

# Interspecies Comparison of Prostate Cancer Gene-Expression Profiles Reveals Genes Associated With Aggressive Tumors

Itai Kela,<sup>1,2</sup> Alon Harmelin,<sup>3</sup> Tova Waks,<sup>1</sup> Avi Orr-Urtreger,<sup>4</sup> Eytan Domany,<sup>2</sup> and Zelig Eshhar<sup>1\*</sup>

<sup>1</sup>Department of Immunology, Weizmann Institute of Science, Rehovot, Israel

<sup>2</sup>Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, Israel

<sup>3</sup>Department of Veterinary Resources, Weizmann Institute of Science, Rehovot, Israel

<sup>4</sup>Genetic Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Prostate cancer (PC) is a heterogeneous disease whose aggressive phenotype is the second leading cause of cancer-related death in men. The identification of key molecules and pathways that play a pivotal role in PC progression towards an aggressive form is crucial. A major effort towards this end has been taken by global analyses of gene expression profiles. However, the large body of data did not provide a definitive idea about the genes which are associated with the aggressive growth of PC. In order to identify such genes, we performed an interspecies comparison between several human data sets and high quality microarray data that we generated from the transgenic adenocarcinoma of mouse prostate (TRAMP) strain. The TRAMP PC mimics the histological and pathological appearance as well as the aggressive phenotype of human PC (huPC). Analysis of the microarray data, derived from microdissected TRAMP specimens removed at different stages of the disease yielded genetic signatures delineating the TRAMP PC development and progression. Comparison of the TRAMP data with a set of genes representing the core expression signature of huPC yielded a limited set genes. Some of these genes are known predictors of poor prognosis in huPC. Interestingly, the modulation of genes responsible for the invasive phenotype of huPC occurs in TRAMP already during the transition to prostate intraepithelial neoplasia (PIN) and onwards to localized tumors. We therefore suggest that critical oncogenic events leading to an aggressive phenotype of huPC can be studied in the PIN stage of TRAMP. *Prostate* 69: 1034–1044, 2009. © 2009 Wiley-Liss, Inc.

**KEY WORDS:** prostate cancer; microarray analysis; gene expression profiles; interspecies comparison

## INTRODUCTION

Prostate cancer (PC) is a leading cause of illness and death among men in the United States and Western Europe [1]. The human disease is diverse in its type (prostatic adenocarcinoma and neuroendocrine (NE) carcinoma) and development (ranging from small slow-growing lesions to aggressive tumors that metastasize rapidly). The difficulty in obtaining repeated prostate biopsies in human patients together with the large genetic heterogeneity between individuals has hindered our understanding the biology of human PC (huPC), and our ability to predict its prognosis and response to treatment, as well as the development of effective therapies. The transgenic adenocarcinoma of mouse prostate (TRAMP) [2,3] that closely mimics

Additional Supporting Information may be found in the online version of this article.

Abbreviations: PC, prostate cancer; huPC, human prostate cancer; TRAMP, transgenic adenocarcinoma of mouse prostate; Tag, T antigen; PIN, prostate intraepithelial neoplasia; NE, neuroendocrine; LCM, laser capture microdissection; FDR, false discovery rate; PCA, principal component analysis.

Grant sponsor: Prostate Cancer Foundation; Grant sponsor: Wolfson Foundation; Grant sponsor: Ridgefield Foundation; Grant sponsor: Moross Cancer Institute; Grant sponsor: Weizmann Institute and Tel Aviv Sourasky Medical Center.

\*Correspondence to: Zelig Eshhar, Department of Immunology, The Weizmann Institute of Science, P.O. Box 26, Rehovot 76100, Israel.

E-mail: zelig.eshhar@weizmann.ac.il

Received 12 January 2009; Accepted 10 February 2009

DOI 10.1002/pros.20950

Published online 2 April 2009 in Wiley InterScience

(www.interscience.wiley.com).

the natural history and the pathology of huPC [3] has been suggested as a model to explore and better understand the molecular progression of PC [4]. TRAMP was generated by specific expression of the simian virus (SV40) small and large T antigens (Tag) under the regulatory sequence of rat probasin (PB) [5]. The PB-Tag transgene is androgen regulated, and its expression is initiated upon sexual maturation of male mice. Progression of the pathology from low grade PIN to high grade PIN, to localized hormone-dependent tumors, and then to androgen-independent tumors is a common feature in both huPC [6] and TRAMP [3].

Another feature that is common to human and TRAMP PC is the NE subtype. The pure NE is extremely rare in the human disease and comprises <0.1% of all PC, while focal NE differentiation represents a common feature occurring in 30–100% of cases [7]. Usually the prevalence of focal NE increases after hormone ablative therapy; NE apparently correlates with tumor progression and poor prognosis at the hormone refractory stage [8]. In TRAMP, the NE phenotype appears spontaneously and is commonly observed in poorly differentiated tumors.

Although several studies have been carried out to characterize the molecular nature of the processes underlying the TRAMP disease [9–12], no rigorous comparison between human and TRAMP PC has been published to date. Using gene expression analysis of well defined tissues obtained from different pathological stages of the TRAMP PC, we identified genetic signatures characteristic of each of the stages that delineate PC development and progression. Interspecies comparison of gene-expression profiles between TRAMP and published huPC data identified a refined subset of genes that are associated with the aggressive phenotype of human PC.

## MATERIALS AND METHODS

### TRAMP and Control Mice

The transgenic founder C57BL/6 females (a kind gift of Dr. Norman Greenberg, Fred Hutchinson Cancer Center, Seattle, WA) were routinely mated with wild type FVB male mice to obtain (C57BL/6x FVB)F1 offspring. The transgene positive males developed tumors, and the negative male F1 mice served as controls. All animal experiments were performed in compliance with Institutional Guidelines for Care and Use of Laboratory Animals.

### Tissue Collection

TRAMP mice were routinely monitored by palpation. At the age of 20–25 weeks, each mouse underwent

surgery to remove a biopsy from the dorsal/lateral lobe of its prostate. After 12 weeks, these mice were sacrificed and samples of the tumor and metastases (lymph nodes, lungs, liver, and kidney) were removed. In addition, specimens were collected from organs in which macro-metastases were not apparent. In parallel, biopsies were taken of normal prostates from littermates that did not express the TRAMP transgene. The specimens were stored in liquid nitrogen.

### Laser-Capture Microdissection (LCM)

Frozen tissues were cut into 8  $\mu$ m thick sections using a cryostat and then stained with hematoxylin and eosin for histological examination. Prostate epithelial cells (cancerous or normal) were isolated selectively using a Laser Capture Microdissection Microscope (P.A.L.M, Microlaser System, Bernried Germany) in accordance with the manufacturer's protocols. Serial sections were used if sufficient cells could not be obtained from a single section. Approximately 20,000 cells were captured for each sample.

