

MOLECULAR ASPECTS OF PROSTATE CANCER: IMPLICATIONS FOR FUTURE DIRECTIONS

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ABSTRACT

Many studies have been developed trying to understand the complex molecular mechanisms involved in oncogenesis and progression of prostate cancer (PCa). Current biotechnological methodologies, especially genomic studies, are adding important aspects to this area. The construction of extensive DNA sequence data and gene expression profiles have been intensively explored to search for candidate biomarkers to evaluate PCa. The use of DNA micro-array robotic systems constitutes a powerful approach to simultaneously monitor the expression of a great number of genes. The resulting gene expressing profiles can be used to specifically describe tumor staging and response to cancer therapies. Also, it is possible to follow PCa pathological properties and to identify genes that anticipate the behavior of clinical disease. The molecular pathogenesis of PCa involves many contributing factors, such as alterations in signal transduction pathways, angiogenesis, adhesion molecules expression and cell cycle control. Also, molecular studies are making clear that many genes, scattered through several different chromosomal regions probably cause predisposition to PCa. The discovery of new molecular markers for PCa is another relevant advance resulting from molecular biology studies of prostate tumors. Interesting tissue and serum markers have been reported, resulting in many cases in useful novelties to diagnostic and prognostic approaches to follow-up PCa. Finally, gene therapy comes as an important approach for therapeutic intervention in PCa. Clinical trials for PCa have been demonstrating that gene therapy is relatively safe and well tolerated, although some improvements are yet to be developed.

Key words: prostatic neoplasms; carcinoma; genomics; molecular markers; micro-array

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INTRODUCTION

Prostate cancer (PCa) is the second most common cause of death from malignancy in American men. Although there are some effective treatment approaches for clinically localized PCa through surgery and radiotherapy, the metastatic form remains incurable. The metastatic potential of tumor cells and its possible dissemination to secondary sites are critical factors related to its mortality rates. In spite of the high incidence and mortality rates, the

molecular mechanisms involved in oncogenesis and progression to PCa are still poorly understood, especially related to the progression to the metastatic form. PCa etiology remains obscure and its tumors vary from indolent forms, with low evolution rates, to extremely aggressive ones, with rapid growing rates. Due to this particularity, the molecular processes that contribute to PCa are under intense investigation. The methods that have been used to characterize the genetic alterations found in this neoplastic disease include familiar studies designed to map some

hereditary loci, chromosomal studies to identify aberrations that could localize oncogenes or tumor suppressor genes and intense studies of gene expression (1,2). These studies reflect many signaling pathways that influence the carcinogenetic process. The use of biotechnological approaches, such as DNA automatic sequencing and DNA micro-arrays allow a systematic study in high through output scale. These and other technologies allow a detailed vision of the biology and pathology of PCa. Altogether, they have the potential to completely characterize the processes involved in this neoplastic disease, turning possible the discovery of new molecular markers for PCa.

The present review describes the approaches currently used for studying the molecular mechanisms that control the onset and progression of PCa. First of all we present the biotechnological methodologies derived from data generated in the Human Genome Project and on its post-genomic stage, with special attention to projects specifically developed for the study of PCa (Genomic Studies). After that, we present some of the available data concerning the mechanisms involved in tumorigenesis and progression of PCa (Molecular Mechanisms of Oncogenesis). Further, we point out the participation of a series of genes involved in the predisposition for the development of PCa (Susceptibility Genes). We also review the current molecular markers for PCa, including the ones presenting the potential to be largely used as additional tools in the diagnosis and follow up of PCa (Molecular Markers). We conclude discussing how the data generated in genomic studies and the new molecular markers for PCa could be used as new therapeutic approaches (Gene Therapy).

GENOMIC STUDIES

The early diagnosis of metastatic PCa as well as the follow up of different therapeutic approaches is very important goals in prostate research. For these reasons, diagnostic and prognostic markers have been extensively investigated (3-6).

