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Microarray Data Mining for Potential Selenium Targets in Chemoprevention of Prostate Cancer

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Abstract. *Background: A previous clinical trial showed that selenium supplementation significantly reduced the incidence of prostate cancer. We report here a bioinformatics approach to gain new insights into selenium molecular targets that might be relevant to prostate cancer chemoprevention. Materials and Methods: We first performed data mining analysis to identify genes which are consistently dysregulated in prostate cancer using published datasets from gene expression profiling of clinical prostate specimens. We then devised a method to systematically analyze three selenium microarray datasets from the LNCaP human prostate cancer cells, and to match the analysis to the cohort of genes implicated in prostate carcinogenesis. Moreover, we compared the selenium datasets with two datasets obtained from expression profiling of androgen-stimulated LNCaP cells. Results: We found that selenium reverses the expression of genes implicated in prostate carcinogenesis. In addition, we found that selenium could counteract the effect of androgen on the expression of a subset obtained from androgen-regulated genes. Conclusions: The above information provides us with a treasure of new clues to investigate the mechanism of selenium chemoprevention of prostate cancer. Furthermore, these selenium target genes could also serve as biomarkers in future clinical trials to gauge the efficacy of selenium intervention.*

Abbreviations: AR, androgen receptor; ARE, androgen responsive element; MSA, methylseleninic acid; PCa, prostate cancer; PSA, prostate specific antigen; TGFβ, transforming growth factor β.

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Supplementation with a nutritional dose of selenium was found to reduce prostate cancer incidence by 50% in a randomized, placebo-controlled cancer prevention trial (1-3). Prostate cancer was actually a secondary endpoint in this study, which was designed originally to evaluate the effect of selenium on non-melanoma skin cancer. We reported previously that a selenium metabolite, in the form of methylseleninic acid or MSA, suppressed the growth of both the androgen-responsive LNCaP and the androgen-refractory PC-3 human prostate cancer cells (4,5). Growth inhibition by MSA was time- and dose-dependent, with an IC_{50} of ~10 μM at 48 hours of treatment. In order to identify the molecular alterations that might be responsible for the growth inhibitory effect of selenium, we profiled gene expression changes in PC-3 cells using the Affymetrix 12K-gene oligonucleotide chip (4,5). Several working hypotheses have been generated from this dataset regarding the mechanisms of selenium action (4,5). In the present study, we completed a similar selenium array analysis in the androgen-responsive LNCaP cells using a 3K custom cDNA array. The smaller array is expected to improve the sensitivity of the assay, although the advantage is compromised by the reduced size of the dataset. Recently, Zhao *et al.* also performed microarray analysis in MSA-treated LNCaP cells using a high-density cDNA array (6). Our goal was to make use of these three selenium datasets and develop a global data mining strategy to earmark putative prostate cancer genes which are sensitive to selenium intervention.

Our approach was to compare the selenium datasets to three recently published prostate cancer microarray datasets generated from human tumor specimen. The first was an Affymetrix oligonucletide array study in 50 normal and 52 prostate cancers reported by Singh *et al.* (7). The second, described by Welsh *et al.* (8), was similar to the first with the exception that fewer samples were examined (9 normal and 25 prostate cancers). The third was an analysis of 41 normal

and 62 prostate cancers by Lapointe *et al.* (9) using a 26K-gene cDNA microarray. These three prostate cancer datasets offer a rich source of information of dysregulated genes implicated in prostate carcinogenesis.

Androgen receptor (AR) signaling is known to play an important role in promoting prostate cancer progression (10). Consequently disruption of AR signaling is an effective means of prostate cancer management. We newly reported that selenium is capable of decreasing the expression and transactivating activity of AR (4). This novel finding underlies the justification of applying microarray analysis to investigate whether the expression of AR-regulated genes might be counteracted by selenium. Recent events have made this query possible. In separate studies by DePrimo *et al.* (11) and Nelson *et al.* (12), LNCaP cells were treated with a synthetic androgen and microarray analyses were then performed to identify genes responsive to androgen stimulation. These two androgen datasets are well suited to serve as a tool to mine the selenium datasets for additional clues. Collectively, the timely publication of a number of prostate cancer and androgen microarrays in the past two years provides an opportunity and sets the stage for the present effort to advance our understanding of selenium chemoprevention of prostate cancer.

Materials and Methods

Our own cDNA microarray analysis of LNCaP cells treated with selenium. The culture conditions of LNCaP cells have been described in detail previously (4). After exposure to 10 μM MSA for 3, 6, 12, 24, 36, or 48 h, total RNA and protein were isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). The RNA collected from three independent experiments was pooled and subjected to microarray analysis using a 3K human cDNA microarray printed at the Microarray and Genomics Core Facility at Roswell Park Cancer Institute. This custom cDNA array was constructed based on the genes which were found to be modulated by selenium in PC-3 cells from our previous study (5). Each gene on this array was spotted in triplicate. Probe generation and array hybridization were conducted according to a protocol developed by the Core Facility (http://microarrays.roswellpark.org/Protocols). The hybridization signals were captured using an Affymetrix 428 array scanner (Affymetrix, Santa Clara, CA, USA), and analyzed using the ImaGene software (BioDiscovery, Inc., Marina Del Ray, CA, USA). Poor quality spots, along with spots with signal levels indistinguishable from background, were disgarded. The extracted image data were then processed by a series of steps including background subtraction, data normalization, ratio calculation, and statistical analysis of replicate spots. Data processing was done with the use of the ImaGene (BioDiscovery, Inc.) and the GeneTraffic software (Iobion Informatics LLC, La Jolla, CA, USA), the statistical package R, and in-house PERL programs. In order to control for the noise introduced by the fluorescent dyes, Cy3 and Cy5, each array experiment was performed twice with the labeling dyes reversed to eliminate dye biases, and the signal ratios from these two experiments were averaged. A $\log_2$-transformed treatment to control signal ratio of ≥ 1 or ≤ -1 was chosen as the criteria for induction or repression, respectively. These threshold values are commonly used in the literature for microarray expression analysis (13,14). Hierarchical clustering analysis was performed using the Hierarchical Clustering Explorer software from the University of Maryland, USA.

Processing of publicly available microarray datasets. The datasets from the six published gene expression profiling studies (cited as references 7-9) were downloaded from the authors' respective websites. Our own selenium PC-3 dataset (cited as reference 5) and selenium LNCaP dataset are available at the Roswell Park website (http://falcon.roswellpark.org/ publication/CIp/dataMining). In view of the fact that the eight microarrays originated from different sources, one must appreciate that different identifiers, including cDNA clone IDs, probe set IDs, and GenBank accession numbers, were used to label the genes. In order to facilitate data comparison, these identifiers were mapped to the UniGene database (Build 136) at the National Center for Biotechnology Information (NCBI). The UniGene Cluster IDs were used to cross-reference genes in different datasets.

Permutation t-test analysis of prostate cancer datasets. For the three prostate cancer datasets (7-9), only samples classified as primary prostate cancer or normal prostate were included in the analysis; all other sample types were excluded from the original datasets. In order to identify genes that are differentially expressed between normal and cancer tissues, permutation *t*-test analysis was performed individually with each dataset. The *t*-statistic of a gene was calculated by the following formula:

$$t = \frac{\mu_1 - \mu_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

where μ_i is the mean expression value of a given gene in the i^{th} group, σ_i^2 is the variance of that gene, and n_i is the sample size of the i^{th} group. The procedure of permutation was carried out on a gene-by-gene basis by randomly assigning each data point to either the normal or cancer group, while maintaining the total sample size of each group. This process was repeated 10,000 times and the *p*-value was defined as the fraction of *t*-statistics generated from randomization that was greater than or equal to the *t*-statistic generated from the actual data points. This method of analysis makes allowance for missing data points; however, anything with less than 5 data points is generally not expected to have sufficient statistical power and is therefore excluded from the analysis. A list of dysregulated genes was compiled based on the following criteria: *p*-values less than 0.001, and consistent changes in at least two out of the three datasets. The false discovery rate (q) was calculated as follows: $q = \frac{pxn}{i}$, where p is the p-value, n is the total number of genes, and i is the number of genes with a p-value less than p. The above analyses were performed with in-house PERL programs.

Merging of datasets. Our selenium LNCaP dataset and Zhao's selenium LNCaP dataset (6) were generated by an essentially identical protocol. The merging of these two datasets or, for that matter, other compatible datasets, would greatly increase the power and precision of the analysis provided that certain key parameters are properly safeguarded. Since the above two array experiments were conducted at multiple time points, it is necessary

to devise a method for categorizing the pattern of expression changes across all time points. The data were filtered first to admit only those changes (induction or repression) that were over the 2-fold threshold *(i.e.* $\log_2$-transformed ratio ≥1 or ≤-1). A decision call of induction or repression was made for each gene only if ≥70% of the filtered data points showed the same direction of change. A consolidated LNCaP dataset was generated by merging the two LNCaP datasets and discarding genes with conflicting decision calls. The two androgen datasets of DePrimo *et al.* (11) and Nelson *et al.* (12) were merged in a similar manner. The above analyses were performed with in-house PERL programs.

Functional annotation of transcripts. Once a gene has been identified to be a target of selenium intervention, we assign it to a functional category for informational purposes. Functional annotation of transcripts was performed by using the Gene Ontology (GO) database and literature review. The UniGene cluster IDs of these genes were used to query the LocusLink database at NCBI (http://www.ncbi.nlm.nih.gov/ LocusLink/) in order to extract the GO terms associated with these genes.

Results

Microarray data mining of genes implicated in prostate carcinogenesis. The three prostate cancer datasets (7-9) were chosen for our investigation because they represent the largest gene expression profiling studies comparing normal and cancerous prostate tissues. No statistical analysis, however, was performed in these three studies to identify putative prostate cancer genes. Since each of these datasets has independent measurements of gene expression in the normal and tumor groups, we undertook a systematic statistical evaluation of their results. Permutation *t*-test was carried out on each dataset, and genes with *p*-values <0.001 were selected as differentially expressed between the normal and cancer groups. Based on this criterion, 5,306 genes were pulled out from the Lapointe study (7-9), 672 from the Singh study (7-9), and 1,527 from the Welsh study (7-9). Our selection method has false discovery rates of 0.005, 0.019, and 0.008, respectively. For cross-validation, we reduced the number of genes to those with the same expression pattern in at least two out of three datasets. This procedure narrowed the list down to 1,067 genes with aberrant expression in prostate cancer. Among these, 497 or 46.6% are up-regulated, and 570 or 53.4% are down-regulated. The top 50 up- or down-regulated genes that appear in all three datasets, ranked by the average ratio, are listed in Tables IA and IB. The complete list can be accessed at our website.

