

Minireview

The Need to Embrace Molecular Profiling of Tumor Cells in Prostate and Bladder Cancer¹

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Abstract

Purpose: Current treatment strategies for urological cancer are still based on empirical formulae as opposed to treatment tailored for each cancer patient. To individualize treatment, the multiple molecular abnormalities within tumor cell populations needs to be mapped out. The aim of this article is to explain molecular profiling (MP) and its associated techniques so that the process is not purely seen as a research tool but as a future adjunctive measure in patient diagnosis and treatment.

Experimental Design: A Medline search of publications relating to MP of prostate and bladder cancer was carried out. A review article was written combining the relevant published literature along with the clinical and scientific experience of both centers.

Results: The advent of MP now provides a strategy by which these molecular abnormalities can be assessed. As well as being of diagnostic and prognostic use, these molecular profiles will identify putative molecular abnormalities within tumor cells that may be appropriate for therapeutic modulation.

Conclusions: In prostate and bladder cancer, mapping out the molecular abnormalities could be translated into a valuable tool to help solve difficult issues regarding patient management decisions.

Introduction

The increasing spectrum of molecular biology techniques over the last 20 years is making a huge impact in the scientific world. The revolution, however, has yet to make significant changes to clinical practice. The lack of understanding of the molecular basis of many of the disease processes and the failure thus far to find a single molecular marker to help clinicians in their work has meant that clinicians have been content for molecular work to remain purely in the laboratory. This may change, and although apprehension is the natural response to any new technology, the MP³ of cells causing disease in the prostate and urinary bladder can only enhance patient diagnosis and treatment.

Survival from urological cancers has not changed dramatically over the last two decades. This lack of improvement is despite the introduction of new diagnostic processes, therapies, and management strategies. Furthermore, the investigation of single molecular targets has yielded important information, but this remains limited in its extension to clinical practice (1, 2). Single gene analysis has not given clinicians conclusive data regarding diagnosis or prognosis. Immunohistochemistry of prognostic markers based on a single parameter has generally proved inadequate, and the relationship between a gene mutation and the expression level of that gene or its protein and disease etiology or extent of disease is often complex. This is not surprising considering that a single gene can encode many proteins through differential splicing. Furthermore, the functionality of the protein will depend on posttranslational modifications. Most researchers are in agreement that oncogenesis is complex, and no individual protein has overall control of either initiation or progression of the oncogenic process (2). It is therefore not that surprising that a laborious single gene by gene analysis or single protein by protein analysis yields limited information. This problem is now being addressed by using novel molecular and biochemical techniques together with bioinformatics and data analysis systems that facilitate multiparameter analysis.

Tumors generally are latent for a long period before they present clinically. This is especially true in relation to prostate cancer in some instances presenting only as metastatic disease. Once a tumor has metastasized, then intervention to achieve a cure is problematic. Consequently, efforts to combat cancer may become more focused on prevention with a drive to support public health doctors who advise the population about individual environment risk strategies to decrease their risk of contracting cancer. This strategy does not help clinicians treating cancer, the problems of which are the same as they were 20 years ago.

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³ The abbreviations used are: MP, molecular profiling; PSA, prostate-specific antigen; MS, mass spectrometry; TOF, time of flight; NCI, National Cancer Institute.

The majority of these unresolved difficulties relate to an inability to accurately diagnose or predict both tumor progression and prognosis, meaning that treatment and prognostication for the patient is suboptimal. To date, interest and understanding of genes has largely been left to academics. This will only be brought into the clinicians world when these novel applications are extended to answer the everyday clinical questions. Before surgery, can tumor activity be predicted? Is it likely to recur after surgery? If so, in an indolent or aggressive manner—to lymph nodes or to other organs? Will it respond to a given therapy? In common with other cancers, histological assessment of stage and grade remains the important factors on which treatment decisions are based. These parameters are subjective assessments, they are not always reproducible, and their value is limited. The challenge for scientists is to supplement stage and grade assessments with more objective clinician friendly assays. Because of the lack of advance from the laboratory bench to the bedside, clinicians continue to rely heavily on histopathology and radiological imaging to assess prostate and bladder tumors.

