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Review

Revealing urologic diseases by proteomic techniques

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Abstract

Proteomics, as the study of the proteomes of tissues and body fluids, has recently been introduced as a tool for revealing urologic diseases. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and surface-enhanced laser desorption/ionzation (SELDI) are two techniques used in proteomic studies. Among the many urologic diseases, the malignancies including prostate cancer, bladder cancer, and renal cancer are the subjects most often selected for proteomic analysis. Poor reproducibility is one of the difficulties that must be overcome in order for proteomic technology to be a robust tool.

Keywords: Review; Proteomics; Urologic diseases

Contents

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1. Introduction

Proteomics, as the study of the proteomes of tissues and body fluids, has presented a new methodological avenue for current biology. Whereas traditional biology deals with a single gene or protein of interest, the new theoretical biology analyses a set of genes (the genome) or proteins (the proteome). The proteome is the group of proteins that are encoded by the genome and expressed in the same biological environment. The development of genomic techniques has provided a better understanding of the molecular signatures of diseases, and these techniques have been used to make more accurate clinical prognoses and to divide diseases into subtypes [1]. However, genomic techniques are limited by the necessity to monitor the expression level of proteins (i.e. the activation state of genes) in a network of biological pathways. Thus, proteome analysis is essential in order to obtain knowledge of the current status of molecular events in an organism.

Proteomes are usually compared, between different states such as disease and health, and various combinations of proteomes may be evaluated according to the purpose of study. The techniques of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and surface-enhanced laser desorption/ionization (SELDI) are the most useful tools for proteomics.

The proteomic approach was introduced for the study of urologic diseases relatively recently, and urologic cancers have been the primary targets. First, this article reviews the currently available proteomic techniques and exemplifies a data analysis. This is followed by an overview of the results from proteomic studies of urologic diseases. Finally, perspectives on the future of proteomics related to urologic diseases are discussed.

2. Techniques for proteome expression analysis

2.1. Two-dimensional polyacrylamide gel electrophoresis

O'Farrell described a method of 2D-PAGE in 1975 for the separation of proteins of *Escherichia coli*. This method used a combination of isoelectric focusing (IEF) and the SDS–PAGE system of Laemmli. In this method, protein separation occurs as a result of the pH gradients generated by the synthetic carrier ampholytes, but reproducibility is extremely difficult to control largely because of the non-fixed nature of the pH gradients in the IEF gels. This problem has been overcome by the development of immobilized pH gradient (IPG) IEF [2]. IPG IEF gels are prepared using Immobilines (Amersham Pharmacia Biotech), a series of acrylamide derivatives that form buffers with different p*K* values. The immobilization of the buffering groups (acrylamide derivatives) through covalent attachment to the polyacrylamide backbone occurs during polymerization. Currently, IPG IEF is the method of choice for the first dimension of 2D-PAGE.

There are a variety of sample preparation methods for two-dimensional electrophoresis (2-DE) separation. Body fluids such as serum, plasma, cerebrospinal fluid and urine are soluble, liquid samples that can be separated by 2-DE using a simple solubilization buffer. Protease inhibitors are sometimes added, but it should be remembered that such reagents can modify proteins, leading to charge artifacts. Albumin and immunoglobulins exist as high abundance proteins and thus can obscure many of the minor component proteins. This can be avoided by removing these proteins on an affinity column, but there remains the possibility that the nonspecific deletion of other proteins can occur. Cells cultured on solid substrates can be harvested by scraping and lysed by the addition of solubilization buffer. Tissue samples that have been frozen in liquid nitrogen can be processed in buffer by using a homogenizer. The heterogeneous nature of tissue samples may cause a problem in analyzing proteomes. To overcome this, laser capture microdissection (LCM) can be usefully employed to obtain a pure population of cells from a tissue section. Briefly, a slide of stained tissue is placed under a microscope, and a transfer film is placed onto the tissue. The area of interest is centered in the field of vision, and a laser is then fired to melt the film, which expands into the target tissue and solidifies as it rapidly cools. The area of pure cells can subsequently be retrieved from the surrounding tissue [3].

One of the most difficult challenges to obtaining reproducible, high-resolution separation of proteins is poor solubilization. The best known method for protein solubilization is that originally described by O'Farrell, which uses a mixture of 9.5 M urea, 4% (w/v) of NP-40, 1% (w/v) of dithiothreitol (DTT), and 2% (w/v) of synthetic carrier ampholytes of the appropriate pH range. While this method works well for many types of samples, it is not universally successful, especially with membrane proteins. Many effective detergents and solubilizing agents, such as a CHAPS/urea–thiourea mixture and SDS, are used to improve solubilization.

The development of basic IPGs with pH values up to pH 12 has made it possible to analyze very alkaline proteins, the introduction of narrow-range IPGs has enabled the separation of proteins with high resolution. Recent developments in fluorescent technology will help researchers to quantify proteins more precisely [4]. Proteins extracted from the spots of 2-DE gels can also be identified easily by mass spectrometry (MS)-based protein identification systems.

2.2. Mass spectrometry

Mass spectrometry is an indispensable tool for the identification and quantification of proteins from complex mixtures such as plasma and tissue [5–7]. For this purpose, it is important to separate the maximum number of proteins. A combination of 2-DE and MS has been one of the most widely used strategies. After proteins are separated by 2-DE and quantitated by the intensity of their staining, selected spots are excised, digested, and identified by peptide mass mapping using matrix-assisted laser desorption ionization-

time-of-flight (MALDI-TOF) or peptide sequence analysis using electrospray ionization (ESI)–MS/MS.

A mass spectrometer consists of an ion source, a mass analyzer, and a detector. The ionized analytes in the ion source are separated in the mass analyzer by their mass to charge ratio (m/z) and registered in the detector by the number of ions of each m/z value. Electrospray ionization and matrix-assisted laser desorption ionization methods are currently the principal soft ionization methods for the MS analysis of peptides or proteins. These techniques have been used with high throughput sample preparation techniques such as liquid chromatography (LC). ESI ionize the analytes out of a liquid phase and thus is readily coupled to LC. At a low flow rate of 500 nl/min or less, which is called, a micro- or nano-electrospray, more sensitive MS spectra can be obtained with less sample consumption [8]. MALDI ionize the analytes out of a co-crystallized dry matrix using energy supplied by a laser. The ion trap, quadrupole, time-of-flight, and fourier transform ion cyclotron (FT-MS) are the major methods used for mass analysis in proteomic research; they can be used either alone or in combination for tandem mass (MS/MS) analysis. A diverse combination of ionization methods and mass analyisis has made possible a number of proteomic approaches of differing sensitivities, resolutions, and applications [9].

Proteins are identified by matching experimental to expected mass data from sequence databases. Isolated proteins can be analyzed by identifying the accurate mass of peptides derived from specific enzymatic cleavages, while more complex mixtures of proteins can be identified by the MS/MS spectra of individual peptides. In general, the former method, known as peptide mapping or fingerprinting, uses MALDI-TOF. The latter method provides a higher level of certainty in the identification of proteins, because, in addition to the peptide mass, the peak pattern in the MS/MS spectrum also provides information about peptide sequence. This method therefore generates information about the type and site of modifications.