Microarray analysis of LNCaP cells treated with selenium. A hierarchical clustering algorithm was applied to group genes according to their expression pattern across six time points following treatment with MSA. The clustering analysis of 762 selenium-responsive genes is shown in Figure 1. The branch points in the dendrogram correspond to each gene, and the length of the branches reflects the degree of relatedness. Red and green squares represent up-regulation and down-regulation, respectively, relative to the control values. Black squares indicate no change, and gray squares signify data of insufficient quality. The genes identified and the raw array data are available at our website. Four distinct clusters emerge from this analysis. Clusters A and C are composed of genes with a gradual or a rapid increase in expression level, respectively. Clusters B and D represent the group of genes with a rapid or gradual reduction in expression level, respectively.

Selenium reverses the expression of genes implicated in prostate carcinogenesis. The cellular responses of the androgen-responsive LNCaP cells and the androgen-nonresponsive PC-3 cells to selenium are very similar. These two cell models represent different stages of prostate cancer progression. In order to identify relevant molecular targets underlying selenium chemopreventive action in incident prostate cancer or late stage relapse, we matched the prostate cancer datasets to the selenium LNCaP and PC-3 datasets. The goal was to identify dysregulated prostate cancer genes which could be reversed or restored to normal by selenium in both LNCaP and PC-3 cells. In this analysis, we compared 1,067 genes that are consistently dysregulated in prostate cancer and 427 genes that are sensitive to selenium modulation in both LNCaP and PC3 cells. We found that there are a total of 71 genes common to both datasets. Among these, 25 are regulated in the same direction, 42 are regulated reciprocally, and 4 are regulated spuriously. Theoretically, when comparing a random list of 1,067 genes with another random list of 427 genes from the human genome (estimated to contain a total of ~30,000 genes), the number of overlap one would expect to obtain is: $\frac{1067}{30000} \times \frac{427}{30000} \times 30000 \approx 15$ genes. Assuming there is a 50% chance of these 15 genes being modulated reciprocally *(i.e.* a random distribution), the number of genes in this category would be reduced by half to 7.5. This number is far less than the 42 reciprocally regulated genes we have identified. Therefore, it is very unlikely that the outcome of our data mining method is due only to chance. These 42 genes are listed in Table II. A negative value denotes down-regulation, while a positive value indicates up-regulation. The flip-flop between the PCa (prostate cancer) column and the two Se columns is self-evident. Three genes, UMPK, SERPINB5, and FOXA1, are also present in Tables IA or IB. It should be noted that the genes in these two tables are only subsets of the cohort of prostate cancer genes used in this analysis.

The genes in Table II are further classified into a number of functional categories. Because of space limitation, it is not possible to elaborate the function of each of these genes.

Table IA. *Top 50 up-regulated genes in prostate cancers.*

Unigene ID	Symbol	Gene description	$\log_2$ transformed ratio#			
			Lapointe	Welsh	Singh	Average
Hs.49598*	AMACR	alpha-methylacyl-CoA racemase	3.31	3.53	3.29	3.38
Hs.118483	MYO6	myosin VI	1.81	2.21	4.36	3.26
Hs.27311	SIM2	single-minded homolog 2 (Drosophila)	1.58	4.00	3.21	3.23
Hs.820	HOXC6	homeo box C6	0.60	3.12	4.08	3.18
Hs.432750*	HPN	hepsin (transmembrane protease, serine 1)	2.51	2.82	3.38	2.95
Hs.458360	UMPK	uridine monophosphate kinase	0.88	2.58	3.93	2.94
Hs.93304	PLA2G7	phospholipase A2, group VII	1.50	2.33	3.69	2.79
Hs.306812	BUCS1	butyryl Coenzyme A synthetase 1	2.70	2.35	3.17	2.78
Hs.412020	BICD1	Bicaudal D homolog 1 (Drosophila)	1.21	2.09	2.87	2.21
Hs.155419	BIK	BCL2-interacting killer (apoptosis-inducing)	0.56	1.60	3.06	2.1
Hs.296638*	PLAB	prostate differentiation factor	0.83	2.83	1.81	2.05
Hs.154103*	LIM	LIM protein (similar to rat protein kinase C-binding enigma)	1.57	1.91	2.22	1.93
Hs.405961	OASIS	old astrocyte specifically induced substance	0.70	1.38	2.67	1.82
Hs.76901	PDIR	for protein disulfide isomerase-related	0.69	2.05	1.99	1.7
Hs.334707	ACY1	aminoacylase 1	0.77	2.00	1.67	1.57
Hs.380460	ICA1	islet cell autoantigen 1, 69kDa	0.70	0.84	2.45	1.57
Hs.360509	FBP1	fructose-1,6-bisphosphatase 1	1.15	1.51	1.82	1.52
Hs.38972	TSPAN-1	tetraspan 1	0.62	1.76	1.83	1.5
Hs.356894	HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	0.78	1.76	1.71	1.48
Hs.278611	GALNT3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)	0.80	1.44	1.76	1.39
Hs.440478	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	1.08	1.79	1.17	1.38
Hs.306251	ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	0.28	1.24	2.05	1.36
Hs.247817	HIST1H2BK	histone 1, H2bk	0.86	1.73	1.34	1.35
Hs.444439*	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	0.79	1.23	1.82	1.34
Hs.83919	GCS1	glucosidase I	0.57	1.32	1.85	1.34
Hs.156682	IGSF4	immunoglobulin superfamily, member 4	0.63	1.76	1.32	1.31
Hs.75139	ARFIP2	ADP-ribosylation factor interacting protein 2 (arfaptin 2)	0.42	1.47	1.71	1.30
Hs.512670	BCAT2	branched chain aminotransferase 2, mitochondrial	0.53	1.31	1.79	1.30
Hs.434243	KIBRA	KIBRA protein	0.71	1.44	1.59	1.29
Hs.297343	CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta	0.87	1.78	0.97	1.26
Hs.387140	FLJ20323	hypothetical protein FLJ20323	0.37	1.18	1.85	1.25
Hs.405410	OGT	O-linked N-acetylglucosamine (GlcNAc) transferase	0.61	1.15	1.75	1.24
Hs.21293*	UAP1	UDP-N-acteylglucosamine pyrophosphorylase 1	1.05	1.27	1.39	1.24
Hs.155040	ZNF217	zinc finger protein 217	0.73	1.43	1.45	1.24
Hs.82280	RGS10	regulator of G-protein signalling 10	1.00	0.85	1.62	1.20
Hs.76285*	DKFZP564B167	DKFZP564B167 protein	0.79	1.29	1.38	1.17
Hs.449815		similar to My016 protein	0.28	0.92	1.85	1.16
Hs.234521	MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	0.47	1.12	1.61	1.14
Hs.2551	ADRB2	adrenergic, beta-2-, receptor, surface	0.52	1.45	1.28	1.13
Hs.357901*	SOX4	SRY (sex determining region Y)-box 4	0.94	1.36	1.05	1.13
Hs.406534	HMG20B	high-mobility group 20B	0.55	1.17	1.50	1.12
Hs.166697	LRIG1	leucine-rich repeats and immunoglobulin-like domains 1	0.77	1.37	1.15	1.12
Hs.118638*	NME1	non-metastatic cells 1, protein (NM23A) expressed in	0.74	1.14	1.39	1.11
Hs.79064	DHPS	deoxyhypusine synthase	0.42	0.48	1.89	1.10
Hs.21894	ARHCL1	ras homolog gene family, member C like 1	0.60	1.59	0.82	1.07
Hs.424551	P24B	integral type I protein	0.54	1.26	1.29	1.07
Hs.75432	IMPDH2	IMP (inosine monophosphate) dehydrogenase 2	0.77	1.01	1.35	1.06
Hs.163484	FOXA1	forkhead box A1	0.33	1.16	1.46	1.05
Hs.291385	ATP8A1	ATPase, aminophospholipid transporter, Class I, type 8A, member 1	0.85	1.19	1.10	1.05
Hs.423095	NUCB2	nucleobindin 2	0.51	1.40	1.10	1.05