Molecular markers have been used to diagnose and monitor prostate cancer for more than 50 years. The discovery of serum marker PSA

(prostate specific antigen) significantly altered the detection and follow up of PCa (7,8). PSA is an androgen-regulated serine protease produced by both prostate epithelial cells and PCa and is the most commonly used serum marker for cancer. It is a member of the tissue kallikrein family, some of the members of which are also prostate specific. PSA is a major protein in semen, where its function is to cleave semenogelins in the seminal coagulum (8). However, although high PSA levels are predictive of advanced PCa, a large fraction of organ-confined cancers present with much lower total PSA values that overlap those levels found in men without PCa (8,9). So, the use of PSA present limitations as a marker mostly related to the PCa diagnosis, generating in many cases both false positive and false negative results. As post surgery marker, however, the use of PSA is of great importance, being its seric levels directly related to PCa progression and/or regression, although it cannot predict tumor metastatic potential. Thus, new biological markers for PCa can be very useful for detection and improvement on the application of different therapeutic options.

Recent advances in genomic studies and biotechnology dramatically increased the amount and accessibility of molecular information relevant to the study of prostate carcinogenesis. An important improvement involves the generation of extensive databases of DNA sequences and gene expression patterns (6,10-12). This information is available in adequate format allowing virtual comparison between normal and cancer cells (<http://www.mbt.washington.edu/PEDB> and <http://www.cgap.nci.nih.gov>). This source has been explored to identify candidate biomarkers to evaluate PCa, based on the homology with known oncogenes. A second important improvement is the use of robotic systems to construct DNA micro-arrays corresponding to thousands of distinct expressed genes in prostate tissues. Such arrays constitute a powerful approach to simultaneously monitor the expression of a great number of genes. Additionally, they result in specific gene expression profiles that can be used as "molecular fingerprints" for tumor diagnosis and staging. Catalogues and indexes of differentially expressed genes and the proteins that

they code have been extensively used to identify informative biomarkers (13-15). In this direction, specifically selected genes coding for proteins differentially expressed in normal and neoplastic prostate tissues emerge as potential molecular markers.

The DNA micro-array analysis has been also used to determine the global biological differences between common PCa pathological properties and to identify genes that anticipate the disease clinical behavior. A group of genes was identified that strongly correlates with prostate tumor differentiation stage, according to the Gleason score measure (16). These authors showed that the gene expression data generated by these DNA micro-arrays profiles predict with accuracy the patient evolution after prostatectomy. These data support the notion that the PCa clinical behavior is related to specific differences in gene expression profile that are detectable at the time of diagnosis.

Gene expression profiles also allow the identification of possible targets for cancer therapy. The study of expression profiles of many malignant and benign prostate tumor samples allowed the identification of a series of differentially expressed genes between tumoral and normal glands. A highly expressed gene in prostate tumor codes a type II transmembrane serine protease called hepsin. In situ hybridization studies showed that hepsin is specifically over-expressed in non-metastatic carcinoma cells and on an independent panel of prostate specimens. A 1.85 Kb hepsin mRNA is expressed in most tissues, with the highest level expressed in liver tissue, and lower levels expressed in other tissues including prostate. Hepsin has been shown to be necessary for normal cell growth and recent data showed that metastatic PCa cell lines over-expressing hepsin show a dramatic reduction in the cell growth and invasion and also present an increase in the cell population undergoing apoptosis. These negative cell growth-regulatory effects of hepsin have unraveled possible cellular and molecular mechanisms that link decrease / loss of hepsin expression with poor prognosis of PCa (17). These findings together with hepsin molecular properties make it a potential target for prostate cancer gene therapy (18).

MOLECULAR MECHANISMS IN PROSTATE CANCER ONCOGENESIS

The molecular pathogenesis of induction and progression of prostate tumor is not completely understood, although many contributing factors (such as alterations in signal transduction pathways, angiogenesis and adhesion molecules) can play important roles in tumor progression. Since defects in cell cycle control can be an early event in cancer evolution, some studies have turned to the study of genetic changes in gene expression of proteins involved in cell cycle. The TERE1 gene, for example, seems to play a role in prostate cancer progression through a growth regulatory pathway, possibly in G1 phase of cell cycle. It was observed a reduced expression of TERE1 protein in metastatic prostate carcinoma, reduced expression of the TERE1 transcript in some invasive PCa and the decreased proliferation of prostate carcinoma cells after over-expression of TERE1 (19). TERE1 is not homologous to any known full-length human gene but is homologous to a number of expressed sequence tags (ESTs). Altogether, these data suggest that TERE1 may be significant in prostate cancer growth regulation and that the down regulation or absence of TERE1 transcript may be an important component of prostate cancer progression.