Large-scale proteome analyses also require high-throughput techniques for searching databases. A multiprocessing algorithm for searching databases has been described which increases the search speed for a large number of spectra. Assembling or filtering algorithms have been developed to analyze search results in order to identify proteins with more confidence [10].

2.3. Surface-enhanced laser desorption/ionization

After matrix-assisted laser desorption/ionization was developed during the late 1980s, surface-enhanced laser desorption/ionization was introduced in the early 1990s. MALDI and SELDI are similar in that both permit a soft ionization of biological molecules such as peptides and proteins. The early, harsh ionization methods could not be used for such fragile biomolecules as they caused too much fragmentation. In addition, both of these techniques are sensitive enough to detect the picomole to fentomole amounts of protein, making them applicable to biological samples requiring highly sensitive analysis. Both MALDI and SELDI involve the spotting of biological samples, pre-mixed with a chemical matrix or an energy-absorbing molecule (EAM), onto a solid surface. After the evaporation of water and solvents from the mixture, the protein samples are placed into the source, which includes a laser of a wavelength that is absorbable by the matrix. The energy absorbed by the EAM is transmitted to nearby proteins and peptides, resulting in the generation of positive ions.

SELDI technology is essentially a modification of the MALDI approach to ionization. SELDI differs from MALDI, however, in that a fraction of proteins from a complex mixture are captured by selective surfaces, whereas all of the proteins are captured in the MALDI technique. Proteins are directly applied to spots on the SELDI ProteinChip® array (Ciphergen Biosystems, Fremont, CA). The spots contain either chemical (anion exchange, cation exchange, reverse phase, or metal affinity) or biochemical (antibody, receptor, DNA, etc.) surfaces designed to capture proteins of interest. The chemical surfaces are employed to capture a class of proteins with the corresponding chemical affinity, and the biochemical surfaces are custom-made by the user to bind the molecule of interest. The bound proteins are treated with wash buffers to remove nonspecifically bound proteins. An energy absorbing molecule such as alpha-cyano-4-hydroxy cinnamic acid (CHCA) or sinapinic acid (SPA) is needed for laser desorption/ionization (LDI) of the proteins on the chip surface. The reader uses pulsed nitrogen laser energy, transmitted through EAM, to ionize proteins from the arrays and measures the mass of each protein species based on its velocity through the time-of-flight analyzer. The SELDI-TOF MS measures the mass-to-charge ratio (m/z) of each protein and quantifies the amount of protein using a detector. Analysis software makes it possible to display protein profiles as a series of peaks or bands.

2.4. Comparison of 2D-PAGE and SELDI

The 2D-PAGE method has been the most widely used protein separation technique. It has the power to separate thousands of proteins simultaneously and to visualize them with a level of sensitivity that makes computer analysis possible. There have been a number of studies on the uniformity and reproducibility of 2-DE gels both within and between laboratories [11]. Nevertheless, it is obvious that many users still experience difficulty in achieving reproducibility. Reproducibility can be defined in terms of both spatial reproducibility (i.e. alignment) and quantitative reproducibility (i.e. intensity). Reproducibility is a critical factor in determining the success or failure of biomarker development. SELDI is known as a good technology for clinical proteomics researchers to use in order to obtain reproducible protein profiles of proteomes. A recent report, however, argues against the complete reproducibility of SELDI [12].

The 2-DE technique has the power to detect a wide range of proteins, depending on their molecular weights (M_r 10,000–150,000). It has some limitations, though, in detecting hydrophobic proteins owing to difficulties with solubilization. SELDI can display only low molecular-weight proteins (M_r 2000–20,000), as relatively small proteins can bind to the chip platform and are ionized more easily than lager ones. SELDI, however, has the ability to detect hydrophobic proteins using hydrophobic ligand-containing chips.

The processes of 2-DE and image analysis require skill and are labor intensive and time consuming. Automation, with which some success has apparently been achieved, will be required to alleviate many of these problems. Conversely, SELDI enables rapid protein profiling of extracts from body fluids, cells, and tissues, and therefore can be used to screen large numbers of samples in a clinical research setting. This advantage is attributable to the rapid processing of the SELDI chip compared with the process of 2-DE.

Protein identification is a significant issue encountered in the SELDI protein profiling approach. The marker proteins and peptides discovered by SELDI analysis are mostly unidentified, and it is not essential that these species are specified at the amino acid-sequence level for the technique to be useful as a diagnostic test [13]. Even so, the identification of the biological nature of the biomarker protein is highly recommended. Pre-fractionation of the sample is a helpful way in which marker proteins can be better identified. On the other hand, protein spots on 2-DE gels can be easily identified using MS-based protein identification techniques.

3. Data analysis for pattern discovery

Various bioinformatics algorithms have been used for the discovery of protein patterns in disease-related samples. These bioinformatics applications are based on the essential proposition that the proteins in a specimen, such as serum or tissue, reflect the status of the disease and therefore have a set of qualitative and quantitative characteristics different from those of the healthy state. A large amount of data is needed to detect differences between the proteome patterns of biological system in healthy and diseased states.

An example of a data analysis for biomarker development is shown here in Table 1. Proteins were selected from a total of 30 serum samples from renal cell carcinoma (RCC) ($n = 15$) and non-RCC ($n = 15$) patients by using strong anion exchange-2 protein (SAX2) chip arrays, and their mass spectra were acquired. The mass data were then randomly divided into two groups, a training set ($n = 24$) and a test set ($n = 6$). A flow diagram can be drawn for the whole analysis process from the protein profiling to the classifier testing (Fig. 1). Peaks were chosen from the crude raw data by using an algorithm with features such as baseline correction, smoothing (or averaging), peak detection, and peak alignment (Fig. 2).

Table 1
Numbers of patients (RCC) and controls (non-RCC) to establish and validate the classification

Sample	RCC	Non-RCC	Total
Training set	12	12	24
Test set	3	3	6

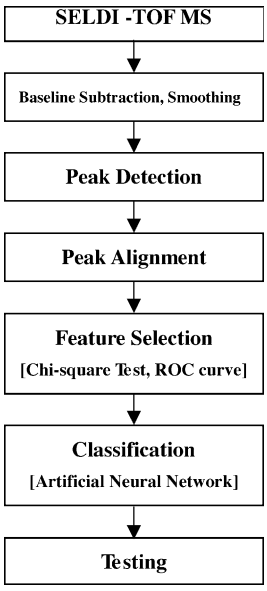

Fig. 1. Flow diagram of the spectrum analysis process.