Table IB. *Top 50 down-regulated genes in prostate cancers.*

Unigene ID	Symbol	Gene description	log_2 transformed ratio			
			Lapointe	Welsh	Singh	Average
Hs.75652	GSTM5	glutathione S-transferase M5	-1.80	-1.47	-∞	-2.21
Hs.77854	RGN	regucalcin (senescence marker protein-30)	-1.05	-2.44	-5.06	-2.10
Hs.339831	PENK	proenkephalin	-1.24	-1.71	-4.21	-1.94
Hs.34114	ATP1A2	ATPase, Na^+/K^+ transporting, alpha 2 (+) polypeptide	-1.37	-2.39	-2.22	-1.92
Hs.80552	DPT	dermatopontin	-1.50	-1.89	-2.35	-1.87
Hs.7357	CLIPR-59	CLIP-170-related protein	-0.70	-2.81	-3.76	-1.85
Hs.301914	DAT1	Neuronal specific transcription factor DAT1	-1.09	-1.83	-3.45	-1.83
Hs.440324	NRLN1	Neuralin 1	-1.41	-2.28	-1.88	-1.81
Hs.78748	RIMS3	regulating synaptic membrane exocytosis 3	-1.17	-1.42	-3.96	-1.76
Hs.448805	GPRC5B	G protein-coupled receptor, family C, group 5, member B	-1.14	-1.55	-3.41	-1.76
Hs.55279	SERPINB5	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 5	-1.38	-2.02	-1.92	-1.75
Hs.78792	NCAM1	neural cell adhesion molecule 1	-1.40	-1.29	-3.20	-1.75
Hs.60177	DZIP1	zinc finger DAZ interacting protein 1	-0.77	-1.82	-4.84	-1.73
Hs.5378	SPON1	spondin 1, (f-spondin) extracellular matrix protein	-1.09	-1.19	-6.12	-1.70
Hs.408	COL4A6	Collagen, type IV, alpha 6	-1.26	-1.97	-1.98	-1.70
Hs.406238	AOX1	Aldehyde oxidase 1	-1.31	-1.96	-1.77	-1.65
Hs.234863	TSPAN-2	tetraspan 2	-0.95	-1.32	-3.90	-1.60
Hs.348387	GSTM4	glutathione S-transferase M4	-1.46	-1.73	-1.62	-1.60
Hs.408767	CRYAB	crystallin, alpha B	-1.62	-1.74	-1.31	-1.55
Hs.411509	GSTP1	glutathione S-transferase pi	-1.24	-1.38	-2.19	-1.55
Hs.101850	RBP1	retinol binding protein 1, cellular	-0.98	-1.41	-2.41	-1.48
Hs.211933	COL13A1	Collagen, type XIII, alpha 1	-0.65	-1.54	-3.28	-1.47
Hs.80395	MAL	mal, T-cell differentiation protein	-0.93	-0.81	-∞	-1.45
Hs.93841	KCNMB1	potassium large conductance calcium-activated channel, subfamily M, beta member 1	-1.37	-1.90	-1.14	-1.44
Hs.74034	CAV1	Caveolin 1, caveolae protein, 22kDa	-1.71	-1.65	-1.05	-1.44
Hs.139851*	CAV2	Caveolin 2	-1.35	-1.34	-1.62	-1.43
Hs.302085	PTGIS	prostaglandin I2 (prostacyclin) synthase	-1.09	-1.52	-1.76	-1.43
Hs.103839	EPB41L3	erythrocyte membrane protein band 4.1-like 3	-0.77	-1.81	-1.92	-1.40
Hs.436657	CLU	Clusterin	-1.35	-1.55	-1.30	-1.40
Hs.421621	COX7A1	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)	-1.23	-1.73	-1.17	-1.36
Hs.79015	MOX2	antigen identified by monoclonal antibody MRC OX-2	-1.08	-1.65	-1.39	-1.36
Hs.131380	SGCD	sarcoglycan, delta (35kDa dystrophin-associated glycoprotein)	-0.76	-1.96	-1.59	-1.35
Hs.5422*	GPM6B	glycoprotein M6B	-0.80	-1.17	-2.47	-1.33
Hs.2006	GSTM3	glutathione S-transferase M3 (brain)	-0.27	-2.01	-2.87	-1.31
Hs.156007*	DSCR1L1	Down syndrome critical region gene 1-like 1	-1.10	-1.73	-1.15	-1.30
Hs.430166	PLS3	plastin 3 (T isoform)	-0.63	-1.09	-3.16	-1.29
Hs.24587	EFS	embryonal Fyn-associated substrate	-1.56	-0.54	-2.25	-1.28
Hs.8022	TU3A	TU3A protein	-1.42	-1.15	-1.27	-1.28
Hs.439040	RPESP	RPE-spondin	-0.99	-1.27	-1.64	-1.28
Hs.2463	ANGPT1	angiopoietin 1	-1.40	-1.41	-0.98	-1.25
Hs.362805*	MEIS2	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse)	-1.12	-1.47	-1.13	-1.23
Hs.300772	TPM2	tropomyosin 2 (beta)	-1.16	-1.80	-0.80	-1.20
Hs.372031*	PMP22	peripheral myelin protein 22	-1.00	-1.79	-0.90	-1.18
Hs.79386	LMOD1	leiomodin 1 (smooth muscle)	-0.76	-2.17	-0.96	-1.18
Hs.414407	HEC	highly expressed in cancer, rich in leucine heptad repeats	-0.80	-1.10	-1.76	-1.17
Hs.137569	TP73L	tumor protein p73-like	-1.36	-1.19	-0.94	-1.16
Hs.75350*	VCL	Vinculin	-1.18	-1.31	-0.99	-1.15
Hs.150358	DPYSL3	dihydropyrimidinase-like 3	-0.94	-1.49	-1.04	-1.14
Hs.79226	FEZ1	fasciculation and elongation protein zeta 1 (zygin I)	-0.33	-1.30	-2.55	-1.13
Hs.81412	LPIN1	lipin 1	-0.69	-1.33	-1.47	-1.12

#log2-tranformed cancer to normal signal ratio. The average is obtained by calculating the mean of the three linear ratios and transforming the mean to log_2 value. – (x) indicates a linear ratio of 0.
*these genes are also present in Rhodes *et al.* (35).

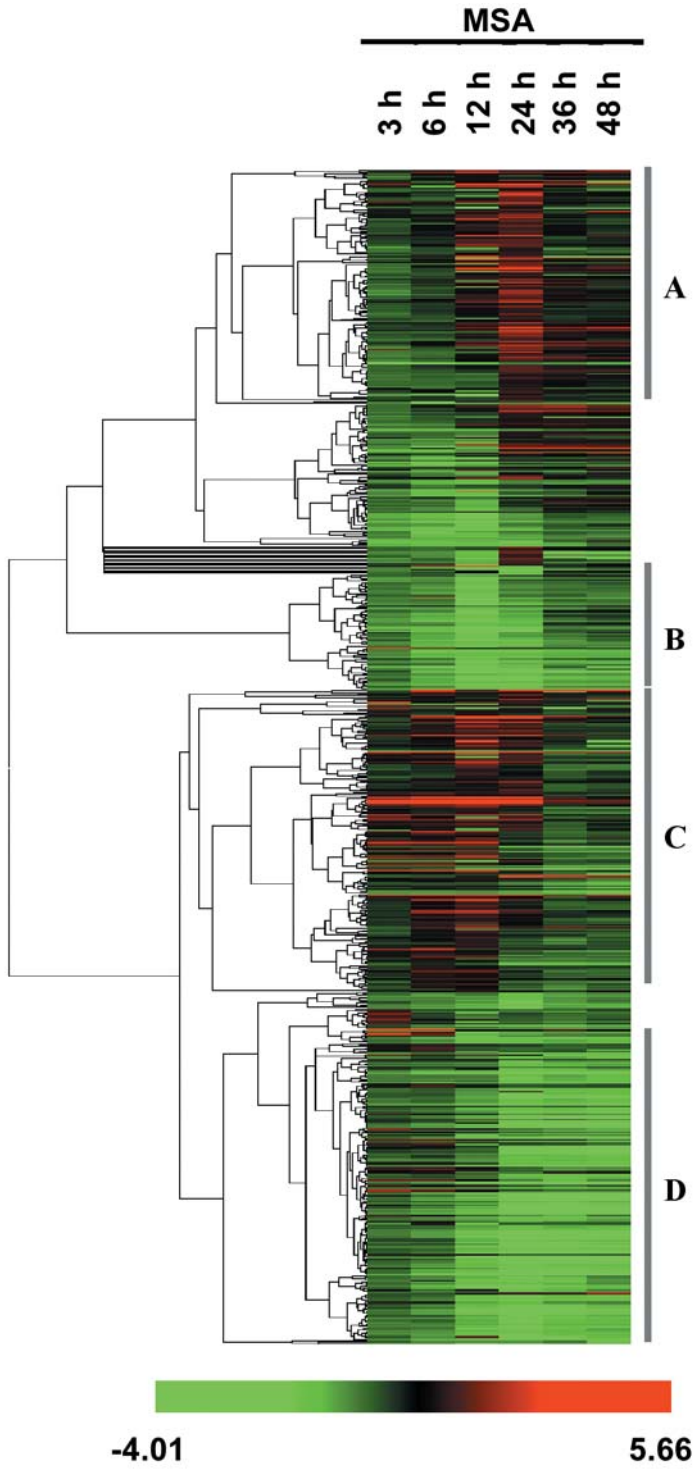

Figure 1. *One-dimensional hierarchical clustering of selenium-responsive genes in LNCaP cells. Rows represent individual genes, and columns represent different time points. Each cell represents the expression level of a gene at a given time point, with red and green indicating up- and down-regulation, respectively, black indicates no change, and gray indicates missing values.*

Suffice it to note that a significant number of them is involved in controlling cell cycle progression and/or cell death, including AHR (15), CHC1 (16), CDKN1C (17), ATF5 (18), and FOXO1A (19-21). Selenium modulates their expression in a way that is consistent with cell growth inhibition, cell cycle block, and apoptosis stimulation. Table II also shows that selenium is able to up-regulate the expression of four genes with tumor suppressing activities. SERPINB5, also known as maspin, is a serine proteinase inhibitor capable of suppressing tumor invasion, apoptosis,

Table II. *Selenium reverses the expression of genes implicated in prostate carcinogenesis: common to LNCaP and PC-3 cells.*

