

2.0

>4

1.5

1.0

-1.5

-2.0

NA

<-4

q-value < 0.01%

S S	LCM BIOPSY SAMPLES						LCM SURGICAL SAMPLES								
0	NP		<b>Ο</b> /11			NP		NP		CAP		NP			
Gene	(ref <sup>29</sup> )		(This Study)			(This Study)		(ref <sup>29</sup> )	(ref <sup>28</sup> )			(ref <sup>28</sup> )			
Symbol	n=12		n=31		15 1145	n=31		n=12	n=37			n=37			
JUN															
TMEM49															
SOCS3												1 1 1 1			
DUSP1															
KLF6															
JUNB															
CXCR4															
CTGF															
BTG2															
CCL4L1	П														
IER2															
MCL1			7												
CLDN4															
XBP1								_							
JUND														111	
FN1															
H3F3B															
NR4A1														101	
RHOB															
TSC22D1															
NORMALIZED															
SIGNAL	בט	327	68 16384	8192	4096	2048	1024	512	256	128	64				
SIGNAL		321	00 10304	0192	4090	2040	1024	312	230	120	04				
D															

В.

(2) (2)		NP (ref <sup>29</sup> ) n=12	CAP (This Study) n=31	NP (This Study) n=31	NP (ref <sup>29</sup> ) n=12	CAP (ref <sup>28</sup> ) n=37	NP (ref <sup>28</sup> ) n=37
<u></u>	NP (ref <sup>29</sup> ) n=12		7.16E-01	5.41E-01	2.73E-04	1.56E-03	2.67E-04
BIOPS	CAP (This Study) n=31	7.16E-01		7.82E-01	4.63E-05	2.66E-04	4.28E-05
<u> </u>	NP (This Study) n=31	5.41E-01	7.82E-01		3.79E-05	2.07E-04	3.55E-05

3.79E-05

2.07E-04

3.55E-05

3.57E-01

9.32E-01

4.63E-05

2.66E-04

4.28E-05

SURGERY

3.57E-01

3.92E-01

9.32E-01 3.92E-01

**BIOPSY** 

2.73E-04

1.56E-03

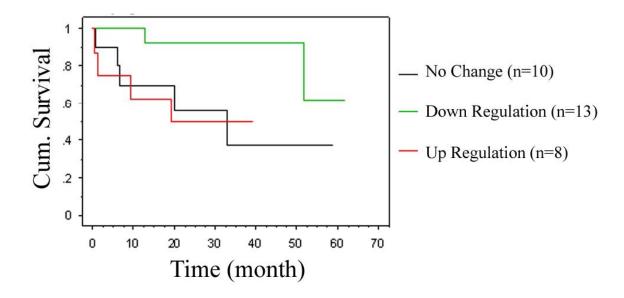
2.67E-04

SURGERY

NP (ref<sup>29</sup>) n=12

CAP (ref<sup>28</sup>) n=37

NP (ref<sup>28</sup>) n=37



#### **Figure Legends**

Supplemental Figure 1. Frozen section of prostate needle biopsy after laser pulsing of benign luminal epithelial cells before (a) and after (b) removal of the cells from the section for RNA extraction. The refractile circles (a) show the sites that the 5 micron diameter laser beam targeted the cells.

Supplemental Figure 2. cDNA microarray gene expression analysis from prostate needle biopsies. Normal and neoplastic epithelium was microdissected individually from needle core biopsies from 31 patients. Following 2 rounds of linear amplification, transcript levels were measured by microarray hybridization. A total of 5910 genes were tested. The heatmap of differentially expressed genes (cancer *vs.* benign) is shown. 457 genes were upregulated, and 497 genes were downregulated, q-values < 0.01%. Red: upregulated in tumor; Green: downregulated in tumor. Row: genes; Column: patients.

Supplemental Figure 3. Comparison of expression levels of ischemia-associated genes identified in Lin, et al. (29) in biopsy and surgical samples. A. Heatmap of expression levels of samples from three different studies. Expression levels are denoted by colors corresponding to signal intensity. Most genes are consistently upregulated in radical prostatectomy samples relative to needle biopsy samples. B. T-tests between groups demonstrate insignificant differences overall comparing only needle biopsy samples, or only surgical samples, and significant differences between needle biopsy samples and surgical samples.

Supplemental Figure 4. The correlation between each patient's pretreatment androgen receptor (AR) mRNA and his biochemical relapse (serum PSA) was analyzed by Kaplan-Meier Plot. No change: there was not any difference in AR mRNA level between benign and cancer epithelium. Downregulation: the patient's benign AR mRNA was 2 fold less than cancer AR. Upregulation: the patient's benign AR mRNA was 2 fold more than cancer AR. X-axis: time to biochemical relapse, Y-axis: % of relapse free survival.