### RNA Amplification and Affymetrix Microarrays

Total RNA was purified from the captured cells with the RNeasy micro kit [13] (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. RNA was tested for quality and quantified by the Agilent 2100 Bioanalyzer (Agilent Technologies) [14]. The yield of RNA was between 25 and 100 ng for each sample.

T7-based linear RNA amplification was performed using the Two-Cycle cDNA Synthesis Kit (Affymetrix, CA) with the biotin-labeling step being substituted by the GeneChip IVT Labeling Kit (Affymetrix). Over 100  $\mu$ g of amplified RNA was generated starting from an initial sample of 25 ng RNA for each sample. The labeled RNA was hybridized on an Affymetrix 430 2.0 mouse array, containing 45,035 probe sets. The microarray data were generated from 28 samples (Table I) including RNA derived from five normal prostates taken from Tag negative mice, and 23 samples taken from Tag positive mice. The tumor samples included eight low grade PIN (LGPIN), seven high grade PIN (HGPIN), five tumors, and three metastases (Met, two from lymph nodes and one from liver).

### Data Preprocessing and Clustering Analysis

The expression value for each gene was calculated by the Affymetrix Microarray Software 5.0 (MAS5). The average intensity difference values were normalized across the sample set. Probe sets that were absent in more than five samples according to Affymetrix flags were removed. All values lower than 30 were replaced by a value of 30, and then log<sub>2</sub> transformed. Six

**TABLE I. Tissue Samples Processed for Microarray**

	Normal	Pre-malignant lesion	Pre-malignant lesion	Tumor	Met
n	5	8	7	5	3
Pathology	Healthy tissue	LGPIN	HGPIN	Two MD, three PD	One MD, two PD
Tissue site	Prostate	Prostate	Prostate	Prostate	Two lymph nodes, 1 liver

Twenty-eight samples were used for the Microarray experiments. Normal samples taken from TRAMP negative mice. The other 23 samples were taken from TRAMP positive mice. LGPIN: low grade prostate intraepithelial neoplasia (PIN) lesion. HGPIN: high grade PIN lesion. Tumor: moderately differentiated (MD) and poorly differentiated (PD) invasive tumor. Met: metastatic tumors.

thousand eight ninety-six genes with the highest variance across the samples were selected for the clustering analysis (for more details see Supplementary data processing file).

The filtered set of genes was clustered by the SPC algorithm [15], and the samples were sorted by the SPIN algorithm [16].

### Comparison Between TRAMP and Human Datasets

A systematic comprehensive comparison (see Supplementary data processing file) was performed between TRAMP and seven human prostate microarray datasets [17–23] that were available in the Oncomine database ([www.oncomine.org](http://www.oncomine.org)).

### Identifying a Set of Aggressive Phenotype Genes

A set of genes previously indicated association with PC, were selected (see Supplementary Information) from six human PC microarray studies [17,19,20, 23–25]. This set consisted of two groups: (1) Genes predicting PC aggressiveness, (2) Genes associated with PC progression. For more details see Supplementary data processing file.

## RESULTS

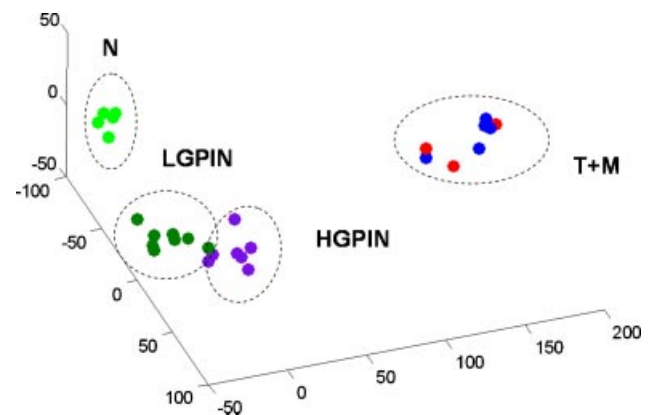
### Profiling of Stage-Related TRAMP Prostate Cancer

To identify the molecular characteristics underlying the oncopathological processes that occur along the progression of the PC in TRAMP, we performed a large scale gene expression analysis of tissue specimens removed at different time points along the course of tumor development. To obtain homogeneous tissue samples and minimize bias in outcome that could be introduced by stromal cells, we used laser-capture microdissection (LCM) and isolated histologically defined malignant and normal prostate epithelial cells. To obtain a global view of the similarities and differences in gene expression at various stages in the TRAMP model, we performed principal component

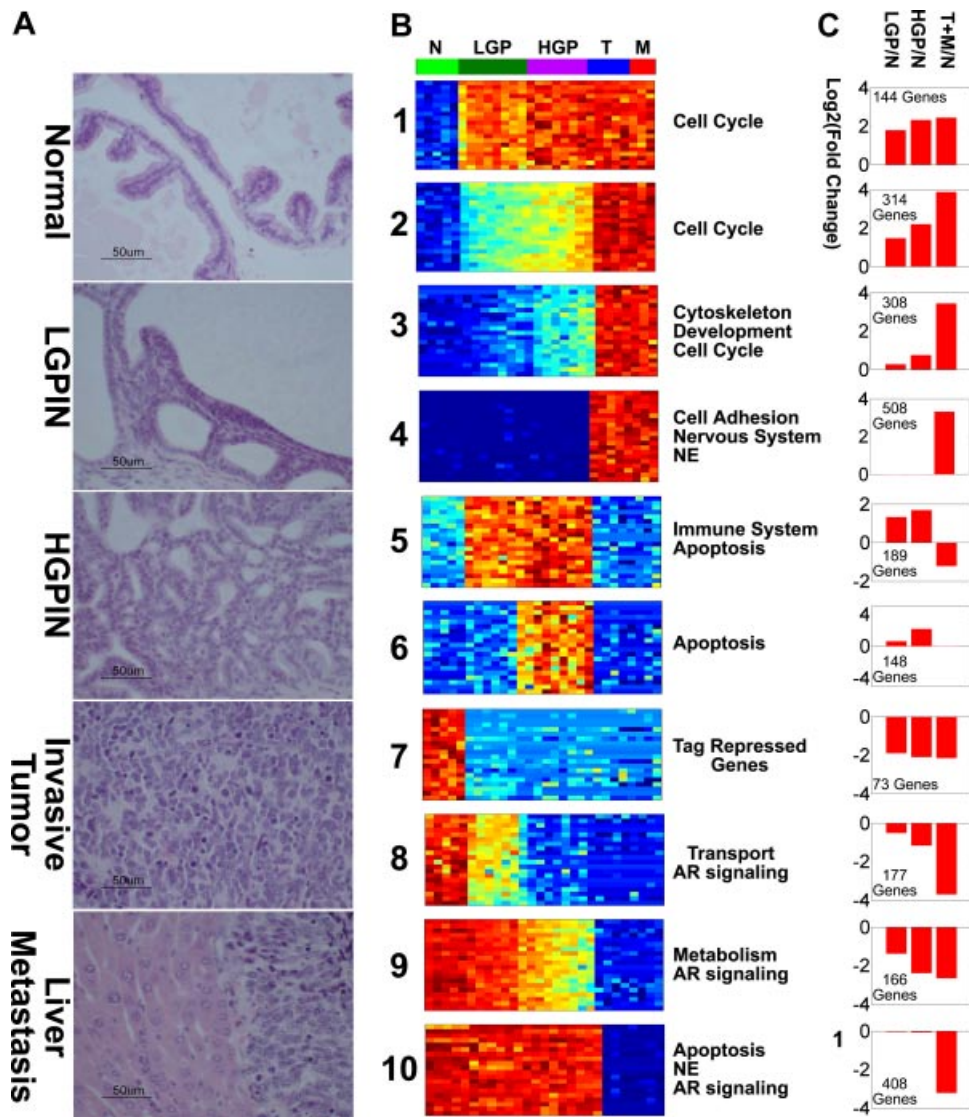
analysis (Fig. 1) based on the filtered set of genes. As seen in Figure 1, the samples were grouped mainly according to their stage of pathology. Nevertheless, the tumor and the metastasis samples formed a single cluster (T+M), which suggests a minor expression differences between these stages in TRAMP PC (detailed below).

