Looking for Biomarkers for Prostate Cancer Early Detection

Bin Lu, PhD, Martin Sanda, MD

[N A J Med Sci. 2009;2(3): 081-083.]

Cancer is a diverse disease and each cancer has different mechanism. To find a highly sensitive and specific biomarker that can reflect a single cancer, is a challenge for detection or therapeutic purpose. Gene expression profiling, proteomics, and immunology are used with increasing frequency as tools for cancer screening. The prostate-specific antigen (PSA) test is widely used as a screening test in prostate cancer. However, low specificity of serum PSA leads to many false-negative and false-positive results. We note that maintaining high specificity (low false-positive rates) is a very high priority for population screening. A small false-positive rate can translate into a large number of people who will suffer unnecessary costly further diagnosis and psychological stress. So, it is necessary that a biomarker of prostate cancer be both sensitive and specific.[1] Beyond prognosis for prostate cancer, it is also very important to find molecular biomarkers correlating to prostate cancer disease progression. True *et al*. reported a molecular signature of 86 genes correlating to the Gleason grading system for prostate adenocarcinoma.[2] Laxman showed a multiplex biomarker analysis of urine, which included six genes for prostate cancer detection.[3] Recently, Chinnaiyan's lab demonstrated a robust pipeline for the discovery of novel gene chimaeras by high-throughput sequencing.[4] Metabolic profiling has shown a potential role for sarcosine in prostate cancer progression by using a combination of high-throughput liquid-and-gas-chromatography-based mass spectrometry in Chinnaiyan's group.[5]

Gene expression microarray has proven effective in identifying target genes and molecular pathways associated with prostate cancer. Profiling of RNA transcripts has been widely utilized to dissect molecular aspects of tumor cell biology as well as to project disease outcome that can be of high prognostic value.[6,7,8] For example, the determination by several such array studies that ERG is commonly overexpressed in prostate cancer, led to identification of novel gene arrangements between TMPRSS2 and ETS transcription factors in prostate cancer.[9,10] Many studies have interrogated differential gene expression between prostate cancer and normal prostate tissue as a means of identifying targets for prostate cancer detection or therapy.[7,8] However, most differentially expressed genes identified via this strategy are also expressed by various other non-prostatic normal human adult tissues. The specificity of such biomarker targets for prostate cancer detection or therapy therefore can be limited by the presence and function of such genes in other (non-prostate) human adult tissues. One strategy to identify and prioritize prostate antigens (that would have greater specificity for prostate cancer early detection or targets for therapy) is to identify genes that are expressed in prostate cancer but not in normal, non-prostate adult tissues. Along this direction, one group has described microarray analyses depicting a 'prostate-specific' transcriptome, in which there is the identification of genes expressed in normal prostate but not in other, extra-prostatic human adult tissues.[11] We sought to extend this strategy by identifying genes expressed in prostate cancer but not expressed in non-prostate human adult tissues as putative targets for prostate cancer detection or therapy.

Toward this goal, we undertook: (A) an expression array experiment using our prostatectomy repository to identify prostate cancer-associated expressed genes; (B) an *in silico* analysis of a publicly available data set to validate genes identified in our array experiment, and (C) a novel analysis *in silico*, interrogating the expression of these PCa-associated antigens in non-prostate human adult tissue (to identify those antigens that are not detectable in adult tissue outside of the prostate). Our array experiment using the Affymetrix U133 array to evaluate gene expression in specimens from our tissue repository identified 1474 genes overexpressed in **prostate**

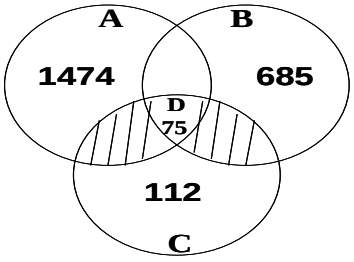

Figure 1. Microarray analysis of prostate cancer-specific antigens.

Bin Lu, PhD, * Martin Sanda, MD
Beth Israel Deaconess Medical Center, Boston, MA 02115

*Corresponding author:
Bin Lu, PhD
Division of Urology
Department of Surgery
Beth Israel Deaconess Medical Center
CLS 4 room 447
3 Blackfan Circle,
Boston, MA 02115
Phone: (617) 735-2105
Fax: (617) 735-2110
E-mail: blu@bidmc.harvard.edu

cancer compared to normal prostate (Figure 1 area A). Validation of genes that were overexpressed in prostate cancer by comparison to the publicly available Stanford prostate cancer array dataset (**Figure 1** area B; 685 transcripts associated with PCa;[8]) implicated 195 transcripts with concordant over-expression between the array datasets. Next, our *in silico* interrogation of the largest publicly available dataset of gene expression in normal human tissues (**Figure 1** area C) showed 112 genes not expressed outside of the human prostate[12] then identified 75 genes that are overexpressed in prostate cancer and are NOT expressed at detectable levels in normal adult human tissue apart from the prostate (**Figure 1** area D).