UniGene ID	Symbol	Gene description	Fold Change (log_2)*		
			PCa	Se/LNCaP	Se/PC3
Cell proliferation/Apoptosis					
Hs.170087	AHR	aryl hydrocarbon receptor	-0.81	3.96	2.46
Hs.9754	ATF5	activating transcription factor 5	0.62	-1.31	-3.14
Hs.106070	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	-0.87	1.16	2.14
Hs.196769	CHC1	chromosome condensation 1	1.06	-1.05	-1.68
Hs.184161	EXT1	exostoses (multiple) 1	-0.53	1.20	1.00
Hs.170133#	FOXO1A	forkhead box O1A (rhabdomyosarcoma)	-0.86	1.17	1.26
Hs.82028	TGFBR2	transforming growth factor, beta receptor II (70/80kDa)	-0.76	1.31	1.2
Signal transduction					
Hs.337774	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	-0.86	1.26	1.85
Hs.116796	DIXDC1	DIX domain containing 1	-1.23	2.31	1.00
Hs.381928	DVL3	dishevelled, dsh homolog 3 (Drosophila)	-0.61	1.88	3.29
Hs.211569	GRK5	G protein-coupled receptor kinase 5	-1.49	2.32	2.10
Hs.436004	JAK1	Janus kinase 1 (a protein tyrosine kinase)	-0.53	1.89	2.46
Hs.79219	RGL1	ral guanine nucleotide dissociation stimulator-like 1	-2.15	1.04	3.81
Transcriptional regulation					
Hs.163484	FOXA1	forkhead box A1	1.46	-1.36	-1.00
Hs.166017	MITF	microphthalmia-associated transcription factor	-0.55	1.13	1.68
Tumor suppressor genes					
Hs.386952	CYLD	cylindromatosis (turban tumor syndrome)	-1.20	1.19	1.20
Hs.446537	GSN	gelsolin (amyloidosis, Finnish type)	-1.38	1.78	1.43
Hs.55279#	SERPINB5	serine (or cysteine) proteinase inhibitor, clade B, member 5	-2.02	1.04	1.81
Hs.152207	SSBP2	single-stranded DNA binding protein 2	-0.68	1.72	1.63
Oncogenes					
Hs.390567	FYN	FYN oncogene related to SRC, FGR, YES	-0.52	2.00	2.04
Hs.223025	RAB31	RAB31, member RAS oncogene family	-1.05	1.94	1.43
Cytoskeleton					
Hs.26208	COL16A1	collagen, type XVI, alpha 1	-1.34	1.35	2.68
Hs.440387	EPB41L2	erythrocyte membrane protein band 4.1-like 2	-0.78	1.25	1.07
Metabolism					
Hs.264330	ASAHL	N-acylsphingosine amidohydrolase (acid ceramidase)-like	1.62	-1.63	-2.74
Hs.303154	IDS	iduronate 2-sulfatase (Hunter syndrome)	-0.43	1.60	1.49
Hs.167531	MCCC2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	2.26	-1.27	-1.43
Hs.458360#	UMPK	uridine monophosphate kinase	3.93	-1.03	-1.00
Other functions					
Hs.408767	CRYAB	crystallin, alpha B	-1.74	1.84	2.17
Hs.8302	FHL2	four and a half LIM domains 2	-1.25	1.13	1.26
Hs.848	FKBP4	FK506 binding protein 4, 59kDa	0.93	-1.22	-1.14
Hs.81361	HNRPAB	heterogeneous nuclear ribonucleoprotein A/B	0.51	-1.26	-1.00
Hs.372571	MBNL2	muscleblind-like 2 (Drosophila)	-1.09	2.93	1.63
Hs.390162	OPTN	Optineurin	-1.94	3.71	1.72
Hs.1501	SDC2	syndecan 2	-0.96	1.01	1.68
Hs.439643	SLC16A7	solute carrier family 16, member 7	-1.20	1.41	1.32
Unknown					
Hs.27621		CDNA FLJ12815 fis, clone NT2RP2002546	-1.49	1.10	1.26
Hs.440808	FNBP1	formin binding protein 1	-1.42	1.16	1.32
Hs.336429	GABARAPL1	GABA(A) receptor-associated protein like 1	-0.68	1.03	1.43
Hs.42322	PALM2	paralemmin 2	-1.00	1.24	1.81
Hs.224262	PJA2	praja 2, RING-H2 motif containing	-0.50	1.36	1.68
Hs.439776	STOM	Stomatin	-0.91	1.50	1.32
Hs.433838	STX12	syntaxin 12	-0.56	1.35	1.00

* For LNCaP and PC-3, the ratio is the maximum value of the data points from all the time- and concentration-series of selenium treatment. For PCa, it is the largest value from three prostate cancer datasets.
# also present in Table IA or IB.

Table III. *Selenium reverses the expression of genes implicated in prostate carcinogenesis: unique to LNCaP cells.*

UniGene ID	Symbol	Gene description	Fold Change (log_2)*	
			PCa	Se
Cell proliferation/Apoptosis				
Hs.77311	BTG3	BTG family, member 3	-0.66	1.42
Hs.95577	CDK4	cyclin-dependent kinase 4	0.42	-1.08
Hs.348153	CUL1	cullin 1	-0.34	1.45
Hs.118638	NME1	non-metastatic cells 1, protein (NM23A) expressed in	1.39	-1.21
Hs.169840	TTK	TTK protein kinase	1.24	-1.13
Signal transduction				
Hs.197081	AKAP12	A kinase (PRKA) anchor protein (gravin) 12	-0.93	1.16
Hs.271809	GPR161	G protein-coupled receptor 161	-0.89	1.01
Hs.433488	GUCY1A3	guanylate cyclase 1, soluble, alpha 3	3.10	-1.53
Hs.149900	ITPR1	inositol 1,4,5-triphosphate receptor, type 1	-1.44	1.53
Transcriptional regulation				
Hs.76884	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	-0.99	4.55
Hs.408222	PBX1	pre-B-cell leukemia transcription factor 1	-1.00	1.29
Hs.360174	SNAI2	snail homolog 2 (Drosophila)	-1.29	1.22
Transporter				
Hs.307915	ABCC4	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	2.56	-1.08
Hs.99865	EPIM	Epimorphin	-1.63	1.05
Hs.14732	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic	-1.04	1.55
Hs.221974	SNAP25	synaptosomal-associated protein, 25kDa	-1.39	1.88
Cytoskeleton				
Hs.403989	ACTG2	actin, gamma 2, smooth muscle, enteric	-1.51	1.02
Hs.440478	ANK3	ankyrin 3, node of Ranvier (ankyrin G)	1.79	-1.51
Hs.446375	MAPRE2	microtubule-associated protein, RP/EB family, member 2	-0.78	1.24
Hs.108924	SORBS1	sorbin and SH3 domain containing 1	-1.28	1.91
Metabolism				
Hs.440117	ALG8	asparagine-linked glycosylation 8 homolog	1.12	-1.25
Hs.75616	DHCR24	24-dehydrocholesterol reductase	1.43	-1.07
Hs.268012	FACL3	fatty-acid-Coenzyme A ligase, long-chain 3	0.83	-1.14
Hs.75485	OAT	ornithine aminotransferase (gyrate atrophy)	-0.77	2.16
Hs.79886	RPIA	ribose 5-phosphate isomerase A (ribose 5-phosphate epimerase)	0.44	-1.46
Protease/Protease inhibitor				
Hs.181350	KLK2	kallikrein 2, prostatic	0.97	-2.27
Hs.171995	KLK3	kallikrein 3, (prostate specific antigen)	0.69	-2.45
Hs.21858	SERPINE2	serine (or cysteine) proteinase inhibitor, clade E, member 2	-0.92	1.23
Other functions				
Hs.237506	DNAJB5	DnaJ (Hsp40) homolog, subfamily B, member 5	-3.65	1.06
Hs.173381	DPYSL2	dihydropyrimidinase-like 2	-1.05	1.14
Hs.315177	IFRD2	interferon-related developmental regulator 2	0.44	-1.23
Hs.5025	NEBL	Nebulette	-0.78	1.57
Hs.131727	PFAAP5	phosphonoformate immuno-associated protein 5	-0.81	1.13
Hs.438582	PRNP	prion protein (p27-30)	-0.94	1.19
Hs.250607	UTRN	utrophin (homologous to dystrophin)	0.88	-1.01
Hs.435800	VIM	Vimentin	-0.61	1.61
Unknown				
Hs.48450		Human mRNA, trinucleotide repeat sequence.	-1.02	1.21
Hs.428112	DEAF1	deformed epidermal autoregulatory factor 1 (Drosophila)	0.82	-1.15
Hs.4747	DKC1	dyskeratosis congenita 1, dyskerin	0.86	-1.11
Hs.112605	DKFZP564O043	hypothetical protein DKFZp564O043	-0.72	1.58
Hs.301839	HABP4	hyaluronan binding protein 4	-0.86	1.33
Hs.278483	HIST1H4J	histone 1, H4j	3.31	-1.98
Hs.157818	KCNAB1	potassium voltage-gated channel, shaker-related subfamily, beta member 1	-1.48	1.38
Hs.408142	KIAA1109	hypothetical protein KIAA1109	-0.56	1.58
Hs.309244	KIAA1579	hypothetical protein FLJ10770	-0.48	2.29
Hs.90797	LOC129642	hypothetical protein BC016005	2.39	-1.40
Hs.270411	PLEKHC1	pleckstrin homology domain containing, family C, member 1	-1.48	1.46
Hs.5957	PTPLB	protein tyrosine phosphatase-like, member b	1.00	-1.06
Hs.356342	RPL27A	ribosomal protein L27a	0.52	-1.21

* For LNCaP, the ratio is the maximum value of the data points from all the time- and concentration-series of selenium treatment. For PCa, it is the average tumor to normal ratio.

and angiogenesis (22-24). It has been reported that the expression of SERPINB5 decreases with increasing prostate cancer malignancy (25). Gelsolin is under-expressed in several cancer types, including prostate (26-29). CYLD is a deubiquitinating enzyme which negatively regulates the activation of NFÎ B, an anti-apoptotic factor (30). Restoring the lost expression of CYLD in prostate cancer cells could conceivably sensitize them to apoptosis induction. SSBP2 is a translocation target in a leukemia cell line and is classified as a tumor suppressor candidate gene (31). It is intriguing that the expression of two oncogenes, FYN and RAB31, is down-regulated in prostate cancer. FYN is a member of the protein-tyrosine kinase oncogene family (32), and RAB31 belongs to the RAS oncogene family (33). The roles of these genes in prostate carcinogenesis are not clear; nonetheless, selenium is found to elevate the expression of both genes.

As a reminder, Table II is produced to highlight the putative prostate cancer genes sensitive to reversal of expression by selenium in both LNCaP and PC-3 cells. For the sake of thoroughness, we also present the analyses of two additional sets of prostate cancer genes which are uniquely modulated by selenium in either LNCaP (Table III) or PC-3 cells (Table IV). Due to the size of these tables, it would be tiresome to go through the data in any comprehensive fashion. Depending on future interests and evolving knowledge, this kind of information has value in seeking out clues and generating hypotheses.

Selenium reverses the effect of androgen on the expression of androgen-regulated genes. In an attempt to identify the androgen-regulated genes of which the expression is opposed by selenium, we compared the list of androgen-regulated genes (422 genes) to the list of selenium-responsive genes in LNCaP (1,031 genes). A partial summary of our analysis is shown in Table V. The AR (androgen-regulated) column shows the genes which are sensitive to androgen. A positive sign means up-regulation, while a minus means down-regulation. A total of 92 genes were found to be present in both datasets. As a control, a list of 1,031 genes were selected randomly from the selenium LNCaP dataset, and compared with the list of androgen-regulated genes to identify genes in common. This process was repeated 10 times, and the number of overlap was 30.4±1.6 (mean±SEM), which is significantly less than the actual number of 92 genes common to the androgen and selenium datasets ($p<0.0005$). Out of these 92 genes, only 38 genes (~41%) are reciprocally modulated by androgen and selenium (Table V). These 38 genes are the ones presented in Table V. In the Discussion, we will offer additional explanation of why only a fraction of AR-targets are oppositely modulated by androgen and selenium, even though selenium is a potent inhibitor of androgen signaling.