To further evaluate and explore the differences in gene transcription along the cancer progression pathway, we clustered the filtered set of genes by the SPC algorithm [15] and sorted the samples using SPIN [16]. The clustering operation yielded ten clusters with expression profiles that were correlated with the histological phenotypes of the samples. The expression matrices of the 20 most significantly modulated genes from each cluster are presented in Figure 2. The full gene-list of each cluster is given in Supplementary Table I.

The 10 clusters presented in Figure 2 summarize the main expression changes during prostate cancer



**Fig. 1.** PCA analysis of the TRAMP samples. PCA analysis of the 28 TRAMP samples based on a set of the filtered probe sets. The samples (each dot represents a sample) are projected on a three-dimensional space; the three axes represent the three largest principal components. The colors refer to the histological phenotypes of the samples. Light green (normal samples), dark green (low grade PIN (LGPIN)), purple (high grade PIN (HGPIN)), dark blue (tumor samples), and red (metastatic samples).



**Fig. 2.** Expression matrices of the 10 clusters in TRAMP PC. Clustering operation of the 6,896 probe sets identified 10 clusters showing different expression profiles correlated with the histology phenotype of the samples. **Frame A** shows the histology of the five stages (normal, low grade PIN, high grade PIN, invasive tumor and metastasis). In **frame B**, the 10 clusters are ordered (from top to bottom) according to their function. Each cluster is represented by the 20 genes that most significantly changed. The upper bar in B represents the five histological stages: Normal (marked N and shown in light green), low grade PIN (marked LGPIN and shown in dark green), high grade PIN (marked HGPIN and shown in purple), tumor (marked T and shown in dark blue), and metastasis (marked M and shown in red). In **frame C**, the average gene expression of each cluster in each of the cancer stages is presented; the Y-axis represents the log<sub>2</sub> fold change increase or decrease of the LGPIN, HGPIN and T + M sample stages (relative to the normal samples).

progression in the TRAMP model. In addition, these profiles highlight the functional expression changes that characterize each stage. For example, the low grade PIN stage is mainly characterized by up-regulation of cell cycle (clusters 1 and 2) and immune related genes (cluster 5); the high grade PIN stage is characterized by up-regulation of pro-apoptotic genes (Cluster 6); and advanced stages (tumors and metastases) are significantly characterized by up-regulation of cell adhesion, cell cycle and NE genes (cluster 4), and by down-

regulation of immune system (cluster 5) and pro-apoptosis genes (clusters 6 and 10).

Among the top ranked genes that are up-regulated in late stages (T + M), are four genes previously linked with nervous system development including *SOX11*, *BASP1*, *DDC*, and *INSM1* (Supplementary Table II). Interestingly, the expression of these four genes was shown to be correlated with an NE phenotype in several cancers including prostate [26,27] and lung cancer [28,29]. Among the down-regulated genes

(Supplementary Table II) is the Microseminoprotein beta (*MSMB*) that strongly decrease in late stages. *MSMB* was recently identified as a candidate PC susceptibility gene whose expression is associated with PC risk [30] and functions as a suppressor of tumor growth [31]. Another interesting down-regulated gene is  $\beta$ -Defensin-1 (*DEFB1*), which is considered to be a candidate tumor suppressor gene for urological cancers [32]. It was shown that *DEFB1* is lost in 82% of PC and its over-expression results in caspase-3-mediated apoptosis.

### Functional Groups of Genes Associated With TRAMP PC Progression

Functional analysis performed using the DAVID (<http://david.abcc.ncifcrf.gov>) and Oncomine ([www.oncomine.org](http://www.oncomine.org)) databases, revealed several functional groups of genes that were significantly enriched ( $P < 0.001$ ) in the gene-clusters, including: cytoskeleton organization (cluster 3), neuroendocrine phenotype (clusters 4 and 10), apoptosis (clusters 6 and 10), androgen signaling (clusters 8–10), metabolism (clusters 9–10), cell cycle (clusters 1–3), immune system (clusters 5 and 6) and cell adhesion (cluster 4). Enrichment (the  $P$ -value for enrichment (over-representation) was calculated based on hypergeometric test [33]) was most significant for the last three functional groups ( $P < 10^{-7}$ ).

**Cell cycle genes are differently modulated along PC progression in TRAMP.** The group of cell cycle genes appears the most prominently altered ones during tumor progression in TRAMP. Three clusters (clusters 1–3) were highly enriched with cell cycle related genes. Cluster 2 (Supplementary Table II) is the highest in cell cycle enrichment ( $P < 10^{-49}$ ), and its expression profile gradually increases during tumor progression ((T + M)/N  $\cong$  20-fold). This cluster contains a large group of cell cycle regulating genes including *CCNE1*, *CCNA2*, *CCNB1*, *E2F8*, *AURKA*, and *MAD2L1*, which are also up-regulated in other cancers. Cluster 1 is mainly enriched with DNA replication genes ( $P < 10^{-25}$ ) displaying increased expression in the low grade PIN stage (*LEPIN*/N  $\cong$  5-fold). Several key proliferation markers appear in this cluster, including *MKI67* (8-fold), *PCNA* (3-fold), and *MCM2* (16-fold change). The increased expression of G1/S and DNA replication genes as early as in the low grade PIN stage may be a result of defects in the normal regulation of these cell cycle phases in the very beginning of the TRAMP PC development. The dramatic increase of G1/S genes present in cluster 2 at the advanced stages suggests a progressive loss of the normal regulation of this cell cycle phase. Cluster 3 is mainly enriched with M-phase

related genes ( $P < 10^{-7}$ ), including the mitotic genes *BUB1*, *TPX2*, and *CDC25C*. This cluster displayed late up-regulation in the tumor and the metastasis samples (16-fold), which may indicate late deregulation of the M-phase genes in TRAMP PC. Interestingly, the significant change in the expression levels of cell cycle genes already in the low grade PIN stage was not described in the huPC [17,34]. In addition, the subdivision of the cell cycle genes to three clusters is a unique feature observed in the TRAMP PC that has not been reported before. In contrast to our data, human cancer gene-expression analyses combined the cell cycle signature into a single expression profile [22,29,35–38]. It is likely that the laser capture microdissection and use of inbred mice yielded more homogenous, lower-noise data that enabled measurement of the behavior of cell cycle genes at a finer resolution. The possibility that the three patterns of cell cycle genes is a result of the Tag oncogenic activity is valid and merits further study.

