PREDICTING PROSTATE CANCER BEHAVIOR USING TRANSCRIPT PROFILES

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ABSTRACT

Purpose: Prostate cancer represents a disease with diverse clinical outcomes. Treatment strategies that optimize benefit and minimize morbidities depend on accurate estimates of disease status and likelihood of progression. Emerging technologies capable of qualitatively and quantitatively profiling genes expressed by neoplastic tissues may provide insights into tumor behavior. This review discusses the use of microarray based transcript expression profiling to stratify human cancers into risk categories.

Materials and Methods: MEDLINE was used to perform a comprehensive literature review of reports describing the assessment of gene expression profiles in malignant diseases. Particular emphasis was placed on studies developing models using individual genes or gene cohorts as predictors of prostate cancer outcome.

Results: Alterations in the expression of individual genes identified by microarray analyses have been used in studies of outcome in cancers of the prostate and other tissue types. Profiles of expressed genes have been used to develop prediction models that stratify cancers into prognostic categories based on relapse rates or responses to therapy.

Conclusions: Gene expression profiles offer an opportunity for acquiring molecular determinants correlating with clinical outcome. With rare exceptions these profiles have yet to be validated or used in prospective studies. Future research will benefit from assessments of intratumor heterogeneity and host factors such as the immune response and hormonal milieu. The prospective validation of predictive models will serve to prove usefulness in the clinical arena.

KEY WORDS: prostate; prostatic neoplasms; gene expressions; prostate-specific antigen; lymphoma, non-Hodgkin

Carcinoma of the prostate exhibits a biology unique among human cancers, characterized by a high prevalence of histologically identifiable cancer and a markedly lower incidence of clinical disease. Despite a variety of clinical and histological parameters used to classify prostate cancer, patients receiving the same diagnosis can have strikingly different disease progression rates and responses to treatment. It is clear that our current taxonomy of prostate cancer fails to stratify accurately prostate cancers into distinct subtypes with predictable clinical phenotypes.

Importantly molecular heterogeneity within individual cancer diagnostic categories is readily observed as chromosomal translocations, deletions of tumor suppressor genes, amplifications or mutations of oncogenes and numerical chromosomal abnormalities. Advances in molecular biotechnology have produced tools capable of analyzing molecular alterations on a comprehensive scale. Recent studies have demonstrated the capability for large-scale gene expression analyses to distinguish subtypes of hematological malignancies, such as lymphoma and leukemia, as well as solid tumors, including breast, lung and prostate carcinoma. It is anticipated that further applications of large-scale gene expression analyses to the study of prostate cancer will not only identify the critical determinants of disease stratification and outcome, but also provide a rational basis for therapeutic intervention.

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INDICATORS OF PROSTATE CANCER PROGNOSIS: CLINICAL, HISTOLOGICAL AND MOLECULAR

Currently decision making for men with a diagnosis of prostate cancer is hampered by a lack of individualized data predicting the natural history of the disease or the expected outcome with intervention. Large cohort studies have attempted to address this through the analysis of clinical and pathological factors that are associated with survival or disease relapse. These cohorts form the basis of extensive work correlating adverse pathological features with pretreatment factors and outcomes following radical prostatectomy.

On the continuum of the therapeutic window there are currently 2 major intervention points where prostate tissue is obtained and prognostic indicators are important. The first point occurs at diagnosis and reflects the clinical stage of the cancer, as determined by physical examination, imaging studies, tumor marker (prostate specific antigen [PSA]) measurements and increasingly prostate biopsy information. The second intervention point occurs at the time of primary treatment and in the case of radical prostatectomy it results in a pathological staging that determines the grade, volume and extent of disease spread. At these 2 intervention points highly accurate information capable of detailing the expected natural history of the cancer as well as the response to treatment would be extremely useful. The objectives for determining prognostic variables or prognostic indicators for prostate cancer are to identify and stratify tumors that 1) should not be treated by virtue of a low incidence of clinical progression, 2) are cured by standard modalities of surgery or radiation therapy, 3) are unlikely to be cured entirely by a single primary procedure but may be cured if additional
neoadjuvant, concurrent or adjuvant therapies are provided and 4) will not be cured by any known therapy and should be considered for palliative care or innovative high-risk therapies.