Before applying artificial intelligence (AI) analysis, a statistical method can be used to select the peaks to be included as inputs for the state classification. This involves ranking the peaks according to their discriminatory power in differentiating between the diseased and healthy states. Computed statistics for the peak ranking, such as the area under the curve (AUC) and the chi-square indicator, were used for the feature selection. The area under the receiver operator characteristic (ROC) curve was computed in order to identify the

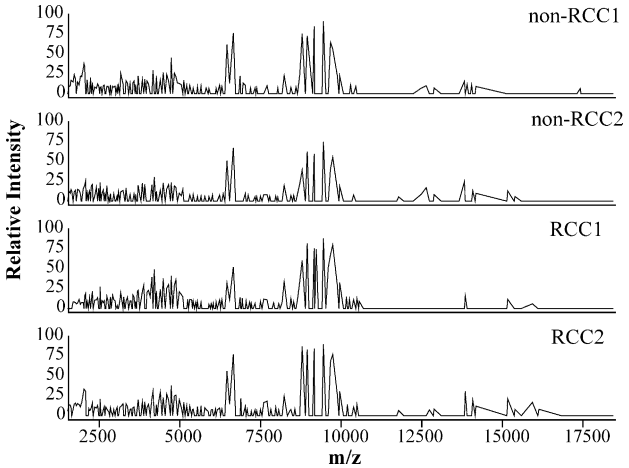

Fig. 2. Examples of the SELDI SAX2 profile of two non-RCC and two RCC samples after baseline subtraction, smoothing (averaging), peak detection, and peak alignment processes.

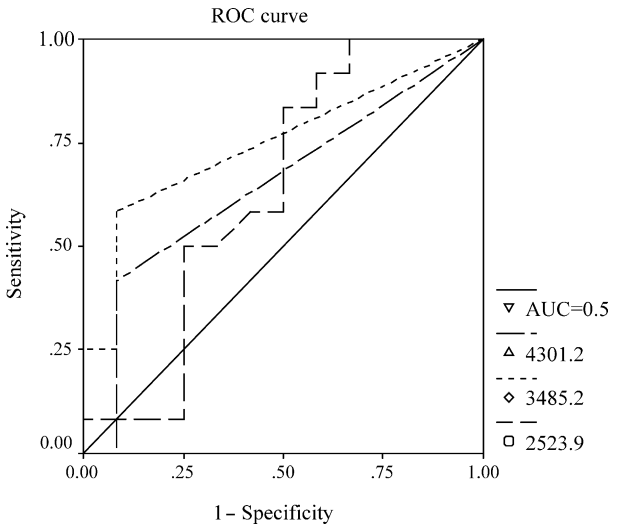

Fig. 3. An example of the receiver operator characteristic (ROC) curve analysis. The ability of the peaks to distinguish between RCC and non-RCC was ranked by the area under the ROC curve (AUC). The peaks with an AUC > 0.63 were used for further artificial neural network (ANN) analysis.

peaks with the highest potential for discrimination between the two groups, based on the assumption that the test results from diseased individuals are more indicative of the disease state than are the test results from non-diseased individuals. When peaks with an AUC < 0.63 were considered to be irrelevant for classification, between three and 12 peaks were identified in each training set (Fig. 3). A 2–2 table for a chi-square test was formed for each input, showing the incidence of peaks against the incidence of cancer in the training set (Table 2). From these, the chi-square indicator for each peak was calculated, and between 2 and 10 peaks were identified with p-value <0.05.

A number of AI algorithms are used for constructing a classification model based on the training data set. Typical algorithms used are decision trees, genetic algorithms with clustering analyses, and neural networks. The validity and accuracy of the classification algorithm is then challenged with a blinded test data set. The sensitivity and specificity of the test is calculated to validate the algorithm. For the example case, two artificial neural networks with back propagation algorithms were constructed with input units that varied according to the results of the feature selection by two statistical methods, five hidden units, and two output units (Fig. 4). Using this neural network, classifications were performed on the training sets, and four independent simulation studies were performed to test this classifier (Table 3).

Table 2
An example of the chi-square test (m/z, 3756.9; $p < 0.05$)

	RCC	Non-RCC	Total
Peak presence	9	2	11
Peak absence	1	8	9
Total	10	10	20

For each peak, the chi-square test for a 2×2 table was used to test the statistical significance of the classification of RCC vs. non-RCC according to peak presence/absence. The peaks with p-values <0.05 were used for further ANN analysis.

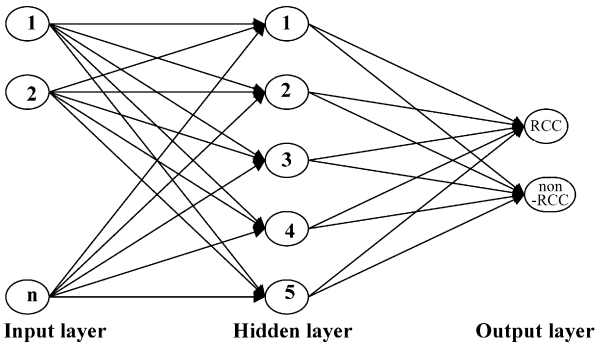

Fig. 4. The architecture of a multi-layer perceptron artificial neural network (ANN). The network was presented with the useful peak data for each of the subjects and was trained using the back-propagation algorithm.

Table 3
Classification results of the training and test sets performed using an artificial neural network with the features as inputs, selected using the ROC curve (AUC > 0.63) and chi-square test (p-value <0.05)

Sample	ROC + ANN		Chi-square test + ANN	
	RCC	Non-RCC	RCC	Non-RCC
(a) Training set				
Study 1	12	12	12	12
Study 2	12	12	12	12
Study 3	12	12	12	12
Study 4	12	12	12	12
Total	48	48	48	48
%	100	100	100	100
(b) Test set				
Study 1	2	2	3	2
Study 2	3	2	3	3
Study 3	1	2	2	3
Study 4	2	1	0	2
Total	8	7	8	10
%	66.67	58.33	66.67	83.33

Finally, in order to make use of this result on a practical platform such as a protein chip, the newly defined biomarker must be biologically validated. This may involve measuring the amount of the protein using a conventional technique such as ELISA or immunochemistry.

4. Proteomic studies of urologic diseases

4.1. Prostate cancer

Prostate cancer is one of the most commonly diagnosed cancers in men throughout the world. Early detection is essential for saving the lives of patients suffering from this malignancy because the success rate of surgery as a treatment for advanced prostate cancer is poor. Currently, the standard method for detecting prostate carcinoma involves

screening for elevated blood levels of prostate-specific antigen (PSA), digital rectal examination, and biopsy of the prostate. Although the measurement of PSA in serum has enhanced the detection rate of prostate cancer, there is uncertainty about its specificity: the specificity in differentiating prostate cancer from benign prostate hyperplasia (BPH) is only 25–30% for PSA concentrations in the range of 4–10 ng/ml. Analyses using 2D-PAGE have led to the discovery of a list of potential diagnostic markers for prostate cancer. Research to explore the clinical implications of their potential as diagnostic markers has progressed so slowly, however, that there is no robust system available for practical use.

A 2D-PAGE analysis of the prostatic fluid from prostate carcinoma patients ($n = 6$) revealed that prostatic acid phosphatase is elevated in BPH and normal controls but is not detected in cancer patients [14]. Some nuclear matrix proteins (NMPs) were shown to be differentially present in prostate cancer tissues when compared with BPH and normal controls [15]. Among those proteins, a M_r 56,000 protein (pI 6.58), designated PC-1, appeared in all prostate cancer specimens ($n = 14$), while it was not detected in any normal prostate ($n = 13$) or BPH tissues ($n = 14$). An antibody against PC-1 was developed, which was validated using an immunohistochemical method with frozen prostate cancer tissue [16]. When 2D-PAGE coupled with MS was used to analyze the proteins from prostate cancer tissues ($n = 10$) and benign controls ($n = 9$), several candidate proteins showed a difference in abundance: tropomyosin 1 (TM1) and prostatic acid phosphatase (PAP) were decreased; heat shock protein 70 (HSP70) was increased [17].