**“Bell-shape” behavior of immune-associated genes in the prostate tissue during TRAMP PC progression.** Another molecular feature that highlights the differences between the pathological stages in TRAMP is the expression pattern of the immune cluster (Fig. 2, cluster 5).

A group of 54 immune modulation genes appears in cluster 5 ( $P < 10^{-12}$ ) display up-regulation in the low and high grade PIN samples (4-fold) and strong down-regulation in the tumor and metastasis samples (11-fold). This group of genes can be subdivided into interferon regulated genes (i.e., *USP18*, *IFIH1*, *IFIT3*, *IFI35*, *IRF7*, *STAT1*, *IFI27*, *IFI44*, *IFI27*, *IFI47*, and *IFIT3*) and antigen presenting genes (i.e., *TAP2*, *TAP1*, *B2M*, *TAPBP*, *H2-D1*, *H2-BL*, *H2-K1*, *H2-T23*, *H2-Q1*, *H2-M3*, and *H2-T22*). The up-regulation of these genes takes place in the absence of massive leukocyte infiltration or inflammation. Although we did not address this issue in our study, it is possible that Tag expression in the TRAMP mice, directly or indirectly induces an interferon (or interferon-like) response in the epithelial cells and this response inhibits cancer progression. As part of PC progression, this response is silenced, which may be an indication of immune escape mechanisms allowing a significant increase in cell cycle activity in the advanced stages, as seen in clusters 2 and 3 (Fig. 2). Comparing these immune response genes to huPC data, we found that in huPC, this cluster behaves differently, and displays gradual decrease along cancer progression, mainly in advanced stages.

**Intensive change in cell adhesion gene expression occurs at the advanced PC stages in TRAMP.** An additional functional group of genes that changes in a

reciprocal direction (e.g., clusters 3, 4, 6, and 10 of Fig. 2) along the progression pattern of the TRAMP PC are those associated with cell adhesion. A large group of cell adhesion related genes are present in clusters 3 and 4 (i.e., *N-Cadherin*, *TRO*, *CDH2*, *NCAM1*, *L1CAM*, *PCDHGC3*, and *TROAP*) show a slight increase of expression in the PIN samples and strong increase of expression (10-fold) in tumors and metastases. In contrast, some adhesion related genes that appear in clusters 6 and 10, exhibit an opposite expression profile. For example, *E-cadherin* (cluster 10) and *AZGP1* (cluster 6) are remarkably down-regulated (by 38- and 343-fold, respectively) during the late stages. These two cell adhesion molecules are both well explored PC genes with expression profiles that are inversely correlated with disease progression [39,40]. Recently, it was shown that reciprocal expression of E-cadherin (decrease) and N-cadherin (increase) is associated with the epithelial to mesenchymal transition, and has been recognized as a feature of aggressive PC tumors [41].

In addition to these aforementioned classes, genes that are involved in apoptosis and androgen signaling were also significantly identified in our data. Pro-apoptotic genes appear mainly in clusters 6 and 10; the expression profiles of these two clusters behave differently in early stages, but both decrease in advanced stages. For example, the genes *TRAF1*, *CASP12*, *CLU*, *CASP4*, and *CASP7* (cluster 6) are up-regulated in the PIN samples, but down-regulated in tumors and metastases. The expression levels of another group of pro-apoptotic genes, including *BNIPL*, *DAP*, *TNFRSF21*, *BCL2L14*, *SCOTIN*, and *TMS1* (cluster 10), are not changed during the transition from normal and PIN samples, but are strongly down-regulated in tumors and metastases. The anti-apoptotic gene, *Survivin* (cluster 2), shows significant up-regulation in the tumor and metastasis samples (33-fold), which is another indication of the loss of the normal apoptotic mechanism at advanced stages in TRAMP. A group of androgen signaling related genes were also observed in our data, in clusters 8 (e.g., *ACPP*, *PYCR1*), cluster 9 (*ABCC4*, *APOD* and *KDEL3*, *TMPRSS2*) and cluster 10 (*AR*, *DDT*, and *NKX3-1*), showing different expression profiles along PC progression.

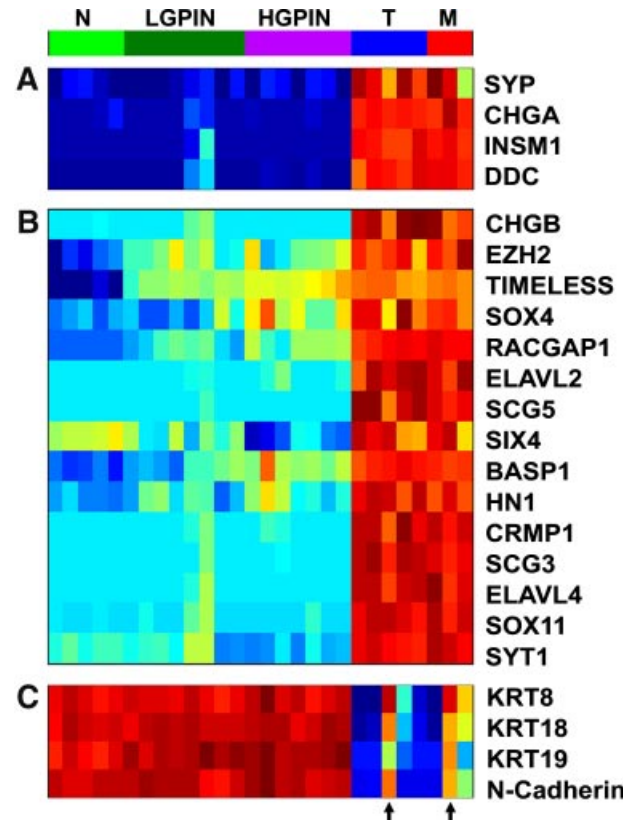
### Dominance of the NE Phenotype in TRAMP

Using the Oncomine resources, we could identify significantly up-regulated (cluster 4, ( $P < 10^{-30}$ ) and down-regulated (cluster 10, ( $P < 10^{-10}$ ) genes that are associated with neuroendocrine tumors (e.g., *SYP*, *CHGA* and *INSM1*, cluster 4).