Another protein involved in cell cycle control with particular expression in PCa is SSeCKS, a major protein kinase C substrate with tumor suppressor activity, is a scaffolding protein for PKC (protein kinase C) and PKA (protein kinase A) signaling pathways. SSeCKS also plays a role in G1→S progression by modulating cyclin D expression and sequestering G1-phase cyclins in the cytoplasm. It was shown that SSeCKS expression is abundant in untransformed human and rat prostate cell line, in normal prostatic epithelial cells and in undifferentiated human prostate cancers in vivo but down regulated in prostate cancer cell lines and in high-grade cancers in vivo. These data suggest a putative role for SSeCKS expression in the onset of prostate cancer metastasis (20).

Telomerase enzyme activation seems to be a critical step in cell immortality and oncogenesis in

PCa. Telomerase is a ribonucleoprotein that is minimally comprised of an integral RNA template (hTR) and a reverse transcriptase protein component (hTERT). In humans, progressive telomere shortening has been implicated as a cause of cellular senescence. Cells capable of bypassing senescence and escaping these events most often reactivate the enzyme telomerase, resulting in stability of telomere ends and continued cellular proliferation. Telomerase directs *de novo* synthesis of telomeric repeats at chromosome ends. In PCa, increased telomerase activity is already evident at the very early stages of the disease, namely prostate *in situ* neoplasia. Indeed, evaluation of telomerase activity in prostate biopsies has become a valuable diagnostic marker for this malignancy in addition to PSA levels (21,22). Telomerase activity has been detected in 90% of prostate carcinomas and is increased in more than 10 fold in tumorigenic conversion. These data show that the absence of telomerase activity may be a strong indicator of a lack of cancer (22).

Alterations in gene expression levels also occur with Gp protein subunits, suggesting their important roles for cell proliferation and neoplastic transformation in human prostate, having a potential prognostic value (23). G-proteins (Guanine nucleotide-binding regulatory proteins) are heterotrimers composed of α , β and γ subunits, and coupled to 7-helix transmembrane receptors (GPCRs). G proteins are involved in many processes, including oncogenic properties, described in studies of G protein regulation of cell growth, differentiation and oncogenesis. The functionality and expression of G proteins subunits are selectively modified in human prostate adenocarcinoma. Low α S and α i subunit levels in prostate cancer suggest an important regulatory role of G proteins for cell proliferation and neoplastic transformation in the human prostate (23).

Human prostatic acid phosphatase (PACp), a major protein tyrosine phosphatase in prostate epithelium, plays a critical role in regulating the growth of prostate cancer cells. It was recently shown that the active form of cellular PACp has a significant suppression effect on the growth of androgen independent prostate cancer cell line, not only in culture but also in mouse xenograft tumor model (24). PACp expression is also decreased in PCa, in such a

way that this enzyme can be involved in PCa progression. Cellular PACp can down regulate prostate cancer cell growth at least partially by dephosphorylating c-ErbB-2 oncogene (25).

An increased immunohistochemistry staining of bcl-2 protein in tumor samples was observed in association with undifferentiated Gleason scores. Bcl-2 is one of the most important regulators of apoptosis and programmed cell death. Elevated levels of bcl-2 protein may contribute to the progression of prostate cancer to a metastatic and hormone-insensitive state characterized by poor responses to chemotherapy. Higher frequency of bcl-2 expression in tumor samples suggests that an increase in this anti-apoptotic protein generally occurs during PCa progression (26).

SUSCEPTIBILITY GENES FOR PROSTATE CANCER

Many chromosomal regions have been shown to be involved in predisposition to development of PCa (27). Predisposition to PCa is probably polygenic and caused by different models of Mendelian inheritance, incomplete penetrance and ethnic variations. A positive family history is among the strongest epidemiological risk factors for PCa (28). Most of the studies based in segregation analysis suggest a dominant autosomic transmission of susceptibility genes (29). Multiple loci situated at chromosomes 1, 10 and 17 were associated with PCa through linkage analysis. Most attention is given to chromosome 1, and it has been proposed that this chromosome contains at least 3 sub regions (HCP1, PACP and CAPB) where possible susceptibility genes for PCa are located (30). Table-1 presents the main described susceptibility genes for PCa.