Prostate-specific membrane antigen (PSMA) is a M_r 100,000 protein which was initially identified from the human prostate cancer cell line LNCaP [18]. This protein, known to be expressed predominantly in prostate tissue, is down-regulated in BPH and up-regulated in prostate cancer [19–23]. It is therefore important to develop a sensitive immunoassay method for quantitation of this protein. Some developments which have proved to be successful include a fluorescence-based sandwich assay to measure the amount of PSMA in tissue [24] and a new detection technology using SELDI MS for the quantitative immunoassay of PSMA [25]. In this study, ProteinChip arrays were used to measure and compare serum PSMA levels in healthy men and patients with either BPH or prostate cancer. PSMA was captured by anti-PSMA on a ProteinChip array and was detected by SELDI-MS; the level of PSMA was quantitated by comparing the peak intensity to a standard curve established using purified recombinant PSMA [26]. According to the data presented in this study, the average PSMA value for prostate cancer (623.1 ng/ml; $n = 17$) was significantly different from that for BPH (117.1 ng/ml; $n = 10$) and all of the samples with a PSA value between 4 and 10 ng/ml were correctly diagnosed as either BPH or prostate cancer.

The mechanism whereby normal cells are transformed into malignant cells can best be understood in in vivo tissue. This is a challenge because it is difficult to obtain relevant cell populations (i.e. normal, prostate intraepithelial neoplasia, and prostate cancer) using the conventional method of homogenizing bulk tissue. Laser capture microdissection, however, allows for the selection of the pure cells of interest, as it is possible to microdissect the cells from frozen tissue sections using the LCM microscope. Some reports claim that LCM cell lysates of prostate cancer display specific protein patterns [27–29]. In one study, pure organ-matched cell populations were obtained using LCM from nine prostectomy specimens and were analyzed by SELDI MS [28]. The relative intensity levels of the protein peaks were then compared among the cell population groups. Statistical analysis was performed using the seven most differentially expressed peaks. High sensitivity and specificity for prostate intraepithelial neoplasia (PIN) and prostate cancer were obtained. One protein from the seven markers was identified as prostate specific antigen.

The serum is believed to reflect the biological state of the human body in the healthy or diseased condition. It has therefore been a good specimen to screen for prostate cancer markers. One of the most notable serum markers is PSA, which cannot be used as a single marker for the detection of early prostate cancer owing to low specificity, as mentioned above. Recently, combining serum protein profiling with artificial intelligence analysis has resulted in the identification of a panel of cancer markers with high sensitivity and specificity. Petricoin et al. trained a genetic algorithm with cluster analysis using a training set ($n = 56$) of known cases, and then tested the discovered pattern against a blinded sample set ($n = 266$) which included prostate cancer and benign diseases (BPH and/or prostatitis; PSA level <4, 4–10, >10 ng/ml) [30]. The proteomic pattern correctly predicted 36 of 38 patients (95%) with prostatic cancer, and 177 of 228 patients (78%) with benign diseases were correctly classified. Most importantly, benign conditions with a PSA level of 4-10 ng/ml were correctly classified in 97 of 137 patients (71%). To put this relatively low specificity in perspective, it has to be taken into account that more than 20% of subjects diagnosed as cancer-free on first biopsy were found to have cancer on a subsequent biopsy [31,32]. Wright et al. [33] showed another encouraging result in the discovery of tumor markers for prostate cancer. They used a decision tree algorithm [34] to train a training data set (167 prostate cancer; 77 BPH; 82 normal) and then tested the trained pattern using a test data set (30 prostate cancer; 15 BPH; 15 normal). A statistical method (i.e. AUC) was used for selecting peaks with a high discriminating power before applying the decision tree analysis. This feature selection process is believed to help train the algorithm more completely. The sensitivity and specificity for prostate cancer versus non-cancer (BPH and normal) groups were 83 and 97%, respectively. With the same training and test data sets, a boosted decision tree analysis was performed, and a result of 100% sensitivity and specificity was obtained [35].

4.2. Bladder cancer

Bladder cancer is the fourth most common malignancy in males, the tenth most common malignancy in females, and the second most common tumor of the urinary tract [36]. With regard to the types of tumors involved, transitional cell carcinoma (TCC) represents >90% of bladder cancers. Approximately 75% of patients have superficial tumors (T_a, T_1), 20% have invasive tumors (T_2–T_4), and 5% have metastasized tumors at the time of diagnosis [37]. The recurrence rate of treated tumors is >70%, and tumor progression to a higher stage or grade develops in 42% of patients after 10 years [38]. The diagnosis is problematic because of the nonspecific nature of the most prevalent symptom of hematuria, which is found in only 4–10% of bladder cancer cases [39].

Cystoscopy represents the gold standard for the detection and monitoring bladder tumors and has a sensitivity of about 70% [40]. This procedure is also useful for resection of tumors and provides specimens for the pathological evaluation of prognostic factors. However, cystoscopy is invasive, painful, and costly, and therefore it is not suitable as a screening test. Urine cytology is a technique for microscopically detecting malignant cells in urine that have detached from a site of bladder cancer; this screening method has a specificity of >90% for TCC diagnosis [41]. However, it has a sensitivity of 20–40% for low-grade and 80% for high-grade tumors [42]. Despite high specificity, the low sensitivity for low-grade tumors allows urine cytology to be used only as an adjunct to cystoscopy.

Research on methods for the early detection of bladder tumors has identified a variety of potential markers. The NMP22 test, an enzyme immunoassay that detects NMP22 in urine, has a sensitivity of >68% [43,44] and a specificity of >61% [45,46]. The fibrin/fibrinogen degradation product (FDP) test recognizes urinary FDP and has a sensitivity of 82% [47] and a specificity of >86% [48]; however, the FDP test is known to lack reproducibility owing to instability in the manufacturing process [49]. The bladder tumor antigen (BTA) test [50] is a latex agglutination test that detects basement membrane complexes in urine. The BTA stat [51] and BTA TRAK [52] are modifications of the BTA test that detects a human complement factor H-related protein (hCFHrp). The best result reported among the BTA series was that of the BTA stat which gave >80% sensitivity and >72% specificity. The telomerase activity test detects the telomerase activity of bladder tumors in urine and has been reported to have a sensitivity of >85% [53,54] and a specificity of 80% [55]. Hyaluronidase and hyaluronic acid are the enzyme and end product generated by cancer cells during the dissolution of the cellular matrix [56]; the reported sensitivity and specificity of both is >86% [57]. The cell surface antigen (ImmunoCyt®) test uses three monoclonal antibodies to detect cell surface antigens of bladder tumors. With this test, both immunostaning and urine cytology can be carried out on the same slide. This combination test has achieved a sensitivity of >86% and a specificity of 90% [58]. In general, all of the biomarkers mentioned above have higher sensitivity and lower specificity than urine cytology, which implies that the strategy of approaching disease detection with a combination of candidate markers would be the best way to discover the most applicable marker.