Discussion

Using prostate cancer chemoprevention as a research problem, Williams and Brooks (34) recently made a poignant commentary that microarray analysis holds great promise in unraveling the mechanisms of anticancer agents. Here we report, for the first time, a data mining approach to gain insight into the mechanisms of selenium utilizing published microarray datasets. The paradigm combines laboratory- and bioinformatics-based research to identify molecular targets or biomarkers of prostate cancer intervention by selenium. We recognize that this approach is only a first step in the discovery process. Nonetheless, the information extracted from this kind of analysis has significant potential in generating new leads to guide future research endeavors.

Rhodes *et al.* recently reported a meta-analysis of four datasets from prostate cancer gene expression profiling studies (35). Our study differs from the Rhodes study in a number of ways. First, two of the largest available datasets by Lapointe *et al.* (9) and Singh *et al.* (7) were not included in their analysis. Second, the Rhodes study compared localized prostate cancer to benign prostate tissue. The latter was inclusive of both normal prostate and benign prostatic hyperplasia (BPH). It has been reported that normal prostate and BPH have distinct gene expression patterns (36,37). Therefore, combining normal prostate and BPH into one single group could obscure some of the differences between normal and cancerous prostate. Third, instead of using a meta-analysis, we performed permutation *t*-test on each of the three datasets because they are large enough to generate independent and statistically verifiable information on their own. As a validation of our approach, the majority (~80%) of the top 40 over- and underexpressed genes of the Rhodes study are also present in our analysis (See our website). Additionally, our analysis picked up a few more genes (not found in the Rhodes paper) that are well known to be deregulated in prostate cancer, such as KLK2, KLK3 (PSA) (see our website), GSTP1, and SERPINB5 (Table IB).

We have identified 42 genes which are dysregulated in prostate cancer and are counter-regulated by selenium in both LNCaP and PC3 cells (Table II). In order to assess the significance of this analysis, we compared the functions of these genes with those of the 25 genes which are similarly regulated in prostate cancer and by selenium and found two major differences. First, there is no tumor suppressor gene modulated in the same direction in prostate cancer and by selenium. In contrast, there are four tumor suppressor genes which are down-regulated in prostate cancer, but are found to be up-regulated by selenium (Table II). Second, there is only one cell cycle regulatory gene modulated in the same direction in prostate cancer and by selenium. In contrast, there are five

Table IV. *Selenium reverses the expression of genes implicated in prostate carcinogenesis: unique to PC-3 cells.*

UniGene ID	Symbol	Gene description	Fold Change (log_2)	
			PCa	Se
Cell proliferation/Apoptosis				
Hs.109752	C6orf108	chromosome 6 open reading frame 108	0.67	-1.14
Hs.282410	CALM1	calmodulin 1 (phosphorylase kinase, delta)	-0.93	3.07
Hs.2132	EPS8	epidermal growth factor receptor pathway substrate 8	-1.29	2.10
Hs.65029	GAS1	growth arrest-specific 1	-1.67	1.14
Hs.370873	IFI16	interferon, gamma-inducible protein 16	-0.68	1.20
Hs.253067	MAEA	macrophage erythroblast attacher	0.34	-1.14
Hs.118630	MXI1	MAX interacting protein 1	-0.98	1.81
Hs.72660	PTDSR	phosphatidylserine receptor	-0.74	1.26
Hs.23582	TACSTD2	tumor-associated calcium signal transducer 2	0.99	-1.07
Cell adhesion				
Hs.415997	COL6A1	collagen, type VI, alpha 1	-1.36	2.81
Hs.79226	FEZ1	fasciculation and elongation protein zeta 1 (zygin I)	-2.55	1.49
Hs.277324	GALNT1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1	1.43	-1.07
Hs.437536	LAMA4	laminin, alpha 4	-1.24	1.00
Hs.436983	LAMB3	laminin, beta 3	-1.73	1.49
Hs.194431	KIAA0992	palladin	-1.35	1.38
Hs.46531	PGM5	phosphoglucomutase 5	-1.46	1.38
Cytoskeleton				
Hs.208641	ACTA2	actin, alpha 2, smooth muscle, aorta	-1.11	1.38
Hs.309415	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1	0.58	-1.00
Hs.65248	DNCI1	dynein, cytoplasmic, intermediate polypeptide 1	-1.46	1.07
Hs.58414	FLNC	filamin C, gamma (actin binding protein 280)	-2.17	1.68
Hs.80342	KRT15	keratin 15	-1.68	1.32
Hs.103042	MAP1B	microtubule-associated protein 1B	-1.37	1.00
Hs.433814	MYL9	myosin, light polypeptide 9, regulatory	-1.56	2.46
Hs.162953	MYRIP	myosin VIIA and Rab interacting protein	1.36	-1.96
Hs.387905	SPTAN1	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	-0.71	1.26
Hs.163111	SVIL	supervillin	-1.34	1.49
Hs.133892	TPM1	tropomyosin 1 (alpha)	-1.35	1.38
Lipid metabolism				
Hs.403436	DCI	dodecenoyl-Coenzyme A delta isomerase	1.13	-1.20
Hs.446676	LYPLA1	lysophospholipase I	1.18	-1.14
Hs.211587	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)	-0.71	1.68
Protease/Protease inhibitor				
Hs.440961	CAST	calpastatin	-0.45	1.14
Hs.83942	CTSK	cathepsin K (pycnodysostosis)	-1.10	1.43
Hs.117874	PACE4	paired basic amino acid cleaving system 4	1.20	-1.43
Hs.41072	SERPINB6	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	0.82	-1.07
Hs.173594	SERPINF1	serine (or cysteine) proteinase inhibitor, clade F, member 1	-1.24	1.32
Signal transduction				
Hs.256398	ADAM22	a disintegrin and metalloproteinase domain 22	-1.23	1.00
Hs.409783	ANK2	ankyrin 2, neuronal	-1.08	1.00
Hs.6838	ARHE	ras homolog gene family, member E	-1.35	1.68
Hs.245540	ARL4	ADP-ribosylation factor-like 4	-0.76	1.20
Hs.444947	C8FW	phosphoprotein regulated by mitogenic pathways	1.38	-3.31
Hs.12436	CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	-1.57	3.92
Hs.458426	CCK	cholecystokinin	-1.37	1.89
Hs.163867	CD14	CD14 antigen	0.88	-1.43
Hs.352554	CDC42EP3	CDC42 effector protein (Rho GTPase binding) 3	-1.11	2.10
Hs.255526	DTNA	dystrobrevin, alpha	-0.69	1.07
Hs.117060	ECM2	extracellular matrix protein 2, female organ and adipocyte specific	-0.93	1.00
Hs.211202	EDNRA	endothelin receptor type A	-1.59	1.20
Hs.82002	EDNRB	endothelin receptor type B	-1.57	1.14
Hs.381870	EFEMP2	EGF-containing fibulin-like extracellular matrix protein 2	-1.25	1.89
Hs.133968	FRAG1	FGF receptor activating protein 1	0.78	-2.51

Table IV. *Continued*

UniGene ID	Symbol	Gene description	Fold Change (log_2)	
			PCa	Se
Hs.74471	GJA1	gap junction protein, alpha 1, 43kDa (connexin 43)	-1.51	1.14
Hs.265829	ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	-1.23	2.54
Hs.188021	KCNH2	potassium voltage-gated channel, subfamily H (eag-related), member 2	-0.82	2.54
Hs.446645	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	0.53	-1.00
Hs.357004	LOC169611	hypothetical protein LOC169611	-2.11	1.00
Hs.21917	LPHN3	latrophilin 3	-2.35	1.43
Hs.155048	LU	Lutheran blood group (Auberger b antigen included)	1.57	-1.63
Hs.370849	MADH7	MAD, mothers against decapentaplegic homolog 7 (Drosophila)	-0.60	1.32
Hs.61638	MYO10	myosin X	1.35	-1.20
Hs.445402	PCTK3	PCTAIRE protein kinase 3	-2.77	1.58
Hs.77439	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	-0.75	1.14
Hs.349845	PRKCB1	protein kinase C, beta 1	-1.36	1.58
Hs.47438	SH3BGR	SH3 domain binding glutamic acid-rich protein	-1.76	1.26
Hs.169300	TGFB2	transforming growth factor, beta 2	-1.53	1.96
Hs.342874	TGFBR3	transforming growth factor, beta receptor III (betaglycan, 300kDa)	-1.47	1.85
Hs.332173	TLE2	transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)	-0.55	1.38
Hs.274329	TP53AP1	TP53 activated protein 1	0.91	-1.00
Hs.459470	WSB2	WD repeat and SOCS box-containing 2	0.88	-1.14
Hs.79474	YWHAE	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	1.10	-1.26
Transcription/Transcriptional regulation				
Hs.356416	CBX7	chromobox homolog 7	-1.12	1.43
Hs.405961	CREB3L1	cAMP responsive element binding protein 3-like 1	2.67	-2.20
Hs.43697	ETV5	ets variant gene 5 (ets-related molecule)	-3.64	1.54
Hs.171262	ETV6	ets variant gene 6 (TEL oncogene)	-0.63	2.00
Hs.331	GTF3C1	general transcription factor IIIC, polypeptide 1, alpha 220kDa	0.90	-1.14
Hs.127428	HOXA9	homeo box A9	1.14	-1.00
Hs.134859	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	-0.87	2.23
Hs.368950	MEF2C	MADS box transcription enhancer factor 2, polypeptide C	-0.88	2.14
Hs.443881	PAXIP1L	PAX transcription activation domain interacting protein 1 like	0.31	-1.26
Hs.3192	PCBD	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	0.79	-1.14
Hs.432574	POLR2H	polymerase (RNA) II (DNA directed) polypeptide H	0.87	-1.00
Hs.78202	SMARCA4	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4	0.67	-1.07
Hs.444445	SMARCD3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	-1.91	2.56
Hs.173911	ZNF24	zinc finger protein 24 (KOX 17)	0.46	-1.58
Hs.419763	ZNF43	zinc finger protein 43 (HTF6)	0.39	-1.07
Transporter				
Hs.374535	ATP2A2	ATPase, Ca^{++} transporting, cardiac muscle, slow twitch 2	-0.65	1.85
Hs.343522	ATP2B4	ATPase, Ca^{++} transporting, plasma membrane 4	-2.23	2.41
Hs.1602	DPYD	dihydropyrimidine dehydrogenase	-0.79	1.58
Hs.31720	HEPH	hephaestin	-1.34	1.38
Hs.188021	KCNH2	potassium voltage-gated channel, subfamily H (eag-related), member 2	-0.82	2.54
Hs.102308	KCNJ8	potassium inwardly-rectifying channel, subfamily J, member 8	-0.92	1.07
Hs.446645	KDELR2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	0.53	-1.00
Hs.101307	SLC14A1	solute carrier family 14 (urea transporter), member 1 (Kidd blood group)	-4.07	1.07
Hs.84190	SLC19A1	solute carrier family 19 (folate transporter), member 1	2.54	-1.14
Hs.417948	TCN2	transcobalamin II; macrocytic anemia	-0.60	1.00
Tumor suppressor gene/Oncogene				
Hs.171262	ETV6	ets variant gene 6 (TEL oncogene)	-0.63	1.07
Hs.65029	GAS1	growth arrest-specific 1	-1.67	1.14
Hs.349470	SNCG	synuclein, gamma (breast cancer-specific protein 1)	-3.68	1.89
Hs.203557	ST7	suppression of tumorigenicity 7	0.35	-2.61
Hs.8022	TU3A	TU3A protein	-1.42	1.14