MOLECULAR MARKERS

Although PSA and the human kalikrein 2 are the available molecular forms for the diagnosis and follow up of PCa, they present insufficient sensitivity and specificity for early detection or staging this neoplastic disease. Many new concepts have been introduced aiming the optimization of the clinical use of PSA. However, all of them present limitations. The

Table 1 – Main susceptibility genes for developing prostate cancer.

Gene	Main Features
Androgen Receptor (AR)	CAG repeats seems to be associated with risk to develop PCa
MSR1 (macrophage scavenger receptor 1)	Seven important gene mutations were found
ELAC2	Gene mutation carriers show high risk to develop PCa
BRCA2	Putative implications in early stages of PCa
RNASEL (codes ribonuclease L)	Heterozygotes men in relation to the mutated allele have a risk around 50% higher to develop PCa
ETV6 (tumor suppressor gene)	Mutational inactivation can occur in prostate carcinoma
AMACR /P504S (alpha methyl-CoA Racemase)	Seventeen sequence variants identified can be associated with high risk of PCa

The main described susceptibility genes for developing for prostate cancer are listed at the left boxes. At the right are presented their most important features related to susceptibility for developing prostate cancer.

PSA molecular forms, especially free PSA, seems to be of utility for the PCa detection in men with total PSA concentrations ranging from 4 to 10 µg/L. New molecular techniques, such as RT-PCR (reverse transcription polymerase chain reaction) for the detection of minimum mRNA that codes for PSA and PMSA (membrane prostate specific antigen), offer new perspectives for the diagnosis, prognosis and possibly for PCa staging (31). Another limitation of PSA is that actually it is not really prostate specific, and a possible role as a prognostic indicator also in woman breast cancer has been described (32).

Due to the mentioned limitations for PSA use as a diagnostic and prognostic marker, much attention has turned to the discovery of new markers in this area (10).

Serum Markers

The serum markers (protein biomarkers) for cancer are among the more desirable form of diagnosis and much effort has been applied in searching for these markers for PCa. The protein biomarkers found in serum offer enormous promises for non-invasive detection, classification and follow up of PCa. Antibody micro-arrays seem to be adequate for the discovery of serum markers, making possible the comparison of relative abundances of hundreds of proteins in the same experiment. Like in DNA micro-array, the antibody micro-array is used to perform

qualitative analysis, comparing the relative abundances of protein in samples of interest. Using the approach of antibody micro-array, 5 proteins were described (Von Willebrand factor, Immunoglobulin M, alpha-1 chymotrypsin, immunoglobulin G and viliin), with significantly higher levels in serum samples of patients with prostate cancer than in normal control serum (33). The DNA micro-arrays also emerges as an alternative for the search of serum markers, where thousand of cDNAs from prostate tumor are applied over micro-array slides and hybridized with RNAs from tumor and normal prostate tissues, with the aim of selecting differentially expressed genes. The proteins coded for these genes, can be evaluated for its immunogenic potential analyzing the presence of antibodies in serum from patients with PCa.

Proteomics, the analysis and characterization of global protein modifications, will add to search for new serum markers, to our understanding of gene function and aid in therapeutic target discovery (34). With the rapid technological advances being made in the field of proteomics, this approach could be integrated with genomics providing a complementary alternative, overcoming disparities between mRNA levels and protein production, and additionally allowing the identification of tumor-associated post-transcriptional modifications (35).

Using proteomics expression profiles of androgen-stimulated prostate cancer cells generated

by two-dimensional electrophoresis (2-DE) and spectrometric analysis a metastasis-suppressor gene NDKA/nm23 was identified, a finding that may explain a marked reduction in metastatic potential when these cells express a functional androgen receptor pathway (36). Another proteomic study was used to map the differences in protein expression profiles expression between normal and malignant prostate tissues, with special regard to proteins lost in malignancy. Comparison of protein maps of normal and malignant prostate were used to identify 20 proteins which were lost in malignant transformation, including the novel finding of NEDD8, calponin and the follistatin-related proteins, whose function warrants further investigation (37). Also, using the approach of SELDI (a protein biochip surface enhanced laser desorption/ionization mass spectrometry) coupled with an artificial intelligence learning algorithm to identify better biomarkers for early detection of PCa, high sensitivity (83%), specificity (97%) and a positive predictive value (96%) results were obtained when comparing PCa versus benign prostate hyperplasia and healthy men groups. These results offer a potential of SELDI proteomic classification system for the early detection and diagnosis of PCa (38).