An extensive 2D-PAGE study on the protein expression profiles of bladder tumors including transitional cell carcinoma and squamous cell carcinoma (SCC) has been implemented by Celis et al. [59–61]. By examining more than 63 TCC cases, they identified four proteins that are expressed in normal urothelium and low-grade TCCs but not in high-grade TCCs. These are adipocyte-type fatty acid binding protein (A-FABP), glutathione *S*-transferase (GST-μ), prostaglandin dehydrogenase (PGDH), and keratin 13 [59]. Six of 150 bladder tumors were diagnosed as SCC, and their proteome analyses showed different expression patterns that depended on the degree of differentiation (i.e. more or less differentiated) [60]. All six SCC patients secreted the protein psoriasin in the urine although this protein was identified only in frozen sections of the more-differentiated SCCs [60]. Moreover, nonkeratinizing metaplastic lesions that exhibit a spectrum of abnormalities were identified and analyzed immunohistochemically using antibodies against the proteins differentially expressed between normal urothelium and SCCs [61].

One SELDI study for the proteome analysis of urine from TCC patients has been performed in a relatively wide range of participants ($n = 94$: 30 TCC, 34 normal, 30 benign urologic diseases) [62]. Among the five potential markers that were expressed differentially in TCCs, one was identified by SELDI immunoassay as defensin. Using an individual marker or a combination of markers, a sensitivity of 43–87% and a specificity of 66–86% have been achieved [62].

4.3. Renal cancer

Although renal cell carcinoma has a relatively low incidence in the general population (8.9 cases per 100,000 people/year), it is one of the most lethal urologic cancers. More than 40% of patients with RCC die, in contrast to the lower mortality rates (20%) associated with prostate and bladder carcinomas [63]. Many RCC masses remain asymptomatic and nonpalpable until they are advanced. Prognosis is mainly related to stage, with a 5-year survival rate of >90% in stage I disease, but only 2–32% for stage IV [64,65]. Currently, there is no satisfactory tumor marker for RCC screening, and early diagnosis relies mainly on unrelated radiographic screening such as abdominal ultrasound, computerized tomography, and magnetic resonance imaging [66].

Some serologic markers such as serum ferritin [67], erythropoietin [68], calcium [69], and renin [70] have been used as determinants of prognosis. Recently, molecular and genomic markers have also been investigated. These include proliferating cell nuclear antigen, Ki-67, silver staining nucleolar organizing regions, cytogenetic alterations, nuclear morphometry, P glycoprotein, p53 and Myc mutations,

β-2 microglobulin levels, interleukin-6, γ-enolase, and E-cadherin. These markers are potentially useful for estimating the biological aggressiveness of a given tumor, but are limited by low specificity and thus are of little use in diagnosing RCC [71–73].

The proteomes of tumor and matched nontumorous kidney tissue from an RCC patient were compared by 2D-PAGE and subsequent immunoblotting with the autologous serum [74]. The autologous serum was allowed to react with proteins in 2-DE gels of tumor and matched normal tissues. Five spots on the tumor protein gel were exclusively reactive with the serum antibody, but the same spots from the control tissue were not reactive. Two of these spot proteins were identified as smooth muscle protein-22-alpha (SM22-α) and carbonic anhydrase I (CAI) [74]. The 2D-PAGE of RCC tissues revealed that five monomeric and two multimeric isoforms of manganese superoxide dismutase (Mn-SOD) were present. In contrast, three monomeric and two multimeric isoforms were not present in normal kidney tissues [75]. Another study on the proteins differentially expressed between RCC and control kidney tissues ($n = 12$) found that four proteins were diminished in tumors [76]; these were identified as enoyl-coA hydratase, α-glycerol-3-phosphate dehydrogenase, aldehyde dehydrogenase I, and aminoacylase-I. Immunoblotting of proteins from RCC and normal kidney epithelium cell lines was carried out with sera obtained from RCC patients and from healthy donors [77]. Distinct differences in antibody reactivity for heat shock protein (HSP) could be detected between the sera from RCC patients and that from healthy controls. A new technique of single gel comparison, in which proteins from an RCC cell line are mixed with radiolabeled proteins from another RCC cell line, has also been introduced [78]. The levels of cytokeratin 8, stathmin, and vimentin on 2D-gels are significantly different between RCC and normal kidney epithelium cell lines, but their histochemical expression patterns are heterogeneous [79]. By screening RCC patients for anti-tumor auto-antibody responses, a number of tumor antigens have been identified in patients, and their relative expression levels have been determined in tumor tissue compared with normal tissue ($n = 6$) [80,81]. The major proteins that are up-regulated in RCC tissue include annexins I and IV, thymidine phosphorylase (TP), triosephosphate isomerase-1, Mn-superoxide dismutase and major vault protein (MVP) [80]. A study of changes in the protein expression profiles after anti-tumor treatment of RCC cells [82] found that many proteins are up- or down-regulated upon treatment with G250 or an anti-RCC antibody. Some of these proteins matched the immunoreactive proteins previously identified by proteome analysis in combination with immunoblotting using sera. When compared with normal renal tissue, the expression of human agmatinase in RCC ($n = 8$) was reduced, as demonstrated by RT-PCR, Western blotting, and immunochemistry [83]. The human urinary proteome was examined by 2-DE after first concentrating urine and removing most of the highly abundant albumin and immunoglobulin G [84]. Approximately 420 protein spots from urine samples were identified by MALDI-MS and LC–MS/MS. In the same report, a preliminary study was performed on the urinary proteomes of low-grade RCC patients before ($n = 6$) and after ($n = 5$) surgery. Three proteins (retinal binding protein, carbonic anhydrase I, and β-2-microglobulin) were increased in abundance and two (mannan-binding lectin serine protease 2, and kininogen) were decreased. By 2-DE gel analysis of the proteomes of RCC and normal kidney tissue ($n = 12$), annexin IV was identified as being up-regulated in tumor cells [85]; this was further characterized by RT-PCR, immunochemistry, and a functional study (Table 4).

The SELDI technique was introduced to determine whether reproducible protein patterns could be identified in archival cytology material for potential diagnostic purposes [86]. In the study, 13 of 15 samples, which included RCC, were identified correctly by their protein pattern. The clinical utility of SELDI profiling in conjunction with neural-network analysis was investigated with urine samples from RCC patients and controls [12]. Samples from patients with RCC ($n = 48$), patients with benign urological diseases ($n = 20$), and normal healthy volunteers ($n = 38$) were used to train neural network models. Using an initial blind group of samples (12 RCC, 9 benign, and 11 healthy) to test the models, sensitivities and specificities of 81.8–83.3% were achieved. However, subsequent testing 10 months later with a different blind group of samples (36 RCC, 13 benign, and 31 healthy) resulted in lower sensitivities and specificities (41.0–76.6%). Factors such as changing laser performance and batch (chip) variability were evaluated to determine whether these contributed to the test results. Another study of SELDI profiling and artificial intelligence analysis (i.e. a decision tree algorithm) of serum samples from RCC patients ($n = 15$) and controls ($n = 21$) [87] found that five independent simulation studies showed a sensitivity and specificity of 85.7 and 86.7%, respectively, for RCC.

