TPH, tryptophan hydroxylase.

enriched approximately 4-fold in LuCaP 49 RNA relative to the other samples. These data confirm the presence of SCG2 transcripts in LuCaP 49 and identify ASCL1 mRNA as a new potential marker for NE-like cells of the prostate. Despite SECRET's enrichment in LuCaP 49, it's presence in other cell-types make it less attractive as a marker.

Since NE and neural cells share many characteristics, we also searched for genes in the PRCA3 library that are enriched in neural tissues. Internexin neuronal intermediate filament protein alpha (INA) is found predominantly in the CNS [40], but a BLAST search of the NCI_CGAP_Lu5 and NCI_CGAP_Lu6 libraries revealed it is also expressed in lung cancers with NE characteristics. Reticulon 3 and 4 transcripts are enriched in the brain and in several other tissues [41–43]. Synaptotagmin 13 (SYT13) belongs to a family of calcium-binding synaptic vesicle proteins [44], while the function of the synaptic vesicle protein 2B homolog (KIAA0737) is unknown. The Kallmann Syndrome 1 (KAL1) and Roundabout homolog 1 (ROBO1) gene products may act in axon guidance [45–47]. Finally, the tryptophan dehydroxylase gene (TPH) encodes an enzyme that catalyses the rate limiting step in serotonin synthesis. Serotonin production is well-documented in prostate NE cells [3,4]. Any of these markers might serve to differentiate Ne-like cells from other cell types in the prostate.

With the exception of RTN3 and RTN4, all of the neural genes identified in Table II are predicted to be differentially expressed when PRCA3/LuCap 49 EST frequencies are compared to those in the PR317 and LNCaP adenocarcinoma libraries. We examined the expression of 6 of the genes (RTN4, NLGN3, ROBO1, INA, SV2B, and TPH) in the cell lines and xenografts described previously. Both RTN4 and NLGN3 were expressed in all of the cell lines tested (data not shown). The ROBO1 gene is unique in being expressed in a subset of cell lines. Furthermore, it is only enriched 3 fold in LuCap 49 relative to whole prostate tissue. In contrast, INA, SV2B and TPH transcripts are present in the LuCaP 49 xenograft and are not detected in the LNCaP, PC3, and DU145 cell lines, all of which are derived from adenocarcinoma metastases (Fig. 2). INA also expresses a transcript that is detected in all cell types tested (data not shown). Hence, we have identified three genes with transcripts that are expressed in LuCap 49, but not in adenocarcinoma-derived samples.

Virtual Expression Analysis of Prostate NE Small Cell Carcinoma

An alternate strategy for finding genes that are relevant to small cell cancer and NE cell biology is to identify transcripts that are highly expressed in NE-like cells relative to other tumor types. This approach does not require any *a priori* knowledge of gene function. We restricted our analysis to transcripts identified in PRCA3/LuCaP 49 that are also expressed in libraries derived from other NE cancers: NCI_CGAP_Lu24 (36609 ESTs), NCI_CGAP_Lu5 (20359 ESTs), or NCI_CGAP_Lu6 (209 ESTs). Lu24 and Lu5 were constructed from NE lung carcinoid tumors; Lu6 was made from a small cell lung carcinoma. Five hundred and ninety-one PRCA3 species were represented in one or more of these cancer libraries. While most of these NE-expressed species are unlikely to represent 'NE-specific' genes, their expression profiles may vary in other cancer types.

We compared the EST frequencies of the 591 NE-expressed transcripts in PRCA3 with the EST frequencies of the same genes in NCI_CGAP_Pr3, a primary adenocarcinoma of the prostate. Using statistical methods [29], 132 of 591 species were predicted to

have significantly different levels of expression in the PRCA3 small cell carcinoma library relative to the Pr3 adenocarcinoma library ($P > 0.9$). Another round of selection was performed by comparing EST frequencies from PRCA3 and a library derived from a prostate cancer adenocarcinoma bone metastasis, NCI_CGAP_Pr12. Forty NE-expressed genes were differentially expressed ($P > 0.9$). A list of these genes is posted at http://www.pedb.org. Since many of the genes have housekeeping functions and may simply reflect differences in metabolic activity, one final round of statistical selection was applied. EST frequencies of the 40 PRCA3 genes were compared to EST frequencies from the LNCaP cell line. This comparison identified 15 genes with a high probability of differential expression ($P > 0.90$; Table III).