A major goal of cancer biology is to identify and understand the genes involved in tumorigenesis. This is not a simple task because each population of cells within a tumor will express a molecular profile that is unique to the subpopulation of cells at that specific stage of tumor evolution. Thus, interpretation of the data from new molecular techniques requires careful consideration of the dynamics of carcinogenesis and the inherent heterogeneity of tumors. Expressed genes do not always give rise to functional proteins so that genetic analysis is insufficient and analysis of proteins within tumor cells is also necessary. Comprehensive global analysis of both mRNA and proteins can be achieved using microarrays and proteomics (3–7). This genetic and protein information will build a fingerprint-type profile that will distinguish individual cancers at each stage of development and progression. It will be possible to identify genes and biochemical pathways that are deregulated in tumors. This will provide an increased understanding of the disease process, as well as new diagnostic and therapeutic targets.

Recent publications have focused on MP in diffuse large B-cell lymphoma, breast cancer, and prostate cancer (8–10). The main benefit of these studies, to date, has been the ability of the MP to allow subclassification of tumors of the same pathological type. Traditional histopathology and single molecular markers are relatively limited in their ability to subclassify tumors, but MP allows us to look more closely at better defined tumor subgroups.

There are, of course, hurdles and challenges to overcome. Molecular biologists face several major issues concerning acquisition and processing methods for the DNA, mRNA, and protein content of the cell populations (6, 7). The ideal method would preserve both the molecular characteristics of the sample and facilitate optimal morphological assessment of the tissue. A major concern is the relevance of snapshot molecular profiles in human cancers because carcinogenesis is a dynamic process that progresses through distinct stages of increasing genetic abnormality. It will be necessary to build up structured progressive genetic and protein profiles on numerous patients to determine the associations between early and late stage patterns (11). Nonetheless, there are many urgent questions, and it is therefore

time for clinicians to move on and to harness new technologies in the search for answers.

Using MP and proteomics it will be possible to generate a global view of DNA alterations, mRNA and proteins in single small samples of tissue (3). These technologies will help clinicians, pathologists, and scientists to compare the molecular anatomy of normal cells with that of progressive stages of cancer; develop new diagnostic and therapeutic targets for clinical intervention; and explain the relationship between genotype and phenotype in humans.

This article will first describe the technology involved in MP and then outline areas in prostate and bladder cancer where it is of potential clinical benefit.

Materials and Methods

The MP Technologies, mRNA Analysis, and Microarrays. The aim of microarrays is to highlight biologically relevant and differentially expressed markers to predict recurrence, progression, and drug resistance by assessing tumor cell mRNA. The sequence complementarity of the DNA duplex has been used in successive ways since Ed Southern used labeled nucleic acids to interrogate nucleic acids on a solid support. Southern and Northern blots that examine DNA and RNA fragments, respectively, and other methods, including *in situ* hybridization, have allowed one gene sequence at a time to be examined. Although the last two decades have generated vast amounts of data in terms of sequencing, which has increased understanding of biological function of many genes, this has had only limited use for the clinician. In the last decade, the latest extension of exploitation of the chemical complementarity of bases to look at the expression of multiple genes at once has been developed via arrays of immobilized nucleic acids (7).

