Hartford Hospital
Tallwood Urology &
Kidney Institute

Identifying Gene Expression to Predict Biochemical Recurrence Following Radical Prostatectomy

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Disclosures

- JRW
  - Medtronic: Consulting
  - Genomic Health: Speaker
  - Decipher Biosciences: Speaker

Background

- For patients undergoing radical prostatectomy, ~1/3 will eventually require additional treatment[1]
- Patients with higher risk prostate cancer may initially be undertreated[2] and miss the treatment window before developing metastatic disease
- The urologic community needs a way to distinguish which patients deserve the most aggressive treatment upfront
- Biomarkers such as Decipher may help determine which patients may benefit from further treatment
- To date, there has been no consensus for a superior predictive model to predict prostate cancer recurrence

1. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. *JAMA*. 1999;281(17):1591–1597.
2. Cooperberg MR, Broering JM, Carroll PR. Time Trends and Local Variation in Primary Treatment of Localized Prostate Cancer. *J Clin Oncol*. 2010;28(7):1117-1123.

Methods

- Between 2008 and 2011, patients undergoing radical prostatectomy at Hartford Hospital were consented to submit specimens for whole genome gene expression as part of the Total Cancer Care Consortium
- RNA isolated from formalin-fixed paraffin-embedded prostates was hybridized to a custom Affymetrix microarray
- Regularized (LASSO) Cox regression was performed with cross-validation to identify a gene expression signature that improves risk prediction
- Recurrence was defined as post-operative PSA >0.2 ng/mL or triggered salvage treatment
- Model performance was assessed using time-dependent ROC curves and survival plots

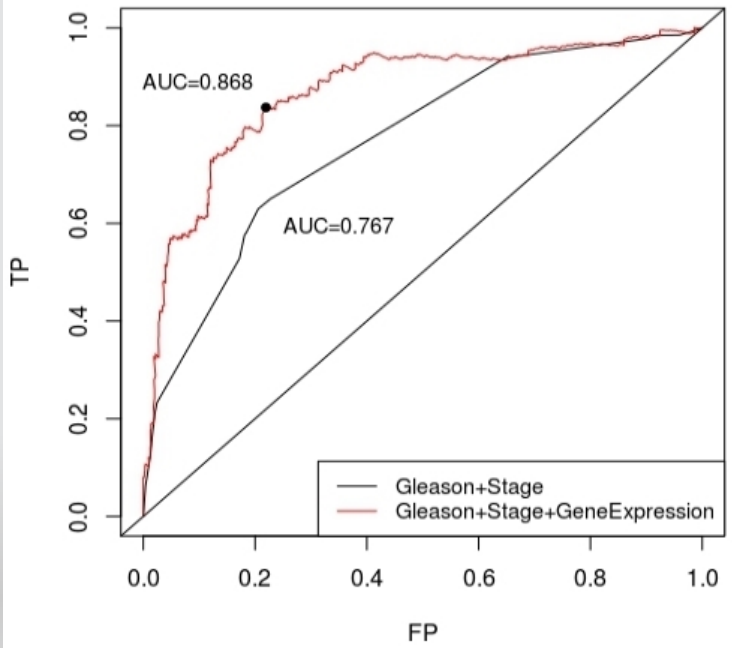
	No BCR (N = 501)	BCR (N = 105)	p-value	
Age	59.8 ± 6.2	61.1 ± 5.8	0.04168	t-test
Race/ethnicity			0.6528	Fisher exact
White	408 (81.4%)	92 (87.6%)		
Black	12 (2.4%)	0		
Hispanic	13 (2.6%)	3 (2.9%)		
Asian	0	0		
Other	68 (13.6%)	10 (9.5%)		
Diagnostic PSA	4.9 (4.1, 6.6)	5.7 (4.2, 7.4)	0.02313	t-test on log
Specimen (grams)	51 ± 16.6	48.7 ± 14.2	0.1849	Mann-Whitney U
Tumor stage			1.752E-11	Fisher exact
pT2a	38 (7.6%)	1 (1%)		
pT2b	12 (2.4%)	1 (1%)		
pT2c	342 (68.3%)	43 (41%)		
pT3a	92 (18.4%)	44 (41.9%)		
pT3b	16 (3.2%)	16 (15.2%)		
pT4	1 (0.2%)	0		
Gleason sum			2.973E-14	Fisher exact
6	153 (30.5%)	9 (8.6%)		
7	327 (65.3%)	66 (62.9%)		
8	10 (2%)	13 (12.4%)		
9	11 (2.2%)	17 (16.2%)		
Positive margins	100 (20%)	30 (28.6%)	0.06818	Chi-squared
D'Amico Risk			6.132E-11	Chi-squared
Low	228 (45.5%)	21 (20%)		
Intermediate	220 (43.9%)	48 (45.7%)		
High	53 (10.6%)	36 (34.3%)		