Despite earlier diagnosis and presumably smaller tumor volumes 35% to 50% of patients with clinically organ confined prostate cancer will be shown to have extraprostatic disease subsequent to radical prostatectomy. The strongest predictive factors for advanced disease are Gleason score, serum PSA and clinical stage. In the context of this discussion PSA is categorized as a clinical indicator rather than as a molecular indicator of outcomes because it is acquired from the patient prior to medical intervention and is not an intrinsic characteristic of the tumor. The Gleason grading system in biopsy or prostatectomy specimens is a measure of biological aggressiveness and it is currently the best correlate for the final pathological stage and subsequent clinical outcome. Several groups have recently combined clinical stage, serum PSA and Gleason score to generate nomograms that predict pathological stage or outcomes and allow prognostic estimates to be made with readily available clinical data.

In an effort to complement clinical and pathological indicators of prognosis prostate cancers have been studied for intrinsic molecular determinants of disease progression. In addition to providing insight into the basic biology of carcinogenesis, the identification of specific genetic alterations can be predictive of clinical behavior and may influence therapeutic intervention. It is important to emphasize that for diagnostic and prognostic purposes it is not necessary to determine if a molecular alteration is the cause or the result of a given neoplastic phenotype, but only that the association is consistent.

Candidate molecular predictors of prostate cancer behavior include genes and gene products involved in a wide variety of cellular functions, including cell cycle regulation, cell signaling and cell adhesion. Immunohistochemistry and other techniques have been used as a means of identifying and quantitating alterations in these gene products to assess the ability of these factors to predict prostate tumor behavior. Molecular alterations shown to have prognostic relevance in prostate cancer include P53, retinoblastoma, p27, chromogranin A and e-cadherin, among others.

Despite the associations of the expression of specific molecular factors with clinical phenotypes of prostate cancer described in each of the studies cited it is clear that taken individually the expression of these genes and proteins cannot predict disease outcome in any given patient. Rather, as with clinical and histological parameters, the percents, odds or likelihood of clinical behavior can be provided. These remain inadequate measures on which to base treatment decisions.

COMPREHENSIVE MEASURES OF GENE EXPRESSION AND TUMOR STRATIFICATION

The Human Genome Project catalyzed several developments that now provide powerful approaches for biological investigations. They include high throughput platforms for acquiring biological information (DNA arrays, DNA sequencing and proteomics), powerful computational, statistical and mathematical techniques for capturing, storing, analyzing and modeling biological information and the idea of discovery science, ie using high throughout tools to define all of the elements in a biological system and place them in a database (eg the sequence of the genome, the transcriptomes [all mRNAs] and the proteomes [all proteins] of individual cells).

These large data sets can be used to model and test hypotheses involving complex biological systems such as cancer. The recognition that it may be extremely useful to determine comprehensively alterations in the genes expressed in normal and diseased cells led to the development of several novel strategies that include the analysis of microarrays, that is chips or slides with spatially defined immobilized DNA fragments representing genes of interest. This technique combines the proven chemistry of nucleic acid hybridization with advanced automation and image analysis technology to monitor quantitatively changes in gene expression patterns. Briefly, individual cDNAs or oligonucleotides representing known or unknown genes are spotted or synthesized on a solid support such as a glass slide. Replicates are made of the arrays using high precision robotics. Complex tissue probes are constructed with radioactive or fluorescent labels and hybridized to the arrays. Individual or groups of transcripts with differential expression signals are identified using quantitative image analysis software. This methodol-

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Prostate cancer outcomes determined by microarray expression profiles.

mRNA is isolated separately from normal and neoplastic prostate tissues, preferably from specific microdissected cell types. RNA samples are labeled directly with fluorescent probes or first converted to cDNA, followed by hybridization to microarrays of spotted or synthesized DNAs representing genes of interest. Gene expression measurements are determined by analyzing fluorescent signal intensity for each gene on microarray and subsequently comparing signal levels between normal and neoplastic cell types, and neoplastic cell types representing different clinical outcomes. Expression data are correlated with known clinical outcomes to determine profile or fingerprint capable of predicting risk of local and distant cancer progression. Patients at minimal risk for progression (ie with indolent disease) would receive no primary intervention but could be considered for dietary alteration or chemoprevention trials. Patients at intermediate risk would receive primary curative therapy such as radical prostatectomy or radiation therapy. Patients at high risk would receive primary therapy with addition of systemic adjuvant or neoadjuvant therapy.