A microarray is the reverse of the Southern blot. This allows known nucleic acid sequences, the probes, to be fixed to a support at defined positions. These are interrogated with cDNA probes synthesized from the mRNA extracted from the sample population of interest. Extraction of sufficient quantity of good quality RNA is imperative to the rest of the procedure. A variety of standard methods are currently used in laboratories, and there are also many kits available commercially, with Affimatrix being one of the first. The RNA is treated with an enzyme DNase to remove contaminating DNA. Purity of RNA can be verified most simply on an agarose gel. A reaction facilitated by reverse transcriptase is carried out during which a marker either radioactive or fluorescent is incorporated to form a cDNA probe. In a hybridization chamber, the probe, which has been synthesized from the RNA of interest, is mixed with the target nucleic acids, which are present on the array. Hybridization will occur if there is a complementary sequence between the analyzed labeled sample and the target nucleic acid, which in turn, allows generation of a detectable signal. The differential expression can then be analyzed in paired samples, *e.g.*, normal and tumor; treated and untreated; and responsive and nonresponsive. Microarrays are broadly either cDNA microchips or oligonucleotide chips. The latter include short (<25) oligonucleotides that can be used for both RNA expression studies and sequence analysis. Using light directed chemistry methods, synthesis of these is fabricated *in situ* to produce high density arrays

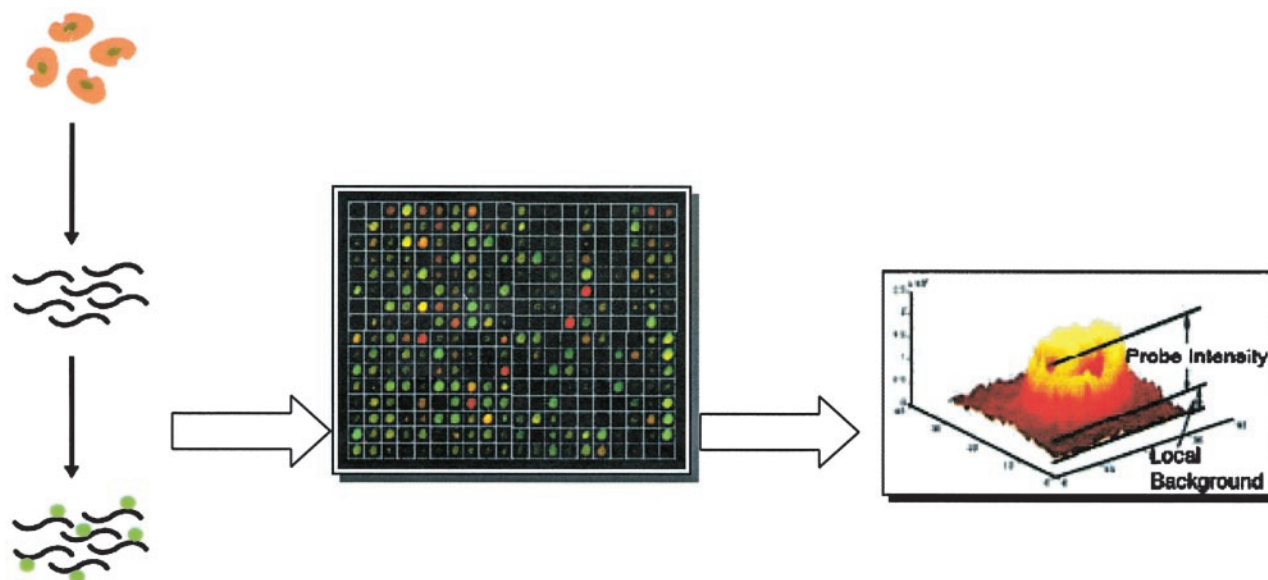


Fig. 1 Microarray chips have a range of cDNA sequences arranged so that complementary binding to the specific target site takes place if the gene is present in the test sample. The level of hybridization is then assayed on the microarray plate by assessing the intensity of fluorescence. The higher the fluorescence detected on each well of the plate reflects increased mRNA expression for that specific gene in the tumor sample.

(Fig. 1). *In situ* synthesis allows consistent high yields over the surface. Thousands of nucleic acid sequences may be immobilized for analysis. These require high resolution detection thus fluorochromes are the preferred detection system (Fig. 1). A major development is the use of a nonporous support glass, which allows use of fluorescent markers. This minimizes background and gives a high resolution facilitating miniaturization. Pletholithographic methods permit high density *in situ* synthesis of oligonucleotides.

cDNA arrays use a larger size of nucleic acids (>100) and are used in RNA expression studies. In this instance, the targets are labeled radioactively with ^{32}P or ^{33}P that is incorporated during first strand synthesis of the reverse transcriptase reaction. The microarrays are usually nylon membranes containing robotically spotted cDNAs or PCR products.