Previous reports highlighted the expression of neuroendocrine (NE) markers in advanced, poorly differentiated, stages of TRAMP [3,42,43]. Similarly,

our data show up-regulation of four NE genes ( $P < 0.001$ , Supplementary Table III) at advanced stage (Fig. 3) of TRAMP PC. For example, mRNAs of the two classical NE markers, Synaptophysin and Chromogranin are up-regulated by 10- and 56 fold, respectively, in the localized and in the metastatic tumors. Other known NE genes, such as *INSM1* and *DDC* [26,29], also show a remarkable up-regulation at the advanced TRAMP PC stages (160- and 290-fold, respectively). In parallel, the expression profiles of a group of epithelial marker genes show a dramatic decrease over the course of tumor development ( $P < 0.01$ , Fig. 3C, Supplementary Table III); for example, the epithelial markers, *KRT8* and *KRT18*, are down-regulated by 136- and 138-fold, respectively, in advanced tumors.

In order to further explore the NE characteristics of TRAMP, we compared its gene expression profiles with the expression profiles of a pure NE carcinoma that develops in Cr2-Tag transgenic mice [27]. Hu et al. [27] identified a list of 30 genes that significantly differentiate between normal prostate samples and the pure



**Fig. 3.** Expression of NE and epithelial marker genes. **A:** Expression profiles of four known NE markers in TRAMP PC. **B:** Expression profiles of the top 15 NE signature genes that were identified by Hu et al. [27]. **C:** Decreased expression of epithelial markers. The two samples that are marked by arrows are moderately differentiated tumors, in contrast to the others that are poorly differentiated.

NE tumors that develop in this strain. Our data show that 22 out of these genes are significantly up-regulated in the poorly differentiated TRAMP tumors ( $P < 0.05$ , Supplementary Table III); the 15 most significant ones are presented in Figure 3B. This observation indicates a spontaneous development (in the absence of any hormonal ablative treatment) of a highly aggressive, homogenous NE phenotype in advanced TRAMP tumors.

### TRAMP as a Model for Aggressive Human PC Phenotype(s)

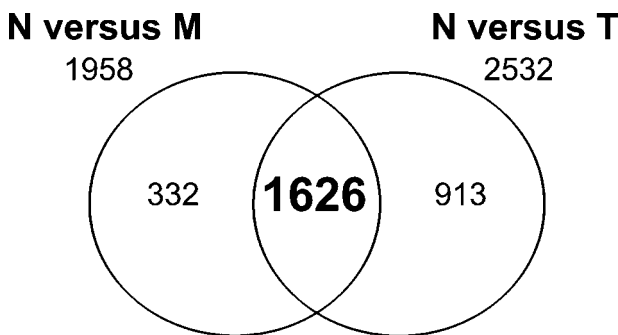
**Lack of a difference in gene-expression between localized, poorly differentiated versus metastatic tumors in TRAMP.** Comparison between localized and metastatic tumors in TRAMP PC did not yield any significant ( $Q < 0.3$ ) expression differences. To further explore the relation between these two stages we used *t*-test to identify the genes that differentiate ( $Q < 0.1$ ) between normal and tumor (2,539 genes) and between normal and metastases (1,958 genes). The Venn diagram (Fig. 4) demonstrates that 83% (1,626 genes,  $P < 10^{-100}$ ) of the genes that differentiate between normal and metastatic samples also differentiate between normal and tumor samples. These results indicate a strong expression similarity between tumor and metastatic samples in TRAMP. In contrast to TRAMP, in human PC, there is a clear shift in gene expression in the transition from poorly differentiated PC (Gleason score 8–9) to its aggressive metastatic stage [17].

To compare between TRAMP and human PC we used seven human prostate microarray datasets [17–23] that were available at the Oncomine database (www.Oncomine.org). For each dataset, we downloaded gene-lists differentiating either normal from

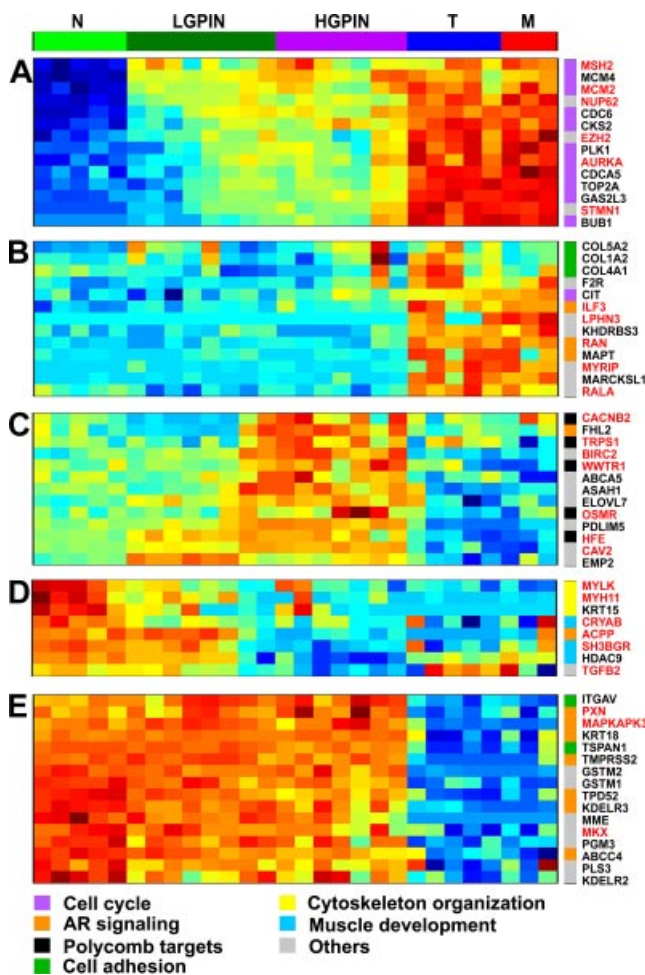
tumor samples (N–T, six datasets) or tumor from metastasis samples (T–M, four datasets). Our meta analysis of human data sets identified 2,909 up and 2,481 down-regulated genes that were significantly ( $Q < 0.1$ ) modulated in the human PC datasets (as described in Materials and Methods Section). In the TRAMP data, we identified 1,236 up and 1,166 down-regulated genes ( $Q < 0.1$ ). The overlap between the human and the TRAMP modulated genes yielded a common set of 469 up and 479 down-regulated genes (a total of 933 unique genes, 15 of which are upregulated from normal to high grade PIN and are then down regulated upon progression to tumor). These subgroups represent the genes that are common to TRAMP and huPC regardless of their classification or importance. Most of the genes, either up or down-regulated, are strongly modulated during the transition from high grade PIN to the tumor stage, with no further change upon transition to the metastatic stage. Some of these genes also show earlier expression changes at the PIN stage (data not shown).