The CD24 antigen and SCG2 genes are predicted to be more highly expressed in prostate NE small cell cancer than in normal prostate, prostate adenocarcinomas, and the LNCaP cell line (Table III), as are the nearly ubiquitously expressed tubulin genes TUBA3 and TUBB. Three other differentially expressed genes are of particular interest with respect to cancer. ALL fused gene from chromosome 1 (AF1Q) is both highly expressed in the thymus and is fused with a variety of other genes in leukemias [48]. Anti-apoptotic-like after growth factor withdrawal (API5L1) is a gene that may protect cells from apoptosis [49], and retinoblastoma binding protein 7 (RBBP7) is found in histone deacetylation complexes and interacts with the BRCA1 protein [50,51].

Other Genes of Interest

NE and malignant NE cells of the prostate have been reported to synthesize and secrete a variety of neuropeptides, including members of the calcitonin gene family, gastrin-releasing-peptide, somatostatin, alpha-human chorionic gonadotropin, thyroid-stimulating hormone (TSH)-like peptide and parathyroid hormone-related protein. Our sample of over 2000 PRCA3 ESTs did not include transcripts from these genes; however, other neuropeptides were found, including calcitonin gene-related peptide-receptor component protein (GCRP-RPC), thyroid-hormone receptor interactor 7 (TRIP7), thyroid receptor interacting protein 15 (TRIP15), and thyroid hormone binding protein p55 (P4HB).

Another area of active research is the role of apoptosis in prostate cancers. ESTs were isolated for programmed cell death 6-interacting protein (PDCD6IP), nerve growth factor receptor (TNFRSF16) associated protein 1 (NGFRAP1), API5-like 1 (API5L1), myeloid-cell leukemia sequence 1, BCL2 related (MCL1), Tax1 binding protein 1 (TAX1BP1), programmed cell death 4 (PDCD4), TGFB1 induced anti-apoptotic factor 1 (TIAF1), apoptosis antagonizing transcription factor

TABLE III. Genes Highly Expressed in LuCaP 49 Relative to Adenocarcinoma-Derived Samples

			cDNA library (tags per million)				
Gene	Description	Unigene Id	PRCA3[a]	PR3[b]	Pr12[c]	LNCaP[d]	Lowest P value[e]
HLA-A	MHC class I-A	Hs.181244	3,817	231	0	0	$0.998 < P < 0.999$
RBBP7	Retinoblastoma binding-protein 7	Hs.31314	3,340	0	0	560	$0.95 < P < 0.96$
SFRS3	Splicing factor arginine/serine rich 3	Hs.167460	2,386	0	0	187	$0.94 < P < 0.95$
CD24	CD24 antigen	Hs.286124	2,386	0	0	0	$0.98 < P < 0.99$
LDHA	Lactate dehydrogenase A	Hs. 2795	2,386	0	0	187	$0.94 < P < 0.95$
AF1Q	ALL1 fused gene from chromosome q1	Hs.75823	1,908	0	0	0	$0.96 < P < 0.97$
SCG2	SCG2	Hs.75426	1,908	0	0	0	$0.96 < P < 0.97$
API5L1	API5-like 1	Hs.227913	1,908	0	0	0	$0.96 < P < 0.97$
	ESTs similar to mucin 2 precursor	Hs.111911	1,431	0	0	0	$0.92 < P < 0.93$
LAPTM4A	Lysosomal associated protein transmembrane 4 alpha	Hs.111894	1,431	0	0	0	$0.92 < P < 0.93$
POLR2H	Polymerase (RNA) II (DNA-directed) polypeptide H	Hs.3128	1,431	0	0	0	$0.92 < P < 0.93$
FLJ20160	Hypothetical protein FLJ20160	Hs.23412	1,431	0	0	0	$0.92 < P < 0.93$
RACGAP1	GTPase activating protein	Hs.23900	1,431	0	0	0	$0.92 < P < 0.93$
TUBA3	Tubulin alpha, brain specific	Hs.272897	6,679	0	0	373	$0.999 < P < 1.00$
TUBB	Tubulin beta polypeptide	Hs.179661	4,771	231	1,142	746	$0.98 < P < 0.99$

[a]PRCA3 2,096 ESTs.
[b]PR3 4,325 ESTs.
[c]Pr12 3,500 ESTs.
[d]LNCaP 5,362 ESTs.
[e]Lowest probability of differential expression among three comparisons.

(DED), and BCL2-like 1 (BCL2L1). The LuCaP 49 xenograft contains foci of necrosis, which may account for the large number of expressed anti- and pro-apoptotic genes [19].