Modeling Approach

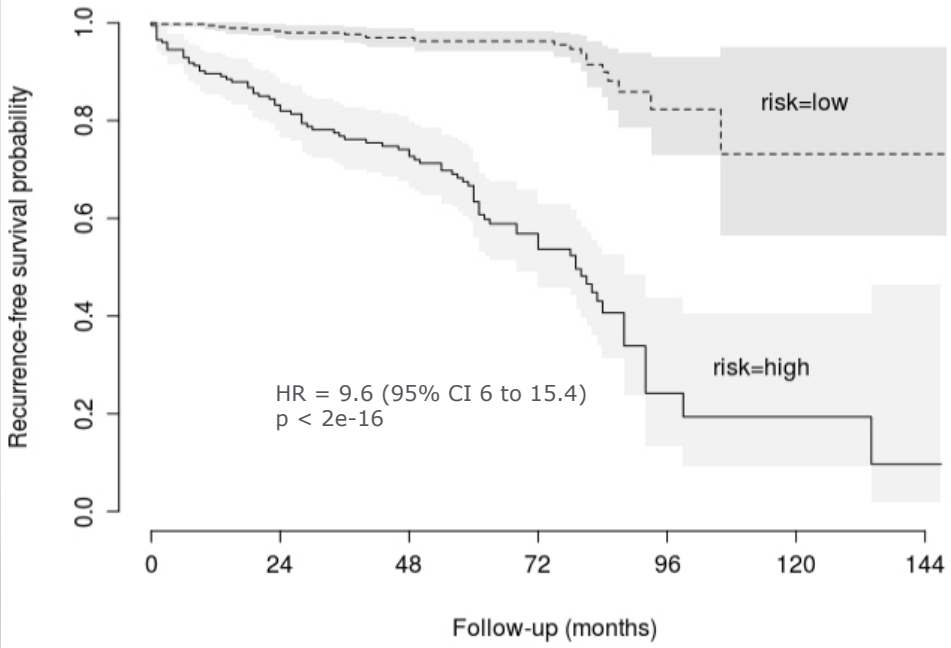
- Use regularized LASSO (least absolute shrinkage and selection operator) Cox regression to identify a gene expression signature that improves risk prediction
  - Start with all genes in the model, use a penalty to shrink coefficients
  - Will shrink coefficients to zero, performing variable selection
  - Provides adjusted coefficients to reduce overfitting

Performance of Unique Genomic Signature

AUC (Path + Signature) v (Path)



PSA Recurrence Low v. High Risk Signature



Risk score = 0.5(Gleason) + 0.32(Stage) - 0.2(CNRIP1) - 0.22(ERP44) + 0.29(MTX2) + 0.23(RHOU) + .21(OXR1)

Genes in LASSO model

- RHOU[3]: Ras homolog family member U
  - Found increased in PCa, silencing in PCa cell lines resulted in growth arrest and cytotoxicity
  - Downstream of JAK/STAT signaling; mediates cell migration in multiple myeloma
- MTX2[4]: Metaxin-2
  - Mitochondrial transport; protein found increased in PCa
- ERP44[5]:Endoplasmic reticulum protein 44
  - protein expressed at higher level in PCa (vs BPH)
  - chaperone in secretory pathway; inhibits lung cancer cell migration
- CNRIP1[6]: Cannabinoid receptor interacting protein 1
  - Non-habit-forming cannabinoid receptor agonists have been suggested for prostate cancer treatment
  - Promoter hyper-methylated in colorectal cancer, correlated with aggressiveness
- OXR1 [7] : Oxidation resistance 1
  - prevents oxidative stress-induced cell death

Risk score = 0.5(Gleason) + 0.32(Stage) - 0.2(CNRIP1) - 0.22(ERP44) + 0.29(MTX2) + 0.23(RHOU) + .21(OXR1)

3-7: References available on request

NCBI

Gene Expression Omnibus

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NCBI > GEO > **Accession Display** Not lo

GEO help: Mouse over screen elements for information.