**Identification of tumor aggressiveness—associated genes shared by human and TRAMP PC.** The changes in the expression of many of the 933 genes identified above may not be directly related to tumor development and aggressiveness. To reveal which genes out of this set serve as causal “driver” or “contributor” to the development of huPC [44] we intersected the 933 genes with a set of 194 human genes (Supplementary Table IV) that represent the core expression of huPC [17,19,20,23–25]. This analysis filtered out many biologically “neutral” genes and yielded a refined subset of 64 genes (Fig. 5 and Supplementary Table V). We suggest these genes to be important in the development of aggressive tumors in both human and TRAMP PC.

These 64 genes (27 up-regulated and 38 down-regulated genes (Fig. 5) were grouped into five clusters that correspond to clusters 2, 3, 4, 5, 10 of TRAMP PC (Fig. 2). A large sub-group of genes of this set (marked in red in Fig. 5) have been previously associated with poor prognosis in PC patients. For example, the genes *STTM1*, *AURKA*, *NUP62*, *MSH2* of cluster A, *ILF3*, *RALA*, *RAN* of cluster B, and the genes *ACPP*, *CRYAB*, *MYLK* of cluster D were previously identified by Varambally et al. [24] as predictors of PC aggressiveness. In addition, the down-regulated genes *CACNB2*, *HFE*, *OSMR*, *TRPS1*, *WWTR1* of cluster C were recently shown by Yu et al. [25] to be targets of the polycomb gene, *EZH2* (which appears in cluster A), and their decreased expression was shown to be correlated with poor outcome in several cancers including breast and PC [25]. Some of these genes were previously implicated in androgen signaling activity, including *ILF3*,



**Fig. 4.** Similarly expressed genes between TRAMP tumors and metastases. The Venn diagram shows that 83% of the genes share a similar expression profile, either in the transition from normal (N) samples to tumors (T) or to metastases (M).



**Fig. 5.** Expression matrix of the human-PC signature genes in TRAMP data. The 64 genes are grouped into five clusters, displaying either increased (clusters A, B) or decreased (clusters C–E) expression levels along cancer progression stages (upper horizontal bar). Among these are genes that were previously implicated as aggressiveness predicting genes in PC patients (gene symbols in red at the right). The vertical bar on the right represents the functional annotation of each gene according to the color spectrum in the bottom of the figure.

*MAPT* and *RAN* of cluster B, *ACPP* of cluster D, and *KRT18*, *MAPKAPK3*, *PXN*, *TPD52* of cluster E [24].

Altogether, we have generated in this study, a high quality gene expression data characterized the PC development and progression in the TRAMP that represents a model for aggressive huPC. Interspecies comparison with the human disease revealed huPC signature genes that are also modulated in TRAMP and are associated with the aggressive phenotype of huPC.

## DISCUSSION

Although human and TRAMP PC are induced by different oncogenic processes, they share gross pathological features at various stages of their develop-

ment [3,10,45,46]. The TRAMP PC is a fast growing tumor, resulting in high incidence of metastasis, while human PC is a slow growing tumor, which in many cases never develops into disseminated disease [1]. We show here that as a model, TRAMP mimics the progression of huPC from low grade PIN to high grade PIN to localized hormone-dependent tumors and further on to androgen independent tumors with focal NE phenotypes [6,47]. To validate the use of the TRAMP model to gain a better understanding of the human disease, we generated here high quality gene expression data from microdissected tissue specimens derived from different stages of PC development TRAMP, and compared the transcription profiles to those of published huPC data. Systematic gene-array analysis of TRAMP PC revealed 10 gene clusters (Fig. 2) that correlate well with histological appearance along the entire development process of this murine model. The functional groups of genes displaying differential expression profiles along this process includes genes involved in cell cycle, cytoskeleton organization, cell adhesion, NE development, immune system, metabolism, apoptosis and others, and allowed the identification of expression signatures that could differentiate between each of the cancer stages. These genes and their functional implications along the various stages of TRAMP PC development and progression are detailed and discussed in the results section above.

The comprehensive comparison between our TRAMP data and seven human prostate cancer microarray datasets [17–23] shows that only 17% of the modulated genes in the human datasets are also significantly modulated in the TRAMP data. The remaining changes in gene expression represent both species-related differences, the consequences of the critical activation of T antigen at the onset of TRAMP PC [5,48,49], as well as technical “noise.” Amongst the shared 933 genes, about 50% are modulated in both TRAMP and huPC during the transition from the normal to tumor stage. The other 50% of the genes are also modulated between the normal and tumor stage in TRAMP, but in the human, are modulated during the transition from tumor to metastases. These findings suggest that the major modulation of the genes responsible for the aggressive phenotype in huPC occurs during the transition from high grade PIN to advanced tumor in TRAMP. Apparently, none of the shared genes are modulated in the transition from poorly differentiated TRAMP PC to metastases. These results suggest that the correct mouse model for the transition of human PC to metastases is the transition from high-grade PIN to advanced tumor in TRAMP PC.

Which genes in the 933 group play a crucial role in the aggressive phenotype of PC and thus serve as causal “driver” or “contributor” genes [44] for the

development of huPC pathophysiology? To this end, the 194 human gene set (Supplementary Table IV) [17,19,20,23–25] representing the core expression of human PC appeared very instrumental and revealing. It identified a refined sub-set of 64 genes (Fig. 5 and Supplementary Table V) that are significantly modulated in TRAMP and are shared by huPC. We suggest these to play an important role in the development of aggressive tumors in both human and in TRAMP PC. Of these, 27 were previously identified [23–25] as predictors of poor prognosis (indicated in red in Fig. 5) in patient PC (amongst the best known ones are *AURKA*, *MCM2*, *EZH2*, *BUB1*, and *RAN*). The behavior patterns of these genes in the TRAMP model do not necessarily reflect similar behavior in huPC. Nevertheless, the fact that these genes have been classified as predictors in huPC and are modulated in TRAMP supports the use of TRAMP as an experimental model to study and validate their role and relevance in huPC development. Although the approach of interspecies comparison may neglect important human PC genes that are not modulated in the TRAMP model, we strongly believe that the 64 genes which were identified by this meta-analysis to be “signature genes” which are highly relevant to the aggressive progression of huPC.

Our data also favors the usage of TRAMP as an adequate model to explore NE development. NE phenotype in huPC (defined as prostatic small cell carcinoma) has been regarded as the most aggressive subtype of the human disease [8]. Earlier studies by Greenberg et al. and Chiaverotti et al. in the TRAMP model, demonstrate by both histology [3,42,43] and gene expression [12] patterns that the NE phenotype dominates the advanced stages of TRAMP, and is rare at the PIN stages. Our gene expression data (cluster 4, Figs. 2 and 3) identified additional NE genes that are modulated in the advanced stages of TRAMP PC. As such, it lands more support that TRAMP strain can serve as a model not only to better understand the origin and development of the aggressive NE subtype but also for the design of specific treatments against the NE form of the aggressive human disease.