From among these 75 candidate prostate cancer-specific antigens, 23 were detected by our specimen U133 arrays and were also confirmed as overexpressed in the publicly available prostate cancer array dataset from Stanford.[8] We then performed quantitative RT-PCR targeting for each of the candidate antigens, and confirmed that 17 of the candidate genes were indeed overexpressed in prostate cancer (compared to normal prostate). The frequency of overexpression in prostate cancer for these antigens ranged from 57% to 86%. From among these 17 prostate cancer specific antigens that were validated by RT-PCR, we selected the gene that was most consistently absent in normal prostate and had the highest frequency of expression in prostate cancer, the single-minded homolog gene (SIM2). We found SIM2 overexpressed in 6 of 7 cancers tested but not in benign prostate tissue, identifying SIM2 as a candidate for further study as a putative prostate cancer associated antigen.

The SIM2 gene is located on the human chromosome 21q22.2 and is a member of the basic helix-loop-helix PAS [per-Arnt-Sim] (bHLH-PAS) family of transcription factors.[13,14] SIM2 was originally thought to contribute to Down's syndrome (DS).[13,15] As a transcription factor (TF), murine SIM2 (mSIM2) mediates gene expression through CNS midline enhancer (CME) element with its dimerization partner ARNT via ARNT carboxy-terminus.[16] SIM2/ARNT complex has been shown to bind hypoxia response elements and affect the transcription process.[17] The transcription factor c-myb regulates SIM2 transcription in glioblastoma cells, and a nuclear localization signal (NLS) mediates nuclear localization of SIM2.[18] A prior *in silico* bioinformatics approach using the Cancer Genome Anatomy Project (CGAP) database of the National Cancer Institute (NCI) identified SIM2 as associated with colon, pancreas and prostate carcinomas, while absent in the corresponding normal tissues.[19] Two different spliced isoforms of SIM2 transcript, SIM2-long (SIM2-l) and SIM2-short (SIM2-s), have been reported while their differential function in humans are not known yet.[13] SIM2-s was specifically expressed in early stages of colon cancer. Inhibition of SIM2 expression by antisense oligos caused growth inhibition and apoptosis in colon cancer cell line RKO and tumor growth in nude mice and also in pancreatic cancer cell line CAPAN-1.[20,21] Apoptosis was induced by Sim2-s inhibition in the RKO colon cancer cell line.[22] However, SIM2-s was not found to have oncogenic activity in breast cancer,[23] indicating that SIM2 function may vary depending on the tissue of origin of a given cancer cell, or cancer cell type.

Although the expression of SIM2 (as measured by immunohistochemistry of prostatectomy specimens) has been associated with aggressive histopathology in prostate cancer,[24] the functional role of SIM2 in prostate cancer is largely unknown. In order to explore the potential for SIM2 as a target for prostate cancer therapy and early detection, we undertook studies to characterize its expression in prostate cancer cell lines, to assess whether SIM2 expression can be regulated by androgens and to determine if SIM2 could be detected in the urine of patients with prostate cancer. The expression of SIM2 in prostate cancer cell lines and immortalized benign prostate cells will be evaluated.

Androgen receptor - regulated gene expression can facilitate prostate cancer onset and progression.[25,26,9,27] It will be interesting to find whether SIM2 expression is regulated by androgens. Literature survey of ARE mapping studies led to a study by Brown's group showing that there are 90 androgen-binding sites on chromosome 21 and 22.[27] Our interrogation of the published CHiP-on-chip data from Wang *et al* indicates 2 putative AR binding sites (b32 and b33) that are close to SIM2 gene, which are about 572.5 kb upstream and 38 kb downstream of SIM2 coding sequence.

Having assembled evidence that SIM2 is associated with prostate cancer and appears to have androgen-responsive expression, it will be also interesting to determine whether SIM2 transcript could be targeted for prostate cancer early detection studies either in human serum or urine. It was recently demonstrated that prostate cancer biomarkers (most of which are not secretory proteins like PSA) can nevertheless be targeted for early detection studies by interrogating whether the cellular fraction of urine specimens (collected after massage of the prostate by digital rectal exam, DRE) contain RNA transcripts of such genes.[3] Antigens triggering autoantibodies could be another potential targets for prostate cancer early detection, based on the evidence that the human immune system can mount aberrant immune responses against self-antigens expressed by tumor cells in several cancer types.[28,29,30]

References

1. Pepe MS, Etzioni R, Feng Z, et al. Phase of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001;93(14): 1054-1061.
2. True L, Coleman I, Hawley S, et al. A molecular correlate to the Gleason grading system for prostate adenocarcinoma. Proc Natl Acad Sci U S A. 2006;103(29):10991-10996.
3. Laxman B, Morris DS, Yu JJ, et al. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. Cancer Research. 2008;68(3):645-649.
4. Maher CA, Kumar-Sinha C, Cao X, et al. Transcriptome sequencing to detect gene fusions in cancer. Nature. 2009;458(7234):97-101.
5. Sreekumar A, Poisson LM, Rajendiran TM, et al. Al Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature. 2009;457(7231):910-914.