Table IV. *Continued*

UniGene ID	Symbol	Gene description	Fold Change (log_2)	
			PCa	Se
Other functions				
Hs.75313	AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	-0.67	1.49
Hs.1227	ALAD	aminolevulinate, delta-, dehydratase	-1.32	2.63
Hs.153591	ALG3	asparagine-linked glycosylation 3 homolog (yeast, alpha-1,3-mannosyltransferase)	0.44	-1.00
Hs.102	AMT	aminomethyltransferase (glycine cleavage system protein T)	-0.80	1.43
Hs.135554	APG-1	heat shock protein (hsp110 family)	-0.90	1.20
Hs.78614	C1QBP	complement component 1, q subcomponent binding protein	0.75	-1.00
Hs.413482	C21orf33	chromosome 21 open reading frame 33	0.67	-1.43
Hs.323053	DKFZp547K1113	hypothetical protein DKFZp547K1113	-0.76	1.20
Hs.444619	DXS9879E	DNA segment on chromosome X (unique) 9879 expressed sequence	0.48	-2.29
Hs.511915	ENO2	enolase 2 (gamma, neuronal)	-1.85	1.49
Hs.412103	FLJ34588	Smhs2 homolog (rat)	0.66	-1.20
Hs.28264	FLJ90798	hypothetical protein FLJ90798	-1.20	1.49
Hs.386567	GBP2	guanylate binding protein 2, interferon-inducible	-0.92	1.32
Hs.121017	HIST1H2AE	histone 1, H2ae	3.67	-2.63
Hs.417332	HIST2H2AA	histone 2, H2aa	1.47	-1.14
Hs.44024	MRPL19	mitochondrial ribosomal protein L19	0.83	-1.26
Hs.9235	NME4	non-metastatic cells 4, protein expressed in	0.87	-1.20
Hs.447045	PPIL2	peptidylprolyl isomerase (cyclophilin)-like 2	0.61	-1.00
Hs.153355	QKI	quaking homolog, KH domain RNA binding (mouse)	-0.49	1.14
Hs.81256	S100A4	S100 calcium binding protein A4	-2.59	2.17
Hs.288215	SIAT7B	sialyltransferase 7 B	-0.95	1.26
Hs.511400	SND1	staphylococcal nuclease domain containing 1	0.97	-1.00
Hs.498154	SNX1	sorting nexin 1	-0.93	1.43
Hs.2943	SRP19	signal recognition particle 19kDa	0.97	-1.63
Hs.326	TARBP2	TAR (HIV) RNA binding protein 2	0.57	-1.07
Hs.8752	TMEM4	transmembrane protein 4	1.00	-1.26
Hs.112986	TMEM5	transmembrane protein 5	0.72	-1.07
Hs.370530	TRIM14	tripartite motif-containing 14	0.65	-1.07
Hs.66708	VAMP3	vesicle-associated membrane protein 3 (cellubrevin)	-0.49	1.68
Unknown				
Hs.148258	BC008967	hypothetical gene BC008967	-1.59	1.32
Hs.277888	CG018	hypothetical gene CG018	-0.9	1.68
Hs.425144	CRA	cisplatin resistance associated	-4.91	3.09
Hs.183650	CRABP2	cellular retinoic acid binding protein 2	-1.93	1.49
Hs.108080	CSRP1	cysteine and glycine-rich protein 1	-1.53	1.32
Hs.200692	DKFZP564G2022	DKFZP564G2022 protein	1.15	-1.32
Hs.75486	FBXL8	F-box and leucine-rich repeat protein 8	-0.81	1.43
Hs.7358	FLJ13110	hypothetical protein FLJ13110	-0.80	2.07
Hs.242271	HHL	expressed in hematopoietic cells, heart, liver	-1.67	1.00
Hs.236774	HMGN4	high mobility group nucleosomal binding domain 4	-0.55	1.07
Hs.18705	KIAA1233	KIAA1233 protein	-1.44	1.00
Hs.234265	LAP1B	lamina-associated polypeptide 1B	-0.51	1.00
Hs.443881	PAXIP1L	PAX transcription activation domain interacting protein 1 like	0.31	-1.26
Hs.78748	RIMS3	regulating synaptic membrane exocytosis 3	-3.96	1.00
Hs.98259	SAMD4	sterile alpha motif domain containing 4	-2.01	1.49
Hs.76536	TBL1X	transducin (beta)-like 1X-linked	-1.07	1.32
Hs.27860		MRNA; cDNA DKFZp586M0723 (from clone DKFZp586M0723)	-2.88	1.38
Hs.458282		Transcribed sequence with strong similarity to protein ref:NP_065136.1 *(H.sapiens)* protocadherin 9 precursor; cadherin superfamily protein VR4-11	-2.62	2.23
Hs.98314		cDNA DKFZp586L0120 (from clone DKFZp586L0120)	-1.86	1.14
Hs.468490		hypothetical protein FLJ20489	-1.25	1.58

*For PC-3, the ratio is the maximum value of the data points from all the time- and concentration-series of selenium treatment. For PCa, it is the average tumor to normal ratio.

Table V. *Selenium reverses the expression of androgen-regulated genes.*

UniGene ID	Symbol	Gene description	Maximum Fold (log_2)#	
			AR	Se
Cell proliferation				
Hs.8230	ADAMTS1	a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 1	2.00	-1.66
Hs.13291	CCNG2	cyclin G2	-2.82	1.46
Hs.405958	CDC6	CDC6 cell division cycle 6 homolog *(S. cerevisiae)*	1.28	-1.99
Hs.374378	CKS1B	CDC28 protein kinase regulatory subunit 1B	1.23	-1.84
Hs.119324	KIF22	kinesin family member 22	1.54	-1.08
Signal transduction				
Hs.197922	CaMKIINalpha	calcium/calmodulin-dependent protein kinase II	-3.43	1.41
Hs.78888	DBI	diazepam binding inhibitor	2.00	-1.69
Hs.433488	GUCY1A3*	guanylate cyclase 1, soluble, alpha 3	1.72	-1.53
Hs.81328	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	1.68	-1.78
Hs.154151	PTPRM	protein tyrosine phosphatase, receptor type, M	1.33	-1.74
Hs.432842	RALGPS1A	Ral guanine nucleotide exchange factor RalGPS1A	-1.28	1.21
Transcriptional regulation				
Hs.55999	NKX3-1	NK3 transcription factor related, locus 1 (Drosophila)	3.90	-1.47
Hs.408222	PBX1*	pre-B-cell leukemia transcription factor 1	-1.98	1.29
Transporter				
Hs.307915	ABCC4*	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	2.96	-1.08
Hs.20952	ATP2B1	ATPase, Ca^{++} transporting, plasma membrane 1	-1.76	2.53
Metabolism				
Hs.75616	DHCR24*	24-dehydrocholesterol reductase	2.58	-1.07
Hs.35198	ENPP5	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)	-1.78	1.01
Hs.268012	FACL3*	fatty-acid-Coenzyme A ligase, long-chain 3	3.98	-1.14
Hs.167531	MCCC2	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	1.98	-1.27
Hs.237323	PGM3	phosphoglucomutase 3	2.07	-1.16
Hs.119597	SCD	stearoyl-CoA desaturase (delta-9-desaturase)	2.56	-2.29
Other functions				
Hs.6790	DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	2.00	-2.05
Hs.173381	DPYSL2*	dihydropyrimidinase-like 2	-1.75	1.14
Hs.181350	KLK2*	kallikrein 2, prostatic	3.17	-2.27
Hs.171995	KLK3*	kallikrein 3, (prostate specific antigen)	3.35	-2.45
Hs.423095	NUCB2	nucleobindin 2	-3.18	1.36
Hs.171952	OCLN	occludin	-2.01	1.34
Hs.188361	RPS6KA3	ribosomal protein S6 kinase, 90kDa, polypeptide 3	1.44	-1.21
Hs.152207	SSBP2	single-stranded DNA binding protein 2	-1.59	1.72
Unknown				
Hs.180197		LOC375504 (LOC375504), mRNA	-1.42	1.63
Hs.22247		CDNA FLJ42250 fis, clone TKIDN2007828	2.40	-1.04
Hs.29189	ATP11A	ATPase, Class VI, type 11A	-2.75	1.27
Hs.512643	AZGP1	alpha-2-glycoprotein 1, zinc	2.49	-1.86
Hs.7557	FKBP5	FK506 binding protein 5	4.67	-1.20
Hs.90797	LOC129642	hypothetical protein BC016005	3.90	-1.40
Hs.298646	PRO2000	PRO2000 protein	3.28	-1.76
Hs.203557	ST7	suppression of tumorigenicity 7	-2.07	1.14

#the maximum value of the data points from all the time- and concentration-series of MSA or R1881 treatment.
*Genes implicated in prostate cancinogenesis.

cell cycle regulatory genes (ATF5, AHR, CDKN1C, EXT1, and CHC1) which are modulated in opposite directions in prostate cancer and by selenium (Table II). More interestingly, selenium alters the expression of these genes in a manner that is consistent with growth inhibition.