Single nucleotide polymorphisms are variations that occur when a single nucleotide is altered in the genome sequence. Tumor DNA can be derived from urine, serum, or plasma in patients with urological malignancies and this affords minimally invasive access to material for microarrays (12). In urological malignancies, different polymorphisms have been investigated, for example, the polymorphisms at the androgen receptor and PSA genes have been examined to assess their association with PSA levels (13) and many polymorphisms, glutathione *S*-transferase, *N*-acetyltransferase, and p53, have already been proven to be associated with bladder cancer. Cells exfoliated in urine have been correlated to the presence of cancer by examining alterations at microsatellite DNA markers (14). Investigation by array methods are gradually replacing current laborious methods to detect allelic imbalances in individual diseases (15) and to profile pathways of response to drugs (16). In diseases with marked heterogeneity, testing for the disease in susceptible individuals may become a reality using arrays to identify and

genotype mutations and polymorphisms. There are already published reports on the use of microarrays to identify prognostic biomarkers in prostate cancer (10). In bladder cancer, Wikman *et al.* (17) evaluated the use of microarrays to screen for p53 mutations and Richter *et al.* (18) examined cyclin E gene abnormalities. However, genes are large, and mutations may be small and scattered and are thus difficult to find and characterize even with microarrays, the principle of which relies on gain or loss of hybridization signals. Determination of differential transcript abundance in two mRNA populations that have been independently labeled permits comparison of the levels of message within the two populations. Differential gene expression in disease compared with normal states, tumor *versus* histologically normal tissue, early *versus* late carcinogenesis can be determined. In cancers, this data may generate signatures for diagnostic purposes and is likely to reveal new insights into tumorigenesis. Most array methods have been validated by Northern blots of the sample. As an internal control, arrays incorporate housekeeping genes and mismatch control probes that are often a perfect match minus 1 base.

Many problems that are encountered involve procedures for obtaining a pure population of cells and optimal preparation of DNA and RNA. Tissue obtained may contain only a small amount of cells of interest with poor quality RNA because routine methods for handling of pathological tissue do not lend themselves to subsequent microarray analysis. All solid tumors are composed of mixed cell lineages and no cell lives in isolation. This means that it is important to examine cells from the living state, not cells from contrived situations such as tissue culture. However, the tumor epithelium is a small fraction of the total cellular load, and it is imperative to separate out the different cell types and to analyze these separately (16). Laser capture microscopy isolates histologically defined cells of in-

terest from stained clinical tissue samples using an infrared laser. Laser capture microdissection enriches cell populations at speed, and this is beneficial because RNA degrades rapidly and also some proteins are labile (19). Microdissected cells from a tissue section are captured on membranes and analyzed. If at least 50,000 cells are microdissected, direct cDNA probe synthesis may be carried out. Unfortunately, often only a small number of cells are available.

Protein Analysis: Proteomics. It was previously believed that one single gene represented one polypeptide, but it has now been recognized that a single gene may code for multiple proteins (20). Often this is attributable to posttranscriptional or posttranslational modifications, and therefore neither gene sequences nor protein detection systems *per se* are sufficient to determine functional consequences. Trafficking of proteins within cells is another confounding factor because it has become evident that proteins may depend on their intracellular location for their functionality. This area is being addressed in cell mapping studies that are often undertaken three dimensionally. There is therefore an imperfect correlation between expression levels of mRNA and corresponding proteins. To understand gene networks, even on a superficial basis, both protein and mRNA information must be integrated. The relevance of this proteomic-genomic interface has been recently reviewed by Unwin *et al.* (21). Complex posttranscriptional control and posttranslational modification cannot be predicted from DNA or mRNA, explaining the shift in emphasis to proteomics (6). The proteome is the complete set of proteins encoded by the genome, and proteomics aims to identify, characterize, and quantitate protein dynamics in healthy tissues and during their response to stress and pathological conditions. There are already published reports of proteomic analysis outlining the steps in squamous differentiation of bladder cancer and also of facilitating diagnosis of transitional cell carcinoma from urine cytology (22).