Number	57
Age	
Median	63
Range	49-74
ECOG PS	
0	52
1	3
PSA	
Median	12.2
Range	1.4-58.6
Clinical Stage (AJCC 2002 criteria)	
T1c	7
T2a	7
T2b	10
T2c	19
T3a	11
T3b	2
T4	1
Biopsy Gleason Score	
6	5
7	22
8	17
9	12
10	1

Supplementary Table S2. Clinical data characteristics of patient samples on microarrays (n=31)

гиррин		Clinical Stage	Surgical	Nodal	•	son Sum S	•	Censored for	Time (months)
ID	Age		margin	status at	Gica	SOII SUIII S	COIC	progression	to progression
		Stage	status	surgery	Biopsy	Surgical	LCM	(PSA > 0.4)	(PSA > 0.4)
6082-02	62	T2b	Negative	Positive	8	9	4+4	0	6.8
6082-03	66	T3a	Negative	Positive	7	7	3+3	0	15.4
6082-04	69	T3a	Positive	Negative	6	7	3+3	1	39.2
6082-07	69	T3a	Negative	Negative	7	7	3+4	1	35.5
6082-08	59	T2c	Negative	Negative	7	6	3+3	1	38.8
6082-10	66	T1c	Negative	Negative	8	6	3+3	1	36.0
6082-13	74	T2b	Negative	Negative	8	8	3+3	0	13.6
6082-14	72	T2a	Negative	Negative	9	8	4+3	1	32.5
6082-15	52	T2a	Positive	Negative	6	7	3+3	1	16.6
6082-16	65	T2a	Negative	Negative	8	7	3+4	1	30.0
6082-19	52	T3a	Negative	Negative	7	7	3+3	1	24.1
6082-22	59	T3a	Negative	Negative	7	7	3+3	1	24.1
6082-23	56	T3a	Negative	Negative	7	8	3+3	1	22.9
6082-24	69	T2a	Positive	Negative	8	8	4+4	1	23.5
6082-25	63	T1c	Negative	Negative	9	7	3+3	0	6.4
6082-28	63	T2b	Positive	Negative	9	7	3+4	1	23.2
6082-31	57	T2c	Negative	Negative	7	7	3+3	1	19.7
6082-32	65	T2c	Negative	Negative	8	6	3+3	1	11.6
6082-34	66	T2c	Negative	Negative	9	9	4+4	0	0.7
6082-36	63	T2c	Positive	Positive	8	9	3+4	1	6.3
KP-01	56	T2c	Negative	Negative	7	6	4+4	1	13.0
KP-02	62	T3b	Negative	Negative	7	7	4+4	1	6.4
KP-03	56	T2b	Negative	Negative	7	7	3+3	1	7.5
KP-04	71	T1c	Positive	Negative	7	7	3+3	1	4.8
KP-05	68	T2b	Positive	Negative	10	10	4+5	1	2.8
UW-01	60	T2c	Positive	Positive	7	7	4+4	0	1.4
UW-03	54	T2c	Positive	Positive	9	9	4+4	0	0.6
UW-05	64	T2c	Negative	Negative	8	7	4+3	1	20.1
UW-09	59	T3b	Negative	Positive	7	NA	4+4	0	10.1
UW-10	58	T2b	Negative	Negative	7	8	3+4	1	10.4
UW-11	63	T2c	Negative	Negative	7	8	3+3	1	9.8

Supplementary Table S3. Comparison of gene expression changes differentiating neoplastic from normal prostate epithelium across four different microarray data sets.

This Study*	True et a	ıl, <sup>28</sup> 2006	Lapointe 6	et al, <sup>15</sup> 2004	Glinsky et al, y 2004		
ton 10/	top 1%	10 (48%)	top 1%	7 (33%)	top 1%	4 (19%)	
top 1% (21 genes)	top 5%	17 (81%)	top 5%	12 (57%)	top 5%	10 (48%)	
(21 genes)	top 10%	19 (90%)	top 10%	15 (71%)	top 10%	13 (62%)	
ton 50/	top 1%	13 (12%)	top 1%	9 (8%)	top 1%	8 (7%)	
top 5% (107 genes)	top 5%	36 (34%)	top 5%	23 (21%)	top 5%	19 (18%)	
(107 genes)	top 10%	57 (53%)	top 10%	35 (33%)	top 10%	34 (32%)	
tom 100/	top 1%	14 (7%)	top 1%	14 (7%)	top 1%	8 (4%)	
top 10% (214 genes)	top 5%	50 (23%)	top 5%	36 (17%)	top 5%	29 (14%)	
(214 genes)	top 10%	81 (38%)	top 10%	56 (26%)	top 10%	54 (25%)	

<sup>\*</sup>Top unique genes (among the 2140 common to all four datasets) identified in this study between normal epithelium and cancer as ranked by SAM t-test score(d).