Prostate cancer outcomes determined by microarray expression profiles. mRNAs are isolated separately from normal and neoplastic prostate tissues, preferably from specific microdissected cell types. RNA samples are labeled directly with fluorescent probes or first converted to cDNA, followed by hybridization to microarrays of spotted or synthesized DNAs representing genes of interest. Gene expression measurements are determined by analyzing fluorescent signal intensity for each gene on microarray and subsequently comparing signal levels between normal and neoplastic cell types, and neoplastic cell types representing different clinical outcomes. Expression data are correlated with known clinical outcomes to determine profile or fingerprint capable of predicting risk of local and distant cancer progression. Patients at minimal risk for progression (ie with indolent disease) would receive no primary intervention but could be considered for dietary alteration or chemoprevention trials. Patients at intermediate risk would receive primary curative therapy such as radical prostatectomy or radiation therapy. Patients at high risk would receive primary therapy with addition of systemic adjuvant or neoadjuvant therapy.

CONCLUSIONS

Based on the widely variable clinical outcomes of prostate cancers characterized by specific tumor grades it is clear that this entity (prostate cancer) actually represents multiple diseases. It is unlikely that a single clinical, histological or molecular biomarker will determine prognosis with high specificity and sensitivity. The nomograms developed for predicting if a given cancer is likely to be organ confined preoperatively or postoperatively use multiple parameters. The nomograms developed for predicting if a given cancer is likely to be organ confined preoperatively or postoperatively use multiple parameters. The continued refinement of strategies that incorporate additional tumor factors (ie molecular alterations) and host factors may allow for more accurate predictors of outcome (see figure).

Gene expression profiles offer an unprecedented view of the diversity and consistency of genetic alterations in prostate tumors. Published reports provide the first glimpse of the potential of this technology to define the molecular pre-
dictors of cancer behavior. However, there clearly are limitations to the approach and significant hurdles to be overcome. All published studies used relatively few samples in the analysis. In part this is due to the lack of suitable samples with well preserved RNA and adequate clinical followup. In addition, the labor and expense of the technology remain limitations. In contrast, nomograms developed for prostate cancer predictions used hundreds to thousands of patients to decipher the relative importance and relationships of clinical characteristics that are best associated with outcome.

It is quite interesting and somewhat disconcerting that cohorts of genes statistically associated with disease outcome in different studies do not correlate with each other. In part this may be explained by the profound heterogeneity of neoplastic prostate tissues. Tissues used in standard analyses may be comprised of various amounts of stroma, inflammatory cells, blood vessels and constituents. While gene expression changes in these cell types may be important predictors, the variable amounts of these cell types among different studies may serve to make interstudy comparisons difficult. One approach to this problem is the use of microdissection techniques that compare only specific cell types. The ultimate end point of this approach is the analysis of expression profiles of individual cells within the tumor environment. While this currently represents a daunting task, great strides in biotechnology do not rule out single cell analysis using high speed flow cytometers and nanotechnology in the near future.

A major confounding factor when assessing tumor outcomes based on expression profiles concerns variables in the host. Published studies have focused on defining molecular determinants within the tumor itself. However, host variables involving immune response, dietary factors and the hormone milieu may have profound influences on the ability and efficiency of tumor cell proliferation, invasion and metastasis. Examples of variation in tumor development and progression in different strains of transgenic mice abound. In the future the predictive power of tumor expression profiles could be enhanced through the inclusion of host expression profiles that define additional variables likely to affect tumor progression and responses to therapy.

Dr. Daniel Lin critically reviewed the manuscript and provided suggestions.

APPENDIX: DIFFERENTIAL GENE EXPRESSION

Chetcuti et al20
Differential gene expression was determined by calculating the ratios of hybridization intensities between normal and neoplastic samples following array normalization. Genes with ratios 3-fold or greater were called differentially expressed.

Chai et al21
Differential gene expression was determined by calculating the ratios of hybridization intensities between normal and neoplastic samples following array normalization. Genes with ratios 2-fold or greater were called differentially expressed.

Luo et al22
Differential gene expression was determined by computing the discriminative weight (w) of each gene to separate cancer from benign tissue with p values assigned based on the (w) values of randomized data.

Dhanasekaran et al15
Several approaches used: t statistics (of prostate cancer vs benign tissue) ranked based on effect size: 200 genes reported. An analysis using fold change identified 1,520 genes with 2-fold changes between 50% of cancer samples and normal adjacent tissue and 1,006 genes with 3-fold change in 75% of cancer samples compared with a normal commercial pool.

Magee et al13
Differential expression between benign and neoplastic prostate tissue; 3-fold or greater change in all 11 tumors compared with 4 benign samples with p values calculated by a 2-tailed t test for independent data sets of unequal size and variance.

Welsh et al23
Differential expression between benign and neoplastic prostate tissue was determined by equally weighing contributions from differences in hybridization intensities, the quotient of hybridization intensities and the result of an unpaired t test between expression levels in tumors and normal tissues.