5. Perspectives

The application of proteomics to the early diagnosis of urologic diseases and their monitoring is a difficult challenge. Owing to the progress in related techniques, the process of biomarker development using proteomics has recently been introduced. Until now, many proteomic technologies, such as 2D-PAGE, SELDI, isotope-coded affinity tags (ICAT), free flow electrophoresis (FFE), and two-dimensional protein fractionation (PF-2D), have been introduced for clinical proteome profiling studies; however, their usefulness is still limited. Thus, the development of new technologies is necessary for the study of protein expression and function.

The 2-DE technique is a powerful method of analysis which can simultaneously resolve up to several thousand proteins. In addition, new methods for the automated characterization of proteins resolved by 2-DE have been developed and are continuously being improved. The automation of 2-DE

Table 4
Major proteins up- or down-regulated in cancer specimens compared with normal specimens

Specimen	Up-regulated	Down-regulated
Prostate cancer		
Prostatic fluid		Prostatic acid phosphatase [14]
Serum	Prostate specific antigen [25]	
	Prostate specific membrane antigen [25]	
Tissue	PC-1 [15,16]	Tropomyosin 1 [17]
	Heat shock protein 70 [17]	Prostatic acid phosphatase [17]
	Prostate specific membrane antigen [19–24]	
	PCa-24 [27]	
Bladder cancer		
Urine	Nuclear matrix protein 22 [43–46]	
	FDP [47,48]	
	BTA stat [51], BTA TRAK [51]	
	Telomerase activity [53–55]	
	Hyaluronic acid and hyaluronidase [57]	
	Psoriasin [60]	
	Defensin [62]	
Tissue		Adipocyte-type fatty acid binding protein (A-FABP) [59]
		Glutathione *S*-transferase (GST-μ) [59]
		Prostaglandin dehydrogenase (PGDH) [59]
		Keratin 13 [59]
Renal cancer		
Serum	Ferritin [67], erythropoietin [68], rennin [70]	
Tissue	Mn-superoxide dismutase [75,80]	Enoyl-coA hydratase [76]
	Cytokeratin 8 [79]	α-Glycerol-3-phosphate dehydrogenase [76]
	Major vault protein [80]	Aldehyde dehydrogenase I [76]
	Thymidine phosphorylase [80]	Aminoacylase-I [76]
	Annexin IV [80,85]	Stathmin [79]
	Annexin I [80]	Vimentin [79]
	Triosephosphate isoisomerase (1) [80]	Lactate dehydrogenase H-chian [80]
		Agmatinase [83]

gel image analysis, protein spot excision, and protein identification by MS-based methods is under development. The rapidly growing commercial interest in this field is responsible for the production of high-performance image analysis, spot-picking robots, and on-line MS/MS analysis technologies.

Multidimensional LC and MS/MS spectrometry, mainly interfaced by ESI, is a new strategy for protein identification [88]. The digestion of proteins creates a hugely complex mixture of peptides, making the resolution of the peptides by high-performance separation techniques necessary prior to entering the MS/MS spectrometer. Various combinations of separation schemes for multiple fractionation have been explored. At present, two-dimensional chromatographic separation, consisting of strong cation exchange and reverse-phase C18 chromatographic methods, is often used for the separation of peptide mixtures, which are frequently pre-fractionated by protein separation method such as 1DE or size exclusion chromatography.

A mixture of peptides from the same proteins of different origins can also be quantitated by the stable-isotope dilution method [89]. The stable isotope tags are introduced to proteins via metabolic labeling, enzymatic transference, or chemical reactions.

For an MS-based protein profiling technique such as SELDI to be used more widely and safely, an even and uniform platform (chip) surface is essential so that reproducible data are obtained. AnchorChip technology is a new MALDI sample preparation technique [90]; the platform is composed of a plain, metal plate that is used for concentrating the matrix/analytes onto a small spot. This technology can be combined with magnetic bead fractionation and high-performance MADI-TOF MS to produce protein profiles.

Recently, proteomics has been accepted as a useful tool in pharmaceutical research and toxicology. Proteome databases can be queried for changes in the concentrations of proteins presenting responses to a group of pharmaceuticals or toxic substances. Such proteins are useful as markers for specific responses. The power of marker proteins in detecting the diagnostic features of a protein expression profile depends largely on the quality of the proteome database. Many pharmaceutical companies have constructed comprehensive databases regarding the molecular effects of drugs. They also offer a service by which anyone can have access to the database. This kind of proteomics application will be expanded to also include urologic diseases.

6. Conclusion

The proteomic approach to revealing urologic diseases, including prostate cancer, bladder tumor, and renal cancer, has only recently been introduced. Many biological targets remain to be studied using this new technology. The 2D-PAGE method coupled with MS-based protein identification is a very powerful tool for proteomic analysis. The proteomes of malignant and benign samples from various urologic diseases have been compared using the 2D-PAGE technique; the disease specific proteins have been identified, and their expressions have been assessed. SELDI, an MS-based protein profiling approach, has been demonstrated to provide a high-throughput technology for urologic proteomics research. Computational analyses of the protein profiles from patients have resulted in the discovery of biomarkers. However, obstacles remain to be overcome for the further development of current technologies for proteome analysis.