6. Dhanasekaran SM, Barrette TR, Ghosh D, et al. Delineation of prognostic biomarkers in prostate cancer. Nature. 2001;412(6849):822-826.
7. Yu YP, Landsittel D, Jing L, et al. Gene expression alterations in prostate cancer predicting tumor aggression and preceding development of malignancy. J Clin Oncol. 2004;22(14):2790-2799.
8. Lapointe J, Li C, Higgins JP, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A. 2004;101(3): 811-816.
9. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005;310(5748):644-648.
10. Tomlins SA, Rubin MA, Chinnaiyan AM. Integrative biology of prostate cancer progression. Annu Rev Pathol. 2006;1(1):243-271.
11. Clegg N, Abbott D, Ferguson C, Coleman R, Nelson PS. Characterization and comparative analyses of transcriptomes from the normal and neoplastic human prostate. Prostate. 2004;60(3):227-239.
12. Su AI, Wiltshire T, Batalov S, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A. 2004;101(16): 6062-6067.
13. Chrast R, Scott HS, Chen H, et al. Cloning of two human homologs of the Drosophila single-minded gene SIM1 on chromosome 6q and SIM2 on 21q within the Down syndrome chromosomal region. Genome Res. 1997;7(6):615-624.
15. Ema M, Hosova T, Mimura J, et al. Mild impairment of learning and memory in mice overexpressing the mSim2 gene located on chromosome 16: an animal model of Down's syndrome. Hum Mol Genet. 1999;8(8):1409-1415.
16. Moffett P, Pelletier J. Different transcriptional properties of mSim1 and mSim2. FEBS LETTER. 2000;466(1):80-86.
17. Woods SL, Whitelaw ML. Differential activities of murine single minded 1 (SIM1) and SIM2 on a hypoxic response element - Cross-talk between basic helix-loop-helix/per-Arnt-Sim homology transcription factors. J Biol Chem. 2002;277(12):10236-10243.
18. Yamaki A, Tochigi J, Kudoh J, Minoshima S, Shimizu N, Shimizu Y. Molecular mechanism of human single-minded 2 (SIM2) gene expression: identification of a promoter site in the SIM2 genomic sequence. Gene. 2001;270(1-2):265-275.
19. Deyoung MP, Scheurle D, Damania H, Zylbeberg C, and Narayanan R. Down's syndrome-associated single minded gene as a novel tumor marker. Anticancer Res. 2002;22(6A):3149-3157.
20. Deyoung MP, Tress M, and Narayanan R. Identification of Down's syndrome critical locus gene SIM2-s as a drug therapy target for solid tumors. Proc Natl Acad Sci U S A. 2003;100(8):4760-4765.
21. Deyoung MP, Tress M, Narayanan R. Down's syndrome-associated Single Minded 2 gene as a pancreatic cancer drug therapy target. Cancer let. 2003;100(1):25-31.
22. Aleman AJ, Deyoung MP, Tress M, Keating P, Perry GW, Narayanan R. Inhibition of Single Minded 2 gene expression mediates tumor-selective apoptosis and differentiation in human colon cancer cells. Proc Natl Acad Sci U S A. 2005;102(36):12765-12770.
23. Kwak HI, Gustafson T, Metz RP, Laffin B, Schedin P, Porter WW. Inhibition of breast cancer growth and invasion by single-minded 2s. Carcinogenesis. 2007;28(2):259-266.
24. Halvorsen ØJ, Rostad K, Oyan AM, et al. Increased expression of SIM2-s protein is a novel marker of aggressive prostate cancer. Clin Cancer Res. 2007;13(3):892-897.
25. Chang C, Kokontis J, Liao S. Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science. 1988; 240(4850):324-326.
26. Heinlein CA, Chang C. Androgen receptor in prostate cancer. Endocr Rev. 2004;25(2):276-308.
27. Wang QB, Li W, Liu X, et al. A hierarchical network of transcription factor governs androgen receptor-dependent prostate cancer growth. Mol Cell. 2007;27(3):380-392.
28. Koziol JA, Zhang JY, Casiano CA, et al. Recursive partitioning as an approach to selection of immune markers for tumor diagnosis. Clin Cancer Res. 2003;9(14):5120-5126.
29. Tan EM. Autoantibodies as reporters identifying aberrant cellular mechanisms in tumorigenesis. J Clin Invest. 2001;108(10):1411-1415.
30. Stockert E, Jager E, Chen YT, et al. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med. 1998;187(8):1349-1354.