Scope: Self Format: HTML Amount: Quick GEO accession: GSE21034

Series GSE21034 Query DataSets for GSE21034

Status	Public on Jun 24, 2010
Title	Whole-transcript and exon-level expression data for human primary and metastatic prostate cancer samples and control normal adjacent benign prostate
Organism	Homo sapiens
Experiment type	Expression profiling by array
Summary	Current knowledge of prostate cancer genomes is largely based on relatively small patient cohorts using single modality analysis platforms. Here we report concordant assessment of DNA copy number, mRNA and microRNA expression and focused exon resequencing in prostate tumors from 218 patients with primary or metastatic prostate cancer with a median of 5 years clinical follow-up, now made available as a public resource. Mutations in known, commonly mutated oncogenes and tumor suppressor genes such as PIK3CA, KRAS, BRAF and TP53 are present but generally rare. However, integrative analysis of mutations with copy number alterations (CNAs) and expression changes reveal alterations in the PI3K, RAS/RAF and androgen receptor (AR) pathways in nearly all metastatic samples and in a higher frequency of primary samples than previously suspected based on single-gene studies. Other new findings include evidence that the nuclear receptor coactivator NCOA2 functions as a driver oncogene in ~20 percent of primaries. Tumors with the androgen-driven TMPRSS2-ERG fusion were significantly associated with a small, previously unrecognized, prostate-specific 3p14 deletion that, through mRNA expression and resequencing analysis, implicates FOXP1, RYBP and SHQ1 as candidate cooperative tumor suppressors. Comparison of transcriptome and DNA copy number data from primary tumors for prognostic impact revealed that CNAs robustly define clusters of low- and high-risk disease beyond that achieved by Gleason score. In sum, this integrative genomic analysis of a substantial cohort of tumors clarifies the role of several known cancer pathways in prostate cancer, implicates several new ones, reveals a previously unappreciated role for CNAs in prognosis and provides a blueprint for clinical development of pathway inhibitors.
Overall design	Human prostate samples were profiled on Affymetrix Human Exon 1.0 ST arrays per manufacturer's instructions.

Model Validation MSKCC data set

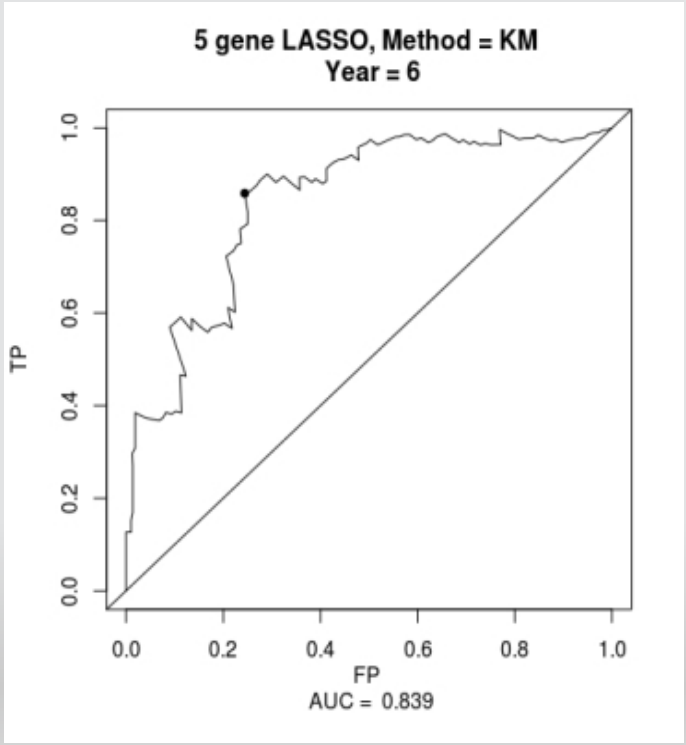
32 samples with recurrence
104 samples no recurrence

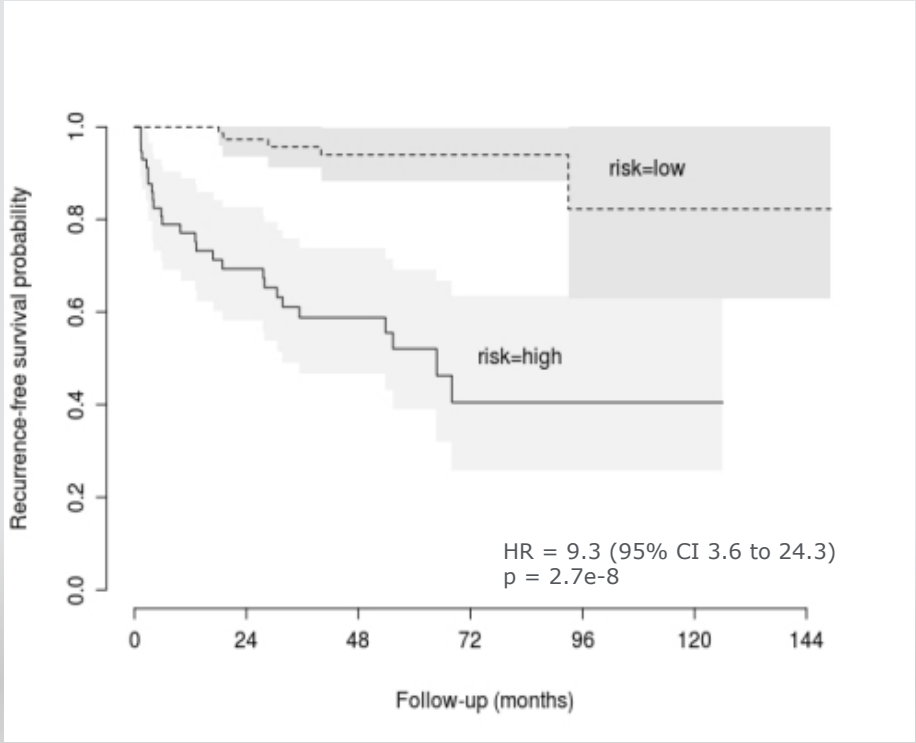
BCR defined as PSA ≥ 0.2 ng/ml on two occasions