Since 1975, two-dimensional gel electrophoresis has been used to separate proteins by charge and remains a popular method (23). Resolution of thousands of individual proteins from a single mixture is possible. Fractionation of peptide mixtures by ion exchange chromatography (link) or affinity fractionation of peptides reduces the complexity of the mix and increases the chances of identifying components (24). Detection of less abundant proteins has been facilitated by the development of immobilized pH gradient strips and by immunoblotting in combination with enhanced chemiluminescence (22). A process of subtraction of gels aided by software allows comparison between two samples. Labeling peptides with isotope-coded affinity tags allows accurate quantitation of levels of differences in expression of proteins. The data (molecular size, isoelectric point, and MS information) from unidentified separated protein spots can then be combined and compared with protein expression databases that are being developed online. Databases can be linked to nucleic acid sequence databases so that the most probable genetic origin of the protein can be predicted. There is now a need for high throughput systems rather than automation of existing traditional approaches.

Surface-enhanced laser desorption/ionization time-of-flight/MS is a novel combination of chromatography and MS in which small amounts of complex protein mixtures are enriched and can then be identified in a very short time. Cell lysates or

body fluids are directly applied onto bait spots. Each spot contains either a chemically or biochemically treated surface. Surfaces treated chemically react with whole classes of proteins, whereas biochemically treated surfaces react with affinity reagents such as antibodies, receptors, or enzymes (25). After enrichment, peptides are ionized and brought into a gaseous phase for analysis of their mass-to-charge ratios using MS (16). Ionized proteins and peptides travel down the vacuum tube in times that vary according to their size. Smaller molecules will have a shorter TOF.

Combined technologies such as surface-enhanced laser desorption-TOF and laser capture microscopy will soon become available to all medical centers. At present, these technologies are still research tools that are used to investigate potential biomarkers, for example, identifying protein fingerprints that could be used to distinguish clinically significant and insignificant carcinomas of the prostate (26–28). In the not too distant future, it is conceivable cancer patients will be fingerprinted from their sera or cells, their profiles will be interpreted using bioinformatic software and the management of their disease will be individualized.

Initially, research is aimed at highlighting proteins in normal cells and to compare these with cells under different conditions or in disease. The ultimate goal must be to determine all of the proteins that interact in the regulatory pathways in cells to maintain organs and systems, and this will provide targets and understanding of how these can be manipulated in disease.

Data Analysis. MP by its very nature is aimed at generating many observations from a given sample of tissue. These observations may relate to the mutational status of hundreds of genes, the expression of hundreds of proteins, and the associated posttranslational modification of these proteins to determine their functionality. This potentially represents an enormous multidimensional dataset that needs to be explored to remove redundant features and uncover useful biomarkers of clinical outcome. Having identified useful and robust biomarkers (there may be many), one then needs to combine these in such a way as to obtain a reliable tumor signature that can be used in clinical management of patients.

Handling and making sense of these enormous and complex datasets requires a range of multivariate statistical and mathematical techniques, and sufficient computing resources are needed to store the data and run the analyses. This field, which combines the use of computing, mathematics, and biology, is termed bioinformatics. It encompasses the development of database software to manage molecular data; the design and use of database search engines to identify annotated sequences such as contigs, promoter sites, and open reading frames; the use of three-dimensional modeling of molecules and its impact on functional genomics; the use of statistical methods such as cluster analysis to identify related features and reduce dimensionality of the data; and the development of artificial intelligence techniques such as neural networks and decision support systems for classification of molecular profiles. Recently, Khan *et al.* (29) successfully used artificial neural networks to help interpret the microarray data from analysis of small round blue cell tumors.