Overall, the data presented here revealed that many gene-expression profiles, associated with specific cellular/biochemical pathways (such as androgen signaling, cell growth and proliferation) observed in advanced huPC, could already be observed at earlier stages in the TRAMP model. Likewise, the gene expression differences between the later stages of the human disease (i.e., localized, poorly differentiated tumors) versus the disseminated tumors are more pronounced than the differences between the corresponding stages of the TRAMP model. Interspecies comparison identified a refined short list of genes

associated with aggressive human PC; many of which were not described in the context of human PC and may play a pivotal role in PC progression towards an aggressive phenotype.

## ACKNOWLEDGMENTS

We are grateful to Dr. Shirley Horn-Saban, Biological Services, Ms Judith Chermesh, Veterinary Resources and Ms Tami Danon, Molecular and Cell Biology for technical assistance, Dr Shelley Schwarzbaum for editorial assistance, Dr. Ulrich Sauer, P.A.L.M. Micro-laser Technologies and Affymetrix technical personnel for instruction and support. This study was supported in part by the Prostate Cancer Foundation, the Wolfson Foundation, the Ridgefield Foundation, by research grants by the Moross Cancer Institute, Ms Marion Achtentuch and Weizmann Institute and Tel Aviv Souraski Medical Center joint grant. I. Kela was partially supported by the Kahn Family Research Cancer for System Biology of the Human Cell. We dedicate this article to the memory of Mr. Michael Dobrin.

## REFERENCES

1. Neal DE, Donovan JL. Prostate cancer: To screen or not to screen? *Lancet Oncol* 2000;1(1):17–24.
2. Greenberg NM, DeMayo FJ, Finegold M, Medina D, Tilley W, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci* 1995; 92:3439–3443.
3. Kaplan-Lefko PJ, Chen TM, Ittmann MM, Barrios RJ, Ayala GE, Huss WJ, Maddison LA, Foster BA, Greenberg NM. Pathobiology of autochthonous prostate cancer in a pre-clinical transgenic mouse model. *Prostate* 2003;55(3):219–237.
4. Martiniello-Wilks R, Dane A, Mortensen E, Jeyakumar G, Wang XY, Russell PJ. Application of the transgenic adenocarcinoma mouse prostate (TRAMP) model for pre-clinical therapeutic studies. *Anticancer Res* 2003;23(3B):2633–2642.
5. Greenberg NM, DeMayo FJ, Finegold M, Medina D, Tilley W, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM. Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci* 1995; 92:3439–3443.
6. Arnold JT, Isaacs JT. Mechanisms involved in the progression of androgen-independent prostate cancers: It is not only the cancer cell's fault. *Endocr Relat Cancer* 2002;9(1):61–73.
7. Cindolo L, Cantile M, Vacherot F, Terry S, de la Taille A. Neuroendocrine differentiation in prostate cancer: From lab to bedside. *Urol Int* 2007;79(4):287–296.
8. Yuan TC, Veeramani S, Lin MF. Neuroendocrine-like prostate cancer cells: Neuroendocrine transdifferentiation of prostate adenocarcinoma cells. *Endocr Relat Cancer* 2007;14(3):531–547.
9. Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, Finegold MJ, Angelopoulou R, Rosen JM, Greenberg NM. Metastatic prostate cancer in a transgenic mouse. *Cancer Res* 1996;56(18):4096–4102.
10. Gingrich JR, Barrios RJ, Kattan MW, Nahm HS, Finegold MJ, Greenberg NM. Androgen-independent prostate cancer progression in the TRAMP model. *Cancer Res* 1997;57(21):4687–4691.