Stamy et al24
Differential expression between BPH and neoplastic prostate tissue; p <0.0005 and expressed in all samples examined.

Luo et al25
Differential expression between benign and neoplastic prostate tissue; p <0.05 by Student's t test; signal intensity greater than 500 U.

Ernst et al26
Differential expression between benign and neoplastic tissue; p <0.05 by Student's t test and fold change greater than 3.

LaTulippe et al27
Differential expression between non recurrent primary prostate cancers and metastasis; fold difference 3-fold or greater.

<table>
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<tr>
<th>No. Genes Studied</th>
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* Estimated by the authors.
Differential expression between normal vs tumor samples was determined using a variation of a signal-to-noise metric. The statistical significance of the gene expression correlations was determined by comparing the observed correlations to the results mined by comparing the observed correlations to the results derived from permutations of the class labels (normal and tumor).

Differential expression between benign and cancer samples was determined by combining equally weighted contributions from differences in hybridization intensities, the quotient of the hybridization intensities and the results of an unpaired t test between expression levels. Selection criteria were further narrowed to a fold change of greater than 2.35 and a p < 0.001 by Student’s t test.

Differential expression between normal and cancer tissue was determined at the p < 0.001 level by 1 sample t test and Wilcoxon ranking.

This report was designed to identify gene expression changes associated with disease progression. Cox proportional hazard survival analysis predicting relapse was used to evaluate each gene (probset). Gene expression predictors of relapse at the p < 0.01 level were reported. A false-discovery rate was calculated to be 23% (61 of the 266 findings were false-positive).

REFERENCES


DISCUSSION

Dr. William G. Nelson. Do you find that there is a greater variance in transcript levels compared with protein levels, perhaps even a greater dynamic range? Do you believe that the protein does not change expression very much but the message still does? Is that going to be a superiority of transcript profiling?

Dr. Peter S. Nelson. I have only heard of 1 set of data that supports a low concordance rate between protein profiles and transcript profiles. In very exquisite studies done in yeast, where you have the entire genome on a chip, the concordance was extremely high in a quantitative sense. There were very few proteins that behaved differently than their transcripts at steady state. I do not think that the power of proteomics lies in its power to discriminate absolute levels, but in looking at functional aspects.

Dr. Anthony V. D'Amico. We know that PSA failure does not correlate with cancer specific death. Is the bioinformatics rigorous enough to come up with an identical solution when tested against different samples? Is the biology changing or is it simply that the solutions that you can get from these profiles, which look at an enormous number of genes, are not unique and, therefore, are clinically questionable?

Dr. Peter Nelson. Each microarray is looking at 100,000 transcripts and you have 100 tumors, so that you are looking at hundreds of thousands of comparisons. With multiple comparison testing there are findings that are statistically significant.

Dr. Neal Rosen. If you have 20,000 variables, you get a reproducible pattern within your set that discriminates tumor from normal tissue. Did that pattern occur randomly and does it have anything to do with tumor vs normal? You can seem to get ordered patterns from random processes. You can have 5 research groups looking at 20,000 genes and getting nice patterns within each group but the concordance among the groups may be very low.

Dr. Peter Nelson. Part of the issue is whether or not you can predict metastatic disease.

Doctor D'Amico. All of these predictors are time to an event—time to PSA failure, time to distant disease. Analysis is confounded by the timing of hormonal therapy. You can not argue with survival as an end point. On another topic, in genomic profiling are you looking at DNA from both the mitochondria and the nucleus in a cell? Could there be differential expression between these 2 compartments in a cell?

Dr. Peter Nelson. Some people are studying mitochondrial DNA.

Dr. William Nelson. RNA that has functions other than encoding genes is not well represented on expression arrays that are printed from genome data bases.

Doctor Rosen. We now know that many neurological degenerative diseases of aging are due to a slow depletion of mitochondria. No one has looked at mitochondria genomically in cancer. You could get differences in expression and also selective loss of mitochondria in various tumors. A lot of what is going on in cancer has to do with mitochondria and bioenergetics. I wanted to ask about the utility of targeted expression array studies.

Dr. Mark A. Rubin. Every expression array study has a different purpose. Many studies were initially done for discovery, to look at specific genes that were differentially expressed, and not necessarily for clinical utility, such as distinguishing tumor from benign tissue.

Doctor Rosen. It depends on what you want to discover. The advantage of global expression array studies is that you get nonbiased information on things that are totally new. The disadvantage may be that you do not get data on some targeted processes that we know are important in cancer.