What is clear is that clinical prediction in cancer will not be based on one or two easily defined parameters. After all, hu-

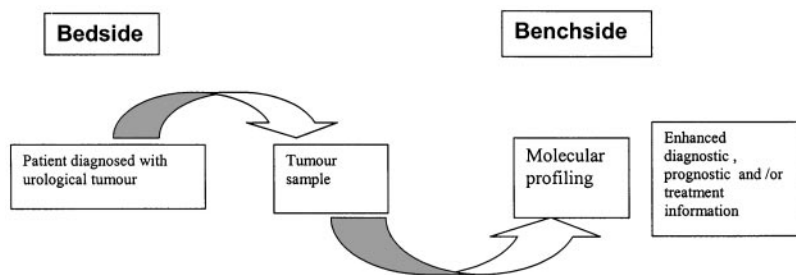


Fig. 2 idealised view of MP linking the benchside data to the bedside management of the patient.

mans and their associated diseases such as cancer represent complex systems, and this will require new and innovative data representation techniques so that complexity can be translated into a classification system that allows patient-targeted management and therapy.

Results

Clinical Application of MP Technology in Genitourinary Cancer. MP can be used to study a patient either in the clinic or in the operating room where tissue is removed to undergo standard histological or cytological examination (Fig. 2). Then the tissue itself or a specific subset of cells from the tissue is analyzed for its biomolecular content.

Current Developments in MP of Urological Tumors. The Cancer Genome Anatomy Project, a large multi-institutional effort, was initiated by the NCI in the United States to identify all abnormal genes in prostate cancer. Prostate cancer cells from radical prostatectomy specimens are processed through a number of steps to identify and sequence the genes expressed.

Aldehyde-based fixatives (*e.g.*, formalin) damage mRNA and other biomolecules. Conversely, frozen tissue specimens permit the study of mRNA from dissected cell populations. Frozen sections are technically difficult, and it requires skill to avoid destruction of the histology. In addition, the frozen sample contains only a limited portion of the tumor. The goal is to develop a universal protocol that is robust but protective of DNA, RNA, and protein while maintaining histological detail.

The NCI Prostate Group has developed a three-dimensional prostate model prototype for this purpose (30). Prostatectomy specimens are processed with a nonaldehyde fixative and a low temperature embedding compound that preserves histological detail and protects mRNA.

Whole mount cross-sections of the entire prostate gland are present in the x- and y-dimensions for viewing and microdissection. Several hundred adjacent serial recut slides are prepared from the tissue blocks at a thickness of 8 μm , and all of the normal histology and pathology in the z-dimension are recreated into a three-dimensional view of the prostate (4). Thus, it is possible to determine the exact physical relationship of the normal ducts, premalignant lesions, and tumor(s) and overlay this with the gene expression data.

Gene expression profiles are incorporated into the NCI Three-Dimensional Model Concept to show that significant changes in gene expression are much more complex than simply

normal *versus* tumor and to provide a complete spectrum of tumor progression in proper context.

Normal epithelium can vary significantly in prostatic ducts and ranges from atrophic to resting to hyperplastic, and each has a unique pattern of gene expression. Also, epithelium adjacent to tumor may not be normal, *i.e.*, although phenotypically normal at the level of the light microscope, it may be genotypically abnormal and/or exhibit an altered gene expression profile because of its physical location near the invading tumor. The genetic profile of prostatic intraepithelial neoplasia is closer to tumor than that of normal tissue. Associated inflammation or proximity to the urethra may also have a significant impact (4). An advantage of the three-dimensional analytical approach is the opportunity to study the complete spectrum of tumor progression in proper context. Prostatectomy specimens resected for cancer contain a fascinating set of biological changes, including hyperplasias, dysplasias, and various grades of invasive tumor. Currently, little is known of the genes or pathways that mediate either the formation or progression of tumors, and virtually nothing is known of the role of stromal cells, inflammatory cells, or microenvironment on this process. The three-dimensional reconstruction approach allows the physical and molecular relationship of these entities to be determined.