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References

[1] L.M. Staudt, Annu. Rev. Med. 53 (2002) 303.
[2] B. Bjellqvist, K. Ek, P.G. Righetti, E. Gianazza, A. Gorg, R. Westermeier, W. Postel, J. Biochem. Biophys. Methods 6 (1982) 317.
[3] N.L. Simone, R.F. Bonner, J.W. Gillespie, M.R. Emmert-Buck, L.A. Liotta, Trends. Genet. 14 (1998) 272.
[4] W.F. Patton, Electrophoresis 21 (2000) 1123.
[5] R. Aebersold, M. Mann, Nature 422 (2003) 198.
[6] D. Lin, D.L. Tabb, J.R. Yates, Biochim. Biophys. Acta 1646 (2003) 1.
[7] P.L. Ferguson, R.D. Smith, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 399.
[8] M. Wilm, M. Mann, Anal. Chem. 68 (1996) 1.
[9] R.J. Simpson, Proteins and Proteomics, Cold Spring Harbor Press, New York, 2002, p. 425.
[10] D.L. Tabb, W.H. McDonald, J.R. Yates, J. Proteome Res. 1 (2002) 21.
[11] J.M. Corbett, M.J. Dunn, A. Posch, A. Gorg, Electrophoresis 15 (1994) 1205.
[12] M.A. Rogers, P. Clarke, J. Noble, N.P. Munro, A. Paul, P.J. Selby, R.E. Banks, Cancer Res. 63 (2003) 6971.
[13] K.P. Rosenblatt, P.B. Greenwood, J.K. Killian, A. Mehta, D. Geho, V. Espina, E.F. Petricoin, L.A. Liotta, Annu. Rev. Med. 55 (2004) 97.
[14] J. Guevara Jr., B.H. Herbert, C. Lee, Cancer Res. 45 (1985) 1766.
[15] A.W. Partin, R.H. Getzenberg, M.J. Carmichael, R. Szaro, A. Oreper, A. Wiesbrock, J. Meyer, D.S. Coffey, J.I. Epstein, Cancer Res. 53 (1993) 744.
[16] A.W. Partin, J.V. Briggman, E.N.P. Subong, R. Szaro, A. Oreper, S. Wiesbrock, J. Meyer, D.S. Coffey, J.I. Epstein, Urology 50 (1997) 800.
[17] A.A. Alaiya, M. Oppermann, J. Langridge, U. Roblick, L. Egevad, S. Brandstedt, M. Hellstrom, S. Linder, T. Bergman, H. Jornvall, G. Auer, Cell. Mol. Life Sci. 58 (2001) 307.
[18] J.S. Horoszewicz, E. Kawinski, G.P. Murphy, Anticancer Res. 7 (1987) 927.
[19] J.K. Troyer, M.L. Beckett, G.L. Wright Jr., Int. J. Cancer 62 (1995) 552.
[20] G.L. Wright, C. Haley, M.L. Beckett, P.F. Schellhammer, Urol. Oncol. 1 (1995) 18.
[21] D.G. Bostwick, A. Pacelli, M. Blute, P. Roche, G.P. Murphy, Cancer 82 (1998) 2256.
[22] D.A. Silver, I. Pellicer, W.R. Fair, W.D.W. Heston, C. Cordon-Cardo, Clin. Cancer Res. 3 (1997) 81.
[23] M. Kawakami, J. Nakayama, Cancer Res. 57 (1997) 2321.
[24] R.L. Sokoloff, K.C. Nkorton, C.L. Gasior, K.M. Marker, L.S. Grauer, Prostate 43 (2000) 150.
[25] Z. Xiao, B.L. Adam, L.H. Cazares, M.A. Clements, J.W. Davis, P.F. Schellhammer, E.A. Dalmasso, G.L. Wright, Cancer Res. 61 (2001) 6029.
[26] Z. Xiao, X. Jiang, M.L. Beckett, G.L. Wright, Protein Expres. Purif. 19 (2000) 12.
[27] Y. Zheng, J.Y. Xu, B. Ye, Lei, M.H. Weinstein, M.P. O'Leary, S.C. Mok, B.C.S. Liu, J.P. Richie, Cancer 98 (2003) 2576.
[28] L.H. Cazares, B.L. Adam, M.D. Ward, S. Nasim, P.F. Schellhammer, O.J. Semmes, G.L. Wright Jr., Clin. Cancer Res. 8 (2002) 2541.
[29] J. Pannek, A.W. Partin, Semin. Urol. Oncol. 16 (1998) 100.
[30] E.F. Petricoin III, D.K. Ornstein, C.P. Paweletz, A. Ardekani, P.S. Hackett, B.A. Hitt, A. Velassco, C. Trucco, L. Wiegand, K. Wood, C.B. Simone, P.J. Levine, W.M. Linehan, M.R. Emmert-Buck, S.M. Steinberg, E.C. Kohn, L.A. Liotta, J. Natl. Cancer. Inst. 94 (2002) 1576.
[31] C.A. Macintosh, M. Stower, N. Reid, N.J. Maitland, Cancer Res. 58 (1998) 23.
[32] C.L. Masters, K. Beyreuther, BMJ 316 (1998) 446.
[33] B.L. Adam, Y. Qu, J.W. Davis, M.D. Ward, M.A. Clements, L.H. Cazares, O.J. Semmes, P.F. Schellhammer, Y. Yasui, Z. Feng, G.H. Wright Jr., Cancer Res. 62 (2002) 3609.
[34] L. Breiman, J.H. Friedman, R.A. Olsen, C.J. Stone, Classification and Regression Trees, Wadsworth International Group, Belmont, 1984.
[35] Y. Qu, B.L. Adam, Y. Yasui, M.D. Ward, L.H. Cazares, P.F. Schellhammer, Z. Feng, O.J. Semmes, G.L. Wright Jr., Clin. Chem. 48 (2002) 1835.
[36] S.L. Johansson, S.M. Cohen, Semin. Surg. Oncol. 13 (1997) 291.
[37] E.M. Messing, T.B. Young, V.B. Hunt, K.W. Gilchrist, M.A. Newton, L.L. Bram, W.J. Hisgen, E.B. Greenberg, M.E. Kuglitsch, J.D. Wegenke, Urology 45 (1995) 387.
[38] H.W. Herr, World J. Urol. 15 (1997) 84.
[39] G.D. Grossfeld, P.R. Carroll, Urol. Clin. N. Am. 25 (1998) 661.
[40] M. Kriegmair, R. Baumgartner, R. Knuchel, H. Stepp, F. Hofstadter, A. Hofstetter, J. Urol. 155 (1996) 105.
[41] R.A. Badalament, D.K. Hermansen, M. Kimmel, H. Gay, H.W. Herr, W.R. Fair, W.F. Whitmore Jr., M.R. Melamed, Cancer 60 (1987) 1423.
[42] M. Burchardt, T. Burchardt, A. Slabsigh, A.D.L. Taille, M.C. Benson, I. Sawczuk, Clin. Chem. 46 (2000) 595.
[43] R. Berezney, J. Cell. Biochem. 47 (1991) 109.
[44] C. Zippe, L. Pandrangi, A. Agarwal, J. Urol. 161 (1999) 62.
[45] V. Serretta, D.L. Presti, P. Vasile, E. Gange, E. Esposito, I. Menozzi, Urology 52 (1998) 793.
[46] N. Miyanaga, H. Akaza, S. Ishikawa, M. Ohtani, R. Noguchi, K. Kawai, K. Koiso, M. Kobayashi, A. Koyama, T. Takahashi, Eur. Urol. 31 (1997) 163.