In androgen-responsive prostate cancer, AR signaling is such a dominant pathway that shutting it down is likely to be sufficient for growth inhibition. Our previous publication showed that selenium markedly down-regulates AR signaling in LNCaP cells (5). Furthermore, we were able to confirm

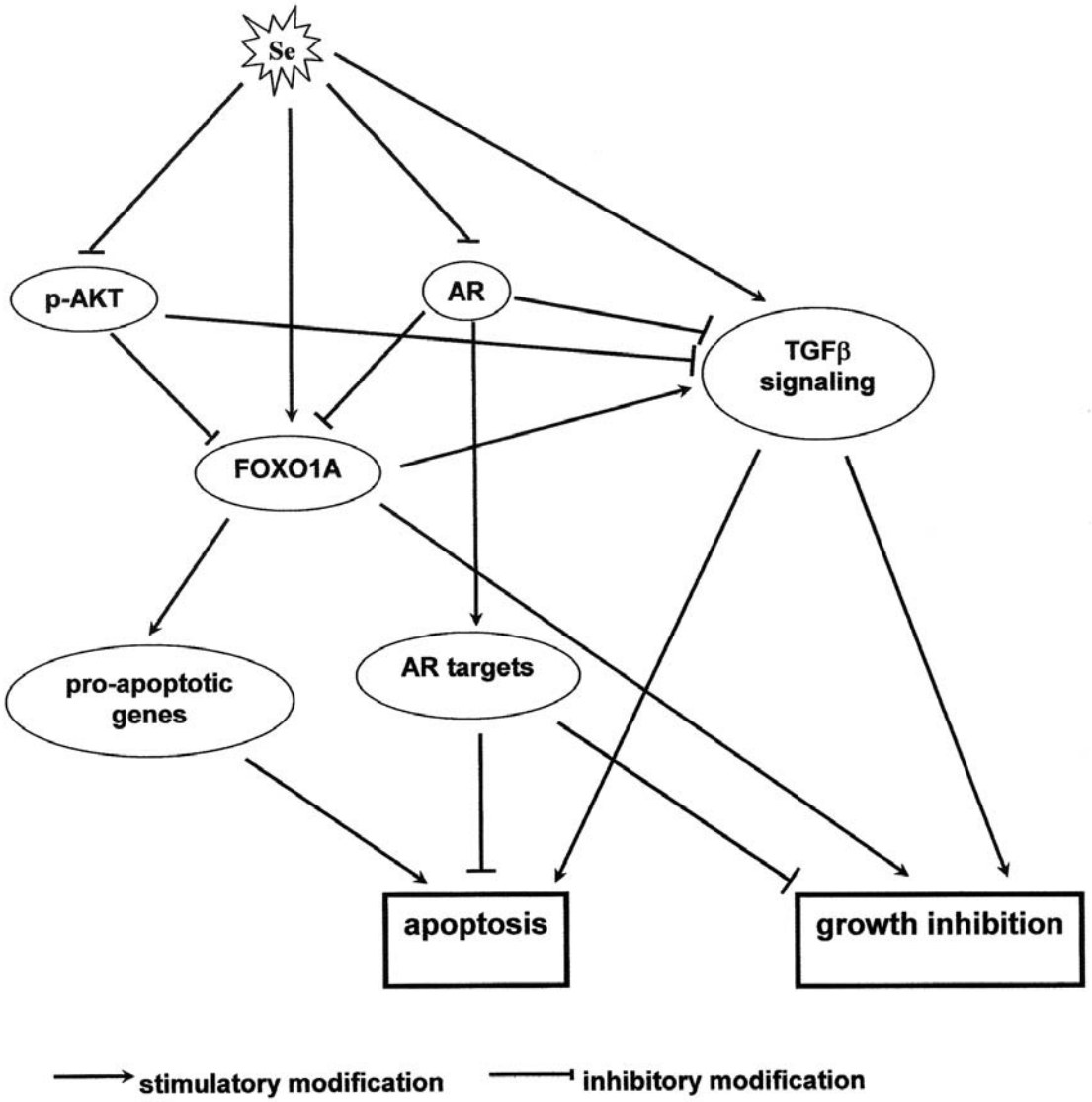

Figure 2. *Proposed model of apoptosis induction and growth inhibition by selenium through the interplay of AKT, AR, and TGFβ signaling pathways.*

that overexpression of AR diminishes the sensitivity to selenium (unpublished data), suggesting that disruption of AR signaling by selenium is biologically relevant. Additionally, selenium is known to modulate a diverse number of cell cycle and apoptosis regulatory molecules, as well as survival signaling molecules, in different cell types regardless of the presence or absence of AR. Different cell types may present both common and unique targets to selenium intervention. Thus, it is apparent that selenium has many targets and there is no one key mechanism to account for the anticancer effect of selenium. The multitude of genes in Table II lends support to common mechanisms for the anticancer activity of selenium in both the androgen-responsive LNCaP cells and the androgen-unresponsive PC-3 cells. However, despite the overall similarity of their cellular responses to selenium, subtle differences exist between the two cell types. For example, selenium slows down cell cycle progression at multiple transition points in PC-3 (5), whereas mostly through G1 arrest in LNCaP (unpublished data). Genes distinctly targeted by selenium in these cells, as presented in Tables III and IV, could be attributable to the above disparities. They might also reflect the difference in genetic background such as response to androgen. Indeed, a noticeable distinction between Table III and Table IV is the presence of androgen-regulated genes in Table III.

Our analysis has identified 92 genes that are regulated by both selenium and androgen. However, only a modest proportion (38 out of 92) of these genes are modulated in

reciprocal directions by selenium and androgen. A possible explanation for this is that genes have multiple regulatory elements, both positive and negative, in their promoter regions. Selenium is known to alter the expression of many transcription factors, co-activators and co-repressors (5). AR regulates the expression of its targets through both direct and indirect mechanisms. In other words, many other transcription factors and co-regulators are likely to be involved by virtue of the rippling effect initiated through AR signaling. Thus, it is to be expected that selenium could counteract the expression of some, but not all, androgen-regulated genes. The litmus test in the future is to study which AR-regulated genes sensitive to selenium reversal are important for modulation of prostate cancer risk.

The induction of forkhead O1A (FOXO1A) by selenium in both LNCaP and PC-3 cells is of special interest to us. FOXO1A is a member of the FOXO family of transcription factors, that induce the expression of pro-apoptotic genes including Fas ligand (38-40), bcl-2 family proteins (19,40,41), and TRAIL (20). Furthermore, FOXO1A is involved in cell cycle arrest (21). FOXO1A is phosphorylated and suppressed by AKT (42,43), which is an important survival molecule for prostate cancer (44). Androgen receptor (AR) also interacts with FOXO1A and inhibits its activation of Fas ligand expression (45). Selenium conveniently down-regulates both AKT and AR signaling (4,46). As shown in Figure 2, the stimulatory effect of selenium on FOXO1A signaling could be due to a direct induction of FOXO1A transcription coupled to an indirect activation of FOXO1A by alleviating the inhibitory modulation through AR and/or AKT.

Three key components of the transforming growth factor β (TGFβ) signaling pathway are consistently repressed in a large set of primary prostate tumors. These genes are TGFβ2, TGFβ receptor type II, and TGFβ receptor type III. Type I and II receptors have serine/threonine protein kinase domains and are directly involved in TGFβ signaling (47). Type III receptor does not have an intrinsic signaling domain; however, it facilitates the binding of all TGFβs, and especially TGFβ2, to the type II receptor (47). TGFβ is a pleiotropic cytokine, but is mainly a growth inhibitor of epithelial cancer, particularly at the early stage of development (48). It has been shown that the type I and II receptors are down-regulated in prostate cancer (49,50) and that loss of expression of the receptors is associated with poor prognosis (51). Therefore, from a prevention standpoint, stimulating TGFβ signaling is likely to produce a desirable outcome. In this study, we found that the expression of TGFβ2, TGFβ type II and III receptors is concertedly up-regulated by selenium. It is also worth mentioning that the expression of TGFβ2 is known to be induced by a forkhead transcription factor closely related to FOXO1A (52) and that both AR and AKT repress TGFβ signaling (53). Thus, the effect of selenium could be amplified by the crosstalk of TGFβ, AKT, and AR signals as illustrated in Figure 2. Our future research efforts will be directed towards elucidating the contribution of these pathways in selenium chemoprevention of prostate cancer.

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References

1 Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL Jr, Park HK, Sanders BB Jr, Smith CL and Taylor JR: Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA *276*: 1957-1963, 1996.

2 Clark LC, Dalkin B, Krongrad A, Combs GF Jr, Turnbull BW, Slate EH, Witherington R, Herlong JH, Janosko E, Carpenter D, Borosso C, Falk S and Rounder J: Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br J Urol *81*: 730-734, 1998.

3 Duffield-Lillico AJ, Dalkin BL, Reid ME, Turnbull BW, Slate EH, Jacobs ET, Marshall JR and Clark LC: Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. BJU Int *91*: 608-612, 2003.

4 Dong Y, Lee SO, Zhang H, Marshall J, Gao AC and Ip C: Prostate specific antigen expression is down-regulated by selenium through disruption of androgen receptor signaling. Cancer Res *64*: 19-22, 2004.

5 Dong Y, Zhang H, Hawthorn L, Ganther HE and Ip C: Delineation of the molecular basis for selenium-induced growth arrest in human prostate cancer cells by oligonucleotide array. Cancer Res *63*: 52-59, 2003.

6 Zhao H, Whitfield ML, Xu T, Botstein D and Brooks JD: Diverse effects of methylseleninic acid on the transcriptional program of human prostate cancer cells. Mol Biol Cell *15*: 506-519, 2004.

7 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 and Sellers WR: Gene expression correlates of clinical prostate cancer behavior. Cancer Cell *1*: 203-209, 2002.

8 Welsh JB, Sapinoso LM, Su AI, Kern SG, Wang-Rodriguez J, Moskaluk CA, Frierson HF Jr and Hampton GM: Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res *61*: 5974-5978, 2001.