Furthermore, the characterization of early dysplastic lesions, in particular, may be critical in determining the fundamental molecular events that initiate prostate tumors.

Both cancerous and noncancerous tissues are heterogeneous. Many patients have multiple independent tumors, only some of which will progress to form metastatic disease. Current work at the NCI is focusing on an area on chromosome 8, which is one of the areas of highest abnormality.

A recent publication from University of Michigan has reported results from cDNA microarray analysis, which assessed the molecular profiles of normal prostatic tissue, benign prostatic hypertrophy, localized, metastatic, and hormone refractory adenocarcinoma. Genes encoding for hepsin and Pim1 were significantly correlated with clinical outcome (10).

In hormone refractory prostate cancer, Bubendorf *et al.* (31, 32) used microarrays to analyze what genes patterns were expressed and found IFGBP2 and HSP27 consistently overexpressed.

Additionally microarrays have been used to identify novel prostate-specific proteins such as prostein (33) and biomarkers for chemoprevention in prostate cancer (34). Elek *et al.* (35)

Table 1 Current molecular profiling studies in urological malignancies

Both prostate and bladder tumor specimens are being analyzed using both microarray and proteomic technology by many research groups. This table gives an outline of some of the published data and some of the ongoing projects in both prostate and bladder cancer.

Prostate cancer		Bladder cancer	
Author/Group	Planned project/Results	Author/Group	Planned project/Results
Cole <i>et al.</i> (Ref. 30; NCI group)	NCI three-dimensional model for radical prostatectomy specimens so that molecular profiling and histology and location in the prostate can be correlated.	Vlahou <i>et al.</i> (Ref. 40; Eastern Virginia Medical School, Virginia)	The diagnostic accuracy of urine cytology can be enhanced by using proteomic analysis for specific tumor biomarkers.
Emmert-Buck <i>et al.</i> (Ref. 4; NCI group)	NCI prostate cancer proteomics project	Celis <i>et al.</i> (Ref. 22; University of Aarhus, Denmark)	Outlined the various stages of urothelial squamous differentiation using proteomics
Dhanasekaran <i>et al.</i> (Ref. 10; University of Michigan)	cDNA arrays of normal and neoplastic prostate show hepsin and Pim-1 are important clinical prognostic markers.	Chen <i>et al.</i> (Ref. 36; Tainan, Taiwan)	Microarray-based changes post genistein therapy
Bubendorf <i>et al.</i> (Ref. 31, 32; Basel, Switzerland)	cDNA arrays of normal and neoplastic prostate show hormone refractory disease is associated with IGFBP2 and HSP27 overexpression.	Duggan <i>et al.</i> (Ref. 2; Belfast, Northern Ireland)	Planned project to assess markers of chemoresistance using cDNA arrays

have also reported using microarrays to identify potential prostate specific proteins.

Although each of these studies are interesting in their own right, there are several issues surrounding the data that can be criticized. The focus of many of the studies is still on finding single genes that are involved in cancer progression, whereas the most likely scenario is that multiple genes are involved and significant heterogeneity exists. The other issue relates to accurate and adequate tissue sampling. In prostate cancer patients, radical prostatectomy specimens are ideal for detailed profiling so that peripheral zone tumors can be compared with central zone lesions and extracapsular disease can be compared with organ confined disease. In metastatic and hormone refractory disease, this is more difficult as there will be heterogeneity between primary and metastatic deposit, and widespread sampling is obviously problematic. The data on prostate cancer is preliminary but exciting and provides a basis for additional research. In bladder cancer, Chen *et al.* (36) have examined changes in bladder tumors after genistein therapy using microarrays, and microarrays are already being used to research the molecular basis of allograft rejection in transplant urology (37).

The NCI have several other initiatives as well, including research on the abnormal proteins expressed in prostate cancer (proteomics project) and karyotypic abnormalities in prostate cancer (Cancer Chromosome Abnormality Project).

A summary of the main publications and planned projects, to date, on microarray and proteomics in prostate and bladder cancer is outlined in Table 1.