DISCUSSION

The role of NE cells in normal prostate development and in the etiology and progression of both adenocarcinoma and small-cell cancers of the prostate is, at present, unclear. To help address these broad issues we have created a cDNA library, PRCA3, from the LuCaP 49 small cell xenograft and have begun characterizing the transcriptome expressed by this tumor type. This report summarizes the identities of 2,096 cDNA clones derived from the PRCA3 library. The sequences assemble into 1,447 distinct transcripts, some of which are highly enriched in NE and NE-like cells. We confirmed the presence of several known, NE markers in LuCaP 49 (ASCL1, CD24, ENO2, CHGB, SCG2, SCG3, and SYP). Eighteen other genes including INA, SV2B, and TPH were found to be highly expressed in LuCaP 49 relative to normal prostate or cell lines derived from prostate adenocarcinomas.

A fundamental concern in describing NE cells in prostate cancer is the extent to which a malignant cell may be considered a NE cell. The dual expression of epithelial markers (such as PSA) and NE markers (such as chromogranin A) has been demonstrated in some cancer cells [3,4]. Similarly, two cell lines derived from adenocarcinomas can be induced to express NE markers [52–54], and chromogranin A is expressed at low frequency in LNCaP cells (http://pedb.org). These phenomena may reflect the ontogeny of prostate cancers. One hypothesis is that basal cells, secretory luminal cells, and NE cells arise from a pluripotent stem cell [38]. Hence, any prostate cancer may be able to express a variety of the markers typically associated with the terminal differentiation of these cell types [38]. Nevertheless, enrichment of a molecular marker in a small cell tumor represents the first step towards identifying potential determinants that will serve to characterize the functional attributes of both NE-like cancers and true NE cells.

The characterization of transcripts expressed in the PRCA3 library revealed nine NE-enriched genes, including one, ASCL1, which had not previously been observed in the prostate. Depletion of ASCL1 transcripts in cultured small cell lung cancers decreases the expression of NE markers [35]. Further, disruption of the mouse homolog of ASCL1, MASH1, prevents NE differentiation in the lung but not in the gut or pancreas [35]. These data suggest that ASCL1 participates in NE cell differentiation, either by affecting cell fate or by modulating the expression of factors important for terminal differentiation. MASH1 null mice die with 24 hr of birth, approximately 2 months before NE cells are detectable in the prostate. Consequently, the role of MASH1 in prostate development has not been described. If ASCL1 plays a role in mediating prostate cellular differentiation, then expressing ASCL1 in non-NE human prostate cancer cell lines may provide insights into the genes it regulates. The cell lines LNCaP and C4-2 may be especially informative as both are competent to express NE markers upon treatment with a variety of physiological and pharmacological agents [52–54]. Alternatively, a transgene under the control of a prostate specific promoter, such as the probasin promoter, make it possible to address this question. A recently described mouse model system for the induction of prostate small cell cancers should permit further investigation of ASCL1's role in progression towards small cell prostate cancer [15].

Eight neural genes were identified among the 1,447 gene species in the PRCA3 library. Three of the six genes studied by Northern analysis were expressed only in LuCaP 49 and not in other non-NE xenografts or prostate cancer cell lines. This suggests that they may be good markers for NE-like cells. Tests with a large sample of primary cancers and normal tissues will be needed to confirm this preliminary conclusion, as primary cancers and cancer cell lines may exhibit diverse gene expression profiles. For example, in our study the gene-encoding Roundabout was expressed in LuCaP 49 and PC3 cells but was not expressed at detectable levels in LNCaP or DU145 cell lines.

In addition to NE markers, 15 genes are expressed at significantly higher levels in PRCA3/LuCaP49 compared to libraries of adenocarcinoma origin. CD24 and SCG2 are expressed in a tissue specific manner, but all of the other genes were found to be expressed in adenocarcinomas of the prostate or in LNCaP cells (see http://www.ncbi.nlm.nih.gov/SAGE/SAGEcid.cgi/). The most highly represented species are the two tubulins TUBA3 and TUBB. The high level of tubulin mRNA may make LuCaP 49 cells particularly susceptible to taxane chemotherapuetic drugs, which induce apoptosis by promoting microtubule polymerization [55]. Taxanes may also act to impair function of anti-apoptotic members of the BCL family of proteins. Expression of bcl-xL, which we observed in PRCA3, is reduced in some cell lines in response to docetaxel. Thus, LuCaP 49 may serve as a good model system for the role of this class of chemotherapeutic drugs on small cell cancers of the prostate.

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