Includes Gleason, pathology stage, PSA progression (with time to recurrence or censor)

*Different microarray

LASSO model: Model validation set GSE21034 MSKCC





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Series GSE94767	Query DataSets for GSE94767
Status	Public on Mar 13, 2017
Title	CancerMap project prostate cancer microarray dataset
Platform organism	Homo sapiens
Sample organisms	Homo sapiens; Mus musculus
Experiment type	Expression profiling by array
Summary	Microarray expression profiling has currently failed to provide a consistent classification for human prostate cancer. Such classifications are important because they provide a framework for the identification of new biomarkers of clinical behavior and for the development of targeted therapies. We hypothesize that previous studies have been unsuccessful because of their failure to take into account the well documented occurrence of prostate cancer multifocality and genetic heterogeneity. We have invented a novel method for collecting whole RNALater preserved 'research slices' from prostatectomy specimens that, for the first time, allows the mapping of multifocality and of genetic heterogeneity in prostate cancer to be integrated with the selection of samples for expression microarray analysis. For each specimen we will construct a map of the regions of cancer and of their ERG gene rearrangement status from whole mount formalin fixed sections immediately juxtaposed to the 'research slice'. Only foci of cancers containing a homogeneous pattern of ERG gene alteration will be selected for study. A pilot study has already demonstrated the feasibility of this approach, and provides initial evidence that cancers may be stratified into at least two prognostically distinct categories. Novel biomarkers defining distinct prostate cancer categories will be verified and validated in future studies linked to clinical trials.
Overall design	236 samples from fresh frozen tissue from the prostatectomies of 154 prostate cancer patients. 185 samples were from malignant tissue and 51 from morphologically benign tissue. 5 samples are from fibroblasts grown on mice.
Contributor(s)	Cooper CS, Neal DN, Brewer DS, Whitaker HC, Edwards S, Burge J, Corcoran M, Dennis N, Dudderidge T, Eeles R, Fisher C, George A, Hazell S, Jameson C, Livni N, Matthews L, Merson S, Ogden C, Stearn S, Ross-Adams H, Russell R, Lamb A
Citation	Bogdan-Alexandru Luca, Daniel S. Brewer, Dylan R. Edwards, Sandra Edwards, Hayley C. Whitaker, Sue Merson, Nening Dennis, Rosalin A. Cooper, Steven Hazell, Anne Y. Warren, The CancerMap Group, Rosalind Eeles, Andy G. Lynch, Helen Ross-Adams, Alastair D. Lamb, David E. Neal, Krishna Sethia, Robert D. Mills, Richard Y. Ball, Helen Curley, Jeremy Clark, Vincent Moulton, and Colin S. Cooper. DESNT: A Poor Prognosis Category of Human Prostate Cancer. European Urology Focus 2017. doi: 10.1016/j.euf.2017.01.016
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Platforms (1)	GPL5175 [HuEx-1_0-st] Affymetrix Human Exon 1.0 ST Array [transcript (gene) version]

Model Validation ICR data set

23 samples with recurrence
57 samples no recurrence

BCR defined as PSA ≥ 0.2 ng/ml or triggered salvage

Includes Gleason, pathology stage, PSA progression (with time to recurrence or censor)