Tissue Markers

It has been shown through gene expression profiles generated by DNA micro-array that the EZH2 gene ("polycom group protein enhancer of zeste homolog 2") is a tissue marker over-expressed in hormone refractory metastatic prostate cancer. It was demonstrated that deregulated expression of EZH2 gene was involved in cancer progression being thus a marker that distinguishes indolent PCa from those with lethal progression (30).

The P504S (alpha methyl-CoA racemase) gene has been recently described as a specific gene for PCa that codes a protein involved in fatty acids beta-oxidation (39). It was shown that the immunohistochemical detection of P504S gene product constitutes a sensitive and specific marker for PCa in phormol paraffin fixed tissues. This marker presents a potential utility for the diagnosis of PCa,

including the ones treated with hormones and radiotherapy.

The molecular detection of circulating tumor cells and micrometastasis also arises as prognostic markers for PCa. The detection of malignant cells has been made through the highly sensitive technique of RT-PCR. These assays are mostly directed against tissue specific prostate markers. In most of the studies in prostate carcinoma, RT-PCR was capable of detecting specific markers of prostate tissues in peripheral blood, bone marrow and lymph nodes of patients with localized or metastatic disease (40).

Tissue micro-array studies also described other biomarkers for PCa, suggesting its role for searching for therapeutic targets and as prognostic factors. Loss or decreased expression detected by tissue micro-array of CD10 (a neutral endopeptidase cell surface marker) is an early and frequent event in human prostate cancer (41). Syndecan-1 (a transmembrane heparan suphate proteoglycan that is involved in cell-cell adhesion, organization, cell-matrix adhesion, and regulation of growth factor signaling) over-expression analyzed by tissue micro-array also predicted early recurrence and was significantly associated with tumor specific survival, high Gleason score, KI67 and bcl-2 over-expression (42). Some members of the annexin family, specifically 1 and 7, were identified by tissue micro-array as potential biomarkers in the development of prostate cancer. The annexins are a group of calcium-binding structural proteins that may play a role in the regulation of membrane trafficking, cell adhesion a cell signaling (43). Tissue micro-array revealed a significant decrease in protein expression of annexins 1 and 7 in hormone refractory PCa as compared to localized PCa. However, no significant differences were detected between the clinically localized PCa and non-cancerous prostate tissues. These findings suggest that down regulation of members of the annexin family may contribute to PCa tumorigenesis (44).

GENE THERAPY

A better understanding of the molecular mechanisms responsible for the onset of the disease

as well as the factors that control the proliferation of PCa allows the identification of fundamental changes in gene expression during cancer progression. Manipulation of genes involved in disease progression represents an important approach for therapeutic intervention in PCa (Gene therapy). In the last few years, significant advances in gene therapy occurred due to improvements in many areas of molecular and cellular biology, including the development of better gene delivery through viral and non-viral systems, discovery of new therapeutic agents, an in-depth comprehension of disease progression mechanisms and exploration of tissue specific DNA promoter sequences (45). The development of new approaches for gene therapy for PCa is a critical step, once no effective treatment for patients in advanced stages is available. The current available strategies for gene therapy for PCa include citoreductive approaches (immunotherapy and cytolytic / pro-apoptotic). The prostate constitutes a tissue that is ideal for gene therapy. It is an accessory organ, offers unique antigens (PSA, PSMA, human glandular kalikrein 2) and is accessible for in situ treatments (46). The clinical trials for prostate cancer demonstrated that gene therapy is relatively safe, although evidences for efficient stable gene therapeutics have yet to be demonstrated (46,47). Recently published studies showed that androgen-independent prostate cancer metastasis showed evidences of gene-therapy induced apoptosis (48).

The greater understanding of the molecular