Discussion

The Potential for Future Study of MP in Genitourinary Cancer

Prostate Cancer. MP has tremendous potential and may hold the key to predicting stage, especially extracapsular exten-

sion, before radical therapy and to predict prognosis and the need for adjuvant therapy. MP in theory should complement the Gleason grading by giving a more objective assessment of the aggressiveness of the disease and also should help elicit the natural history of prostatic intraepithelial neoplasia. An additional potential use is in the diagnosis of imminent hormone resistance, and this relates to Bubendorf *et al.* (31) findings of increased IGFBP2 and HSP27. A question that is often asked by many urologists, both in early stage and metastatic prostate cancer is: what to do in the event of a rising PSA? We would predict that in several years, a molecular profile of the tumor cells will be the clinical test of choice. This will give predictive information on the biological potential of an individual patients tumor.

Bladder Cancer. Ideally, MP would provide a state of the art assessment of urine cytology for bladder tumor cells. This would allow its use as a tool for follow-up of transitional cell carcinoma of the bladder or TCC of the upper urinary tract. This role of MP as an adjunct to cytopathology is interesting and may in the future pave the way for more accurate urine cytology (38).

Potentially, it could help predict progression and time to invasion, thus helping to decide timing of cystectomy. As with prostate cancer, MP of a wide range of bladder tumors would provide information on the spectrum of disease within bladder cancer:

Normal epithelium; no mutations → minimal change

→ Ta → T1 → aggressive disease

Urologists are familiar with the concept that lamina propria invasion, high-grade disease, multiplicity, and size of the presenting tumor are associated with progression. However, combining these histopathological and clinical factors with single

molecular markers is still insufficient to predict accurately which tumors will progress.

Recurrence and progression of bladder cancer is known to be related to several genetic factors such as chromosomal aberrations and p53 mutation (39). A molecular profile predicting progression would be advantageous to patients with aggressive disease. The available evidence would suggest that the tumors with multiple aberrations tend to progress (39).

Use of MP to Direct Therapeutic Intervention. If MP is to offer new opportunities to overcome some of the current limitations in cancer diagnosis and treatment, their added value over traditional approaches must be systematically assessed through transitional studies and clinical studies. The key to the future success of MP is establishing streamlined experimental protocols and standardized quality control procedures so that a strong evidence base can be built to convince clinicians of its use.

The concept of individualization of cancer therapy based on MP is attractive. At present, chemotherapy and radiotherapy are not patient specific and MP could enhance more individual targeting of therapy. Anticancer drug discovery will have an increasing set of targets because of the large expansion in knowledge of molecular alterations in cancer cells. However, it is probably more important to know the order or pattern of the molecular alterations and how each of these affect the tumor cell phenotype. After DNA damage is induced by chemotherapy or radiotherapy, the genotype of a cell has a major influence on the decision-making process. If antiapoptotic proteins predominate or DNA repair is successful, then the tumor cell clone will survive. Apoptosis will only be initiated if there is a drug target interaction followed by triggering of proapoptotic genes within the tumor cells.

One area where genetic modulation may help is with modulation of tumor chemosensitivity. Development of therapeutic agents that are able to revert key oncogenic steps back to an apoptotically sensitive tumor phenotype would give current cancer therapies a much greater potency. The benefits of targeting particular genes in tumor cells is that because of the specificity of the target, only one protein molecule will be eliminated. Also, if we understand the tumorigenesis pathway well enough, we can target the gene therapy at specific steps, *e.g.*, 9q therapy to prevent tumor initiation and p53 therapy to prevent invasion in bladder cancer.

However, all such molecularly specific therapies are limited by several facts: (a) the complex circuitry in tumor cell signaling is only currently being elucidated; (b) uptake strategies and gene inactivation strategies are still in development phase; and (c) redundancy of molecular pathways.

An important goal of at this time should be the MP initiative so that the techniques that will develop facilitate integration of genomic and protein signatures into patient data sets. Such projects will allow a comprehensive analysis of the status of genes and gene products in urological cancer.

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