*Different microarray

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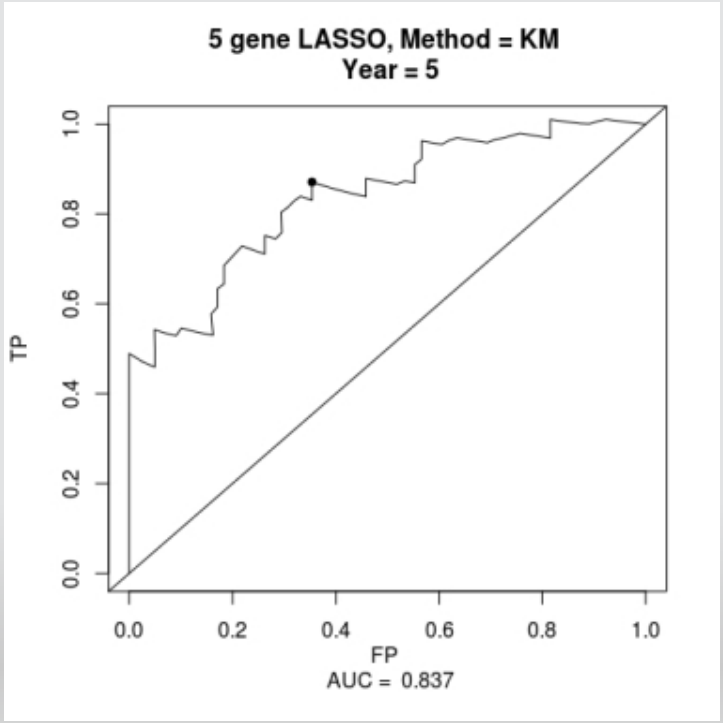
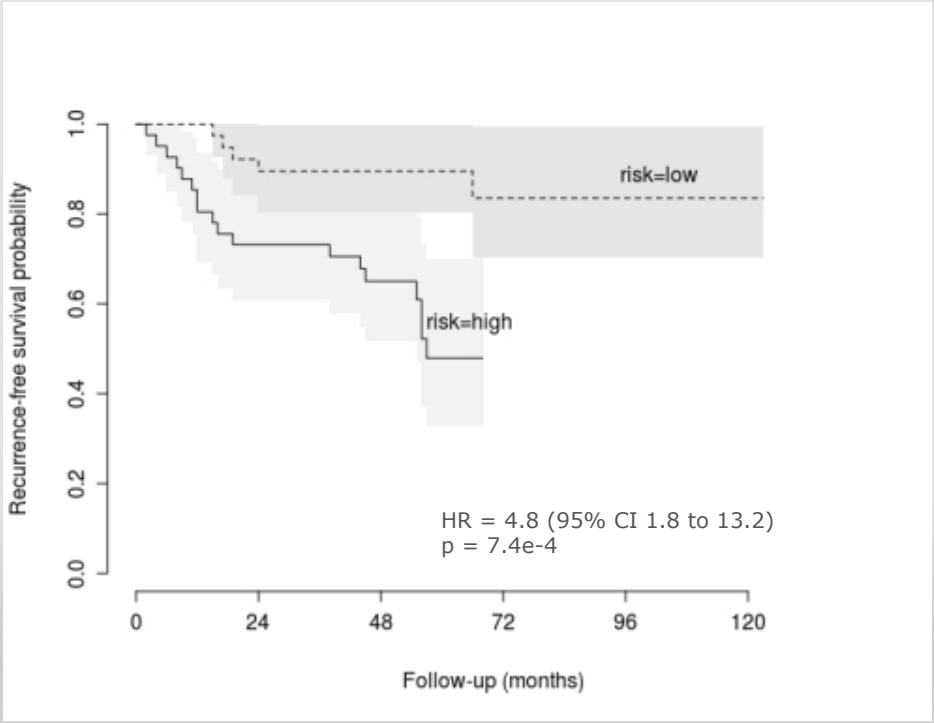
LASSO model: Model validation set GSE94767 ICR





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Discussion

- PSA threshold for recurrence: MSKCC used classic value of 0.2. We (and ICR) used 0.2 or salvage treatment (75% of our patients currently receive salvage radiation with a PSA less than 0.2)
- Median follow up time was 60 months
- Sample population underwent radical prostatectomy between 2008 and 2011; many patients today would choose AS over RALP

Conclusion

- Using a large sample of radical prostatectomy specimens, a gene expression signature was identified that predicts BCR
- The prediction model was validated on two independent gene expression data sets and surpassed Gleason grade and tumor stage alone
- The prediction model has a similar AUC to commercially available tests that incorporate pathologic parameters and gene signatures; further studies are required to compare the results of our study against other predictive models and other genomic tests
- The five gene signature includes three genes that have previously been identified as associated with high risk prostate cancer as well as two additional genes that to our knowledge have not been previously identified
- Potential for gene targeted therapy

Thank You