[47] B. Johnston, A. Morales, L. Emerson, M. Lundie, J. Urol. 158 (1997) 2098.
[48] B.S. Schmetter, K.K. Habicht, D.L. Lamm, A. Morales, N.H. Bander, H.B. Grossman, M.G. Hanna Jr., S.R. Silberman, B.T. Butman, J. Urol. 158 (1997) 801.
[49] S. Ramakumar, J. Bhuiyan, J.A. Besse, S.G. Roberts, P.C. Wollan, M.L. Blute, D.J. O'Kane, J. Urol. 161 (1999) 388.
[50] M.F. Sarosdy, World J. Urol. 15 (1997) 103.
[51] M.F. Sarosdy, M.A. Hudson, W.J. Ellis, M.S. Soloway, R.D. White, J. Sheinfeld, M.V. Jarowenko, P.F. Schellhammer, E.W. Schervish, J.V. Patel, G.W. Chodak, D.L. Lamm, R.D. Johnson, M. Henderson, G. Adams, B.A. Blumenstein, K.R. Thoelke, R.D. Pfalzgraf, H.A. Murchison, S.L. Brunelle, Urology 50 (1997) 349.
[52] W.J. Ellis, B.A. Blumenstein, L.M. Ishak, D.L. Enfield, Urology 50 (1997) 882.
[53] K. Yoshida, T. Sugino, H. Tahara, A. Woodman, J. Bolodeoku, V. Nargund, G. Fellows, S. Goodison, E. Tahara, D. Tarin, Cancer 79 (1997) 362.
[54] H. Kinoshita, O. Ogawa, Y. Kakehi, M. Mishina, K. Mitsumori, N. Itoh, H. Yamada, T. Terachi, O. Yoshida, J. Natl. Cancer Inst. 89 (1997) 724.
[55] E. Kavaler, J. Landman, Y. Chang, M.J. Droller, B.C.S. Liu, Cancer 82 (1998) 708.
[56] T.B. Csoka, G.I. Frost, R. Stern, Invasion Metastasis 17 (1997) 297.
[57] H.T. Pham, N.L. Block, V.B. Lokeshwar, Cancer Res. 57 (1997) 778.
[58] C. Mian, A. Pycha, H. Wiener, A. Haitel, M. Lodde, M. Marberger, J. Urol. 161 (1999) 1486.
[59] J.E. Celis, M. Ostergaard, B. Basse, A. Celis, J.B. Lauridsen, G.P. Ratz, I. Andersen, B. Hein, H. Wolf, T.F. Orntoft, H.H. Rasmussen, Cancer Res. 56 (1996) 4782.
[60] M. Ostergaard, H.H. Rasmussen, H.V. Nielsen, H. Vorum, T.F. Orntoft, H. Wolf, J.E. Celis, Cancer Res. 57 (1997) 4111.
[61] J.E. Celis, P. Celis, M. Ostergaard, B. Basse, J.B. Lauridsen, G. Ratz, H.H. Rasmussen, T.F. Orntoft, B. Hein, H. Wolf, A. Celis, Cancer Res. 59 (1999) 3003.
[62] A. Vlahou, P.F. Schellhammer, S. Medrinos, K. Patel, F.I. Kondylis, L. Gong, S. Nasim, G.L. Wright Jr., Am. J. Pathol. 158 (2001) 1491.
[63] S.H. Landis, T. Murray, S. Bolden, P.A. Wingo, Can. Cancer J. Clin. 49 (1999) 8.
[64] A.J. Pantuck, A. Zisman, A.S. Belldegrun, J. Urol. 166 (2001) 1611.
[65] L.S. Zellos, D.J. Sugarbaker, Semin. Oncol. 29 (2002) 41.
[66] O. Kessler, E. Mukamel, H. Hadar, G. Gillon, M. Konechezky, C.J. Servadio, Surg. Oncol. 57 (1994) 201.
[67] A. Essen, H. Ozen, A. Ayhan, A. Ergen, C. Tasar, F. Remzi, J. Urol. 145 (1991) 1134.
[68] G. Sufirn, E.A. Mirand, R.H. Moore, T.M. Chu, G.P. Murphy, J. Urol. 117 (1977) 433.
[69] A. Gotoh, S. Kitazawa, Y. Mizuno, A. Takenaka, S. Arakawa, O. Matsumoto, R. Kitazawa, T. Fujimori, S. Maeda, S. Kamidono, Cancer 71 (1993) 2803.
[70] G. Sufrin, S. Chasan, A. Golio, G.P. Murphy, Semin. Urol. 7 (1989) 158.
[71] A. Novick, S. Campbell, P. Walsh, A. Retik, E.D. Vaughn, A. Vine, Campbell's Urology, WB Saunders, Philadelphia, 2002, Chapter 75.
[72] R.O. Northway, C. Ritenour, F.F. Marshall, AUA Update Ser. (2001) 193.
[73] T. Shimazui, E. Oosterwijk, H. Akaza, P.P. Bringuier, E. Ruijter, H.V. Berkel, J.O. Wakka, A.V. Bokhoven, F.M.J. Debruyne, J.A. Schalken, Clin. Cancer Res. 4 (1998) 2419.
[74] C.S. Klade, T. Voss, E. Krystek, H. Ahorn, K. Zatloukal, K. Pummer, G.R. Adolf, Proteomics 1 (2001) 890.
[75] C. Sarto, C. Deon, G. Doro, D.F. Hochstrasser, P. Mocarelli, J.C. Sanchez, Proteomics 1 (2001) 1288.
[76] S. Balabanov, U. Zimmermann, C. Protzel, C. Scharf, K.J. Klebingat, R. Walther, Eur. J. Biochem. 268 (2001) 5977.
[77] R. Lichtenfels, R. Kellner, J. Bukur, J. Beck, W. Brenner, A. Ackermann, B. Seliger, Proteomics 2 (2002) 561.
[78] A. Spandidos, T.H. Rabbitts, J. Mol. Biol. 318 (2002) 21.
[79] R. Kellner, R. Lichtenfels, D. Atkins, J. Bukur, A. Ackermann, J. Beck, W. Brenner, S. Melchior, B. Seliger, Proteomics 2 (2002) 1743.
[80] R.D. Unwin, P. Harnden, D. Pappin, D. Rahman, P. Whelan, R.A. Craven, P.J. Selby, R.E. Banks, Proteomics 3 (2003) 45.
[81] R. Lichtenfels, R. Kellner, D. Atkins, J. Bukur, A. Ackermann, J. Beck, W. Brenner, S. Melchior, B. Seliger, BBA 1646 (2003) 21.
[82] B. Seliger, M. Menig, R. Lichtenfels, D. Atkins, J. Bukur, T.M. Halder, M. Kersten, A. Harder, A. Ackermann, J. Beck, B. Muehlenweg, W. Brenner, S. Melchior, R. Kellner, F. Lottspeich, Proteomics 3 (2003) 979.
[83] K. Dallmann, H. Junker, S. Balabanov, U. Zimmermann, J. Giebel, R. Walther, Int. J. Cancer 108 (2004) 342.
[84] R. Pieper, C.L. Gatlin, A.M. McGrath, A.J. Makusky, M. Mondal, M. Seonarain, E. Field, C.R. Schatz, M.A. Estock, N. Ahmed, N.G. Anderson, S. Steiner, Proteomics 4 (2004) 1159.
[85] U. Zimmermann, S. Balabanov, J. Giebel, S. Teller, H. Junker, D. Schmoll, C. Protzel, C. Scharf, B. Kleist, R. Walther, Cancer Lett. 209 (2004) 111.
[86] P.A. Fetsch, N.L. Simone, P.K.B. Greenwood, F.M. Marincola, A.C. Filie, E.F. Petricoin, L.A. Liotta, A. Abati, Am. J. Clin. Pathol. 118 (2002) 870.
[87] Y.G. Won, H.J. Song, T.W. Kang, J.J. Kim, B.D. Han, S.W. Lee, Proteomics 3 (2003) 2310.
[88] A.J. Link, J. Eng, D.M. Schieltz, E. Carmack, G.J. Mize, D.R. Morris, B.M. Garvic, J.R. Yates, Nat. Biotechnol. 17 (1999) 676.
[89] S.P. Gygi, B. Rist, S.A. Gerber, F. Turecek, M.H. Geib, R. Abersold, Nat. Biotechnol. 17 (1999) 994.
[90] W. Pusch, M.T. Flocco, S.M. Leung, H. Thiele, M. Kostrzewa, Pharmacogenomics 4 (2003) 463.