9 Lapointe J, Li C, Higgins JP, van de RM, Bair E, Montgomery K, Ferrari M, Egevad L, Rayford W, Bergerheim U, Ekman P, DeMarzo AM, Tibshirani R, Botstein D, Brown PO, Brooks JD and Pollack JR: Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci USA *101*: 811-816, 2004.

10 Jenster G: The role of the androgen receptor in the development and progression of prostate cancer. Semin Oncol *26*: 407-421, 1999.

11 DePrimo SE, Diehn M, Nelson JB, Reiter RE, Matese J, Fero M, Tibshirani R, Brown PO and Brooks JD: Transcriptional programs activated by exposure of human prostate cancer cells to androgen. Genome Biol *3*: RESEARCH0032, 2002.

12 Nelson PS, Clegg N, Arnold H, Ferguson C, Bonham M, White J, Hood L and Lin B: The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci USA *99*: 11890-11895, 2002.

13 Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D and Heller RA: Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. Proc Natl Acad Sci USA *97*: 1778-1783, 2000.

14 Wang Y, Rea T, Bian J, Gray S and Sun Y: Identification of the genes responsive to etoposide-induced apoptosis: application of DNA chip technology. FEBS Lett *445*: 269-273, 1999.

15 Puga A, Xia Y and Elferink C: Role of the aryl hydrocarbon receptor in cell cycle regulation. Chem Biol Interact *141*: 117-130, 2002.

16 Ohtsubo M, Kai R, Furuno N, Sekiguchi T, Sekiguchi M, Hayashida H, Kuma K, Miyata T, Fukushige S, Murotsu T *et al.*: Isolation and characterization of the active cDNA of the human cell cycle gene (RCC1) involved in the regulation of onset of chromosome condensation. Genes Dev *1*: 585-593, 1987.

17 Matsuoka S, Edwards MC, Bai C, Parker S, Zhang P, Baldini A, Harper JW and Elledge SJ: p57KIP2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. Genes Dev *9*: 650-662, 1995.

18 Persengiev SP, Devireddy LR and Green MR: Inhibition of apoptosis by ATFx: a novel role for a member of the ATF/CREB family of mammalian bZIP transcription factors. Genes Dev *16*: 1806-1814, 2002.

19 Gilley J, Coffer PJ and Ham J: FOXO transcription factors directly activate bim gene expression and promote apoptosis in sympathetic neurons. J Cell Biol *162*: 613-622, 2003.

20 Modur V, Nagarajan R, Evers BM and Milbrandt J: FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. J Biol Chem *277*: 47928-47937, 2002.

21 Nakamura N, Ramaswamy S, Vazquez F, Signoretti S, Loda M and Sellers WR: Forkhead transcription factors are critical effectors of cell death and cell cycle arrest downstream of PTEN. Mol Cell Biol *20*: 8969-8982, 2000.

22 Cher ML, Biliran HR Jr, Bhagat S, Meng Y, Che M, Lockett J, Abrams J, Fridman R, Zachareas M and Sheng S: Maspin expression inhibits osteolysis, tumor growth, and angiogenesis in a model of prostate cancer bone metastasis. Proc Natl Acad Sci USA *100*: 7847-7852, 2003.

23 Zou Z, Anisowicz A, Hendrix MJ, Thor A, Neveu M, Sheng S, Rafidi K, Seftor E and Sager R: Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. Science *263*: 526-529, 1994.

24 Bass R, Fernandez AM and Ellis V: Maspin inhibits cell migration in the absence of protease inhibitory activity. J Biol Chem *277*: 46845-46848, 2002.

25 Chen Z, Fan Z, McNeal JE, Nolley R, Caldwell MC, Mahadevappa M, Zhang Z, Warrington JA and Stamey TA: Hepsin and maspin are inversely expressed in laser capture microdissectioned prostate cancer. J Urol *169*: 1316-1319, 2003.

26 Lee HK, Driscoll D, Asch H, Asch B and Zhang PJ: Downregulated gelsolin expression in hyperplastic and neoplastic lesions of the prostate. Prostate *40*: 14-19, 1999.

27 Asch HL, Head K, Dong Y, Natoli F, Winston JS, Connolly JL and Asch BB: Widespread loss of gelsolin in breast cancers of humans, mice, and rats. Cancer Res *56*: 4841-4845, 1996.

28 Dosaka-Akita H, Hommura F, Fujita H, Kinoshita I, Nishi M, Morikawa T, Katoh H, Kawakami Y and Kuzumaki N: Frequent loss of gelsolin expression in non-small cell lung cancers of heavy smokers. Cancer Res *58*: 322-327, 1998.

29 Tanaka M, Mullauer L, Ogiso Y, Fujita H, Moriya S, Furuuchi K, Harabayashi T, Shinohara N, Koyanagi T and Kuzumaki N: Gelsolin: a candidate for suppressor of human bladder cancer. Cancer Res *55*: 3228-3232, 1995.

30 Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A and Mosialos G: CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature *424*: 793-796, 2003.

31 Castro P, Liang H, Liang JC and Nagarajan L: A novel, evolutionarily conserved gene family with putative sequence-specific single-stranded DNA-binding activity. Genomics *80*: 78-85, 2002.

32 Semba K, Nishizawa M, Miyajima N, Yoshida MC, Sukegawa J, Yamanashi Y, Sasaki M, Yamamoto T and Toyoshima K: yes-related protooncogene, syn, belongs to the protein-tyrosine kinase family. Proc Natl Acad Sci USA *83*: 5459-5463, 1986.

33 Chen D, Guo J, Miki T, Tachibana M and Gahl WA: Molecular cloning of two novel rab genes from human melanocytes. Gene *174*: 129-134, 1996.

34 Williams ED and Brooks JD: New molecular approaches for identifying novel targets, mechanisms and biomarkers for prostate cancer chemopreventive agents. Urology *57*: 100-102, 2001.

35 Rhodes DR, Barrette TR, Rubin MA, Ghosh D and Chinnaiyan AM: Meta-analysis of microarrays: interstudy validation of gene expression profiles reveals pathway dysregulation in prostate cancer. Cancer Res *62*: 4427-4433, 2002.

36 Luo J, Dunn T, Ewing C, Sauvageot J, Chen Y, Trent J and Isaacs W: Gene expression signature of benign prostatic hyperplasia revealed by cDNA microarray analysis. Prostate *51*: 189-200, 2002.

37 Prakash K, Pirozzi G, Elashoff M, Munger W, Waga I, Dhir R, Kakehi Y and Getzenberg RH: Symptomatic and asymptomatic benign prostatic hyperplasia: molecular differentiation by using microarrays. Proc Natl Acad Sci USA *99*: 7598-7603, 2002.

38 Kavurma MM and Khachigian LM: Signaling and transcriptional control of Fas ligand gene expression. Cell Death Differ *10*: 36-44, 2003.

39 Suhara T, Kim HS, Kirshenbaum LA and Walsh K: Suppression of Akt signaling induces Fas ligand expression: involvement of caspase and Jun kinase activation in Akt-mediated Fas ligand regulation. Mol Cell Biol *22*: 680-691, 2002.

40 Dijkers PF, Medema RH, Lammers JW, Koenderman L and Coffer PJ: Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. Curr Biol *10*: 1201-1204, 2000.
41 Tang TT, Dowbenko D, Jackson A, Toney L, Lewin DA, Dent AL and Lasky LA: The forkhead transcription factor AFX activates apoptosis by induction of the BCL-6 transcriptional repressor. J Biol Chem *277*: 14255-14265, 2002.
42 Tang ED, Nunez G, Barr FG and Guan KL: Negative regulation of the forkhead transcription factor FKHR by Akt. J Biol Chem *274*: 16741-16746, 1999.
43 Guo S, Rena G, Cichy S, He X, Cohen P and Unterman T: Phosphorylation of serine 256 by protein kinase B disrupts transactivation by FKHR and mediates effects of insulin on insulin-like growth factor-binding protein-1 promoter activity through a conserved insulin response sequence. J Biol Chem *274*: 17184-17192, 1999.
44 Lin J, Adam RM, Santiestevan E and Freeman MR: The phosphatidylinositol 3'-kinase pathway is a dominant growth factor-activated cell survival pathway in LNCaP human prostate carcinoma cells. Cancer Res *59*: 2891-2897, 1999.
45 Li P, Lee H, Guo S, Unterman TG, Jenster G and Bai W: AKT-independent protection of prostate cancer cells from apoptosis mediated through complex formation between the androgen receptor and FKHR. Mol Cell Biol *23*: 104-118, 2003.
46 Jiang C, Wang Z, Ganther H and Lu J: Distinct effects of methylseleninic acid *versus* selenite on apoptosis, cell cycle, and protein kinase pathways in DU145 human prostate cancer cells. Mol Cancer Ther *1*: 1059-1066, 2002.
47 Massague J: TGF-beta signal transduction. Annu Rev Biochem *67*: 753-791, 1998.
48 Moses HL, Yang EY and Pietenpol JA: Regulation of epithelial proliferation by TGF-beta. Ciba Found Symp *157*: 66-74, 1991.
49 Eastham JA, Truong LD, Rogers E, Kattan M, Flanders KC, Scardino PT and Thompson TC: Transforming growth factor-beta 1: comparative immunohistochemical localization in human primary and metastatic prostate cancer. Lab Invest *73*: 628-635, 1995.
50 Williams RH, Stapleton AM, Yang G, Truong LD, Rogers E, Timme TL, Wheeler TM, Scardino PT and Thompson TC: Reduced levels of transforming growth factor beta receptor type II in human prostate cancer: an immunohistochemical study. Clin Cancer Res *2*: 635-640, 1996.
51 Kim IY, Ahn HJ, Lang S, Oefelein MG, Oyasu R, Kozlowski JM and Lee C: Loss of expression of transforming growth factor-beta receptors is associated with poor prognosis in prostate cancer patients. Clin Cancer Res *4*: 1625-1630, 1998.
52 Samatar AA, Wang L, Mirza A, Koseoglu S, Liu S and Kumar CC: Transforming growth factor-beta 2 is a transcriptional target for Akt/protein kinase B *via* forkhead transcription factor. J Biol Chem *277*: 28118-28126, 2002.
53 Chipuk JE, Cornelius SC, Pultz NJ, Jorgensen JS, Bonham MJ, Kim SJ and Danielpour D: The androgen receptor represses transforming growth factor-beta signaling through interaction with Smad3. J Biol Chem *277*: 1240-1248, 2002.

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