11. Wikstrom P, Lindahl C, Bergh A. Characterization of the autochthonous transgenic adenocarcinoma of the mouse prostate (TRAMP) as a model to study effects of castration therapy. *Prostate* 2005;62(2):148–164.
12. Morgenbesser SD, McLaren RP, Richards B, Zhang M, Akmaev VR, Winter SF, Mineva ND, Kaplan-Lefko PJ, Foster BA, Cook BP, Dufault MR, Cao X, Wang CJ, Teicher BA, Klinger KW, Greenberg NM, Madden SL. Identification of genes potentially involved in the acquisition of androgen-independent and metastatic tumor growth in an autochthonous genetically engineered mouse prostate cancer model. *Prostate* 2007;67(1):83–106.
13. Deng MY, Wang H, Ward GB, Beckham TR, McKenna TS. Comparison of six RNA extraction methods for the detection of classical swine fever virus by real-time and conventional reverse transcription-PCR. *J Vet Diagn Invest* 2005;17(6):574–578.
14. Burgemeister R, Gangnus R, Haar B, Schutze K, Sauer U. High quality RNA retrieved from samples obtained by using LMPC (laser microdissection and pressure catapulting) technology. *Pathol Res Pract* 2003;199(6):431–436.
15. Marcelo Blatt SW. Eytan Domany. Superparamagnetic clustering of data. *Phys Rev Lett* 1995;76(18):3251–3254.
16. Tsafirir D, Tsafirir I, Ein-Dor L, Zuk O, Notterman DA, Domany E. Sorting points into neighborhoods (SPIN): Data analysis and visualization by ordering distance matrices. *Bioinformatics* 2005;21(10):2301–2308.
17. Tomlins SA, Mehra R, Rhodes DR, Cao X, Wang L, Dhanasekaran SM, Kalyana-Sundaram S, Wei JT, Rubin MA, Pienta KJ, Shah RB, Chinnaiyan AM. Integrative molecular concept modeling of prostate cancer progression. *Nat Genet* 2007;39(1):41–51.
18. Vanaja DK, Cheville JC, Iturria SJ, Young CY. Transcriptional silencing of zinc finger protein 185 identified by expression profiling is associated with prostate cancer progression. *Cancer Res* 2003;63(14):3877–3882.
19. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K, Ferrari M, Egevad L, Rayford W, Bergerheim U, Ekman P, DeMarzo AM, Tibshirani R, Botstein D, Brown PO, Brooks JD, Pollack JR. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. *Proc Natl Acad Sci USA* 2004;101(3):811–816.
20. Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson HF Jr, Hampton GM. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. *Cancer Res* 2001;61(16):5974–5978.
21. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, Tamayo P, Renshaw AA, D'Amico AV, Richie JP, Lander ES, Loda M, Kantoff PW, Golub TR, Sellers WR. Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell* 2002;1(2):203–209.
22. Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA, Chinnaiyan AM. Delineation of prognostic biomarkers in prostate cancer. *Nature* 2001;412(6849):822–826.
23. Yu YP, Landsittel D, Jing L, Nelson J, Ren B, Liu L, McDonald C, Thomas R, Dhir R, Finkelstein S, Michalopoulos G, Becich M, Luo JH. Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. *J Clin Oncol* 2004;22(14):2790–2799.
24. Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, Shah RB, Chandran U, Monzon FA, Becich MJ, Wei JT, Pienta KJ, Ghosh D, Rubin MA, Chinnaiyan AM. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* 2005;8(5):393–406.
25. Yu J, Rhodes DR, Tomlins SA, Cao X, Chen G, Mehra R, Wang X, Ghosh D, Shah RB, Varambally S, Pienta KJ, Chinnaiyan AM. A polycomb repression signature in metastatic prostate cancer predicts cancer outcome. *Cancer Res* 2007;67(22):10657–10663.
26. Margiotti K, Wafa LA, Cheng H, Novelli G, Nelson CC, Rennie PS. Androgen-regulated genes differentially modulated by the androgen receptor coactivator L-dopa decarboxylase in human prostate cancer cells. *Mol Cancer* 2007;6:38.
27. Hu Y, Ippolito JE, Garabedian EM, Humphrey PA, Gordon JI. Molecular characterization of a metastatic neuroendocrine cell cancer arising in the prostates of transgenic mice. *J Biol Chem* 2002;277(46):44462–44474.
28. Koh T, Yokota J, Ookawa K, Kina T, Koshimura K, Miwa S, Ariyasu T, Yamada H, Osaka M, Haga H, Hitomi S, Sugiyama T, Takahashi R. Alternative splicing of the neurofibromatosis 1 gene correlates with growth patterns and neuroendocrine properties of human small-cell lung-carcinoma cells. *Int J Cancer* 1995;60(6):843–847.
29. Taniwaki M, Daigo Y, Ishikawa N, Takano A, Tsunoda T, Yasui W, Inai K, Kohno N, Nakamura Y. Gene expression profiles of small-cell lung cancers: Molecular signatures of lung cancer. *Int J Oncol* 2006;29(3):567–575.
30. Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK, Mulholland S, Leongamornlert DA, Edwards SM, Morrison J, Field HI, Southey MC, Severi G, Donovan JL, Hamdy FC, Dearnaley DP, Muir KR, Smith C, Bagnato M, Arden-Jones AT, Hall AL, O'Brien LT, Gehr-Swain BN, Wilkinson RA, Cox A, Lewis S, Brown PM, Jhavar SG, Tymrakiewicz M, Lophatananon A, Bryant SL, Horwich A, Huddart RA, Khoo VS, Parker CC, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Fisher C, Jamieson C, Cooper CS, English DR, Hopper JL, Neal DE, Easton DF. Multiple newly identified loci associated with prostate cancer susceptibility. *Nat Genet* 2008;40(3):316–321.
31. Beke L, Nuytten M, Van Eynde A, Beullens M, Bollen M. The gene encoding the prostatic tumor suppressor PSP94 is a target for repression by the Polycomb group protein EZH2. *Oncogene* 2007;26(31):4590–4595.
32. Sun CQ, Arnold R, Fernandez-Golarz C, Parrish AB, Almekinder T, He J, Ho SM, Svoboda P, Pohl J, Marshall FF, Petros JA. Human beta-defensin-1, a potential chromosome 8p tumor suppressor: Control of transcription and induction of apoptosis in renal cell carcinoma. *Cancer Res* 2006;66(17):8542–8549.
33. Barash B, Friedman N. A simple hyper-geometric approach for discovering putative transcription factor binding sites. *Lecture Notes Comput Sci* 2001;249:278–293.
34. Quinn DI, Henshall SM, Sutherland RL. Molecular markers of prostate cancer outcome. *Eur J Cancer* 2005;41(6):858–887.
35. van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415(6871):530–536.
36. Dai H, van't Veer L, Lamb J, He YD, Mao M, Fine BM, Bernards R, van de Vijver M, Deutsch P, Sachs A, Stoughton R, Friend S. A cell proliferation signature is a marker of extremely poor outcome in a subpopulation of breast cancer patients. *Cancer Res* 2005;65(10):4059–4066.
37. Rosty C, Sheffer M, Tsafirir D, Stransky N, Tsafirir I, Peter M, de Cremoux P, de La Rochefordiere A, Salmon R, Dorval T, Thiery JP, Couturier J, Radvanyi F, Domany E, Sastre-Garau X. Identification of a proliferation gene cluster associated with

- HPV E6/E7 expression level and viral DNA load in invasive cervical carcinoma. *Oncogene* 2005;24(47):7094–7104.
38. Beer DG, Kardia SL, Huang CC, Giordano TJ, Levin AM, Misek DE, Lin L, Chen G, Gharib TG, Thomas DG, Lizyness ML, Kuick R, Hayasaka S, Taylor JM, Iannettoni MD, Orringer MB, Hanash S. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med* 2002;8(8):816–824.
  39. Henshall SM, Horvath LG, Quinn DI, Eggleton SA, Grygiel JJ, Stricker PD, Biankin AV, Kench JG, Sutherland RL. Zinc-alpha2-glycoprotein expression as a predictor of metastatic prostate cancer following radical prostatectomy. *J Natl Cancer Inst* 2006; 98(19):1420–1424.
  40. Umbas R, Isaacs WB, Bringuier PP, Schaafsma HE, Karthaus HF, Oosterhof GO, Debruyne FM, Schalken JA. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Res* 1994;54(14):3929–3933.
  41. Gravdal K, Halvorsen OJ, Haukaas SA, Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res* 2007;13(23):7003–7011.
  42. Chiaverotti T, Couto SS, Donjacour A, Mao JH, Nagase H, Cardiff RD, Cunha GR, Balmain A. Dissociation of epithelial and neuroendocrine carcinoma lineages in the transgenic adenocarcinoma of mouse prostate model of prostate cancer. *Am J Pathol* 2008;172(1):236–246.
  43. Huss WJ, Gray DR, Tavakoli K, Marmillion ME, Durham LE, Johnson MA, Greenberg NM, Smith GJ. Origin of androgen-insensitive poorly differentiated tumors in the transgenic adenocarcinoma of mouse prostate model. *Neoplasia* 2007; 9(11):938–950.
  44. Chin L, Gray JW. Translating insights from the cancer genome into clinical practice. *Nature* 2008;452(7187):553–563.
  45. Gingrich JR, Barrios RJ, Foster BA, Greenberg NM. Pathologic progression of autochthonous prostate cancer in the TRAMP model. *Prostate Cancer Prostatic Dis* 1999;2(2):70–75.
  46. Bethel CR, Bieberich CJ. Loss of Nkx3.1 expression in the transgenic adenocarcinoma of mouse prostate model. *Prostate* 2007;67(16):1740–1750.
  47. Steiner MS. High-grade prostatic intraepithelial neoplasia and prostate cancer risk reduction. *World J Urol* 2003;21(1):15–20.
  48. Jiang D, Srinivasan A, Lozano G, Robbins PD. SV40 T antigen abrogates p53-mediated transcriptional activity. *Oncogene* 1993; 8(10):2805–2812.
  49. DeCaprio JA, Ludlow JW, Figge J, Shew JY, Huang CM, Lee WH, Marsilio E, Paucha E. DM L. SV40 large tumor antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell* 1988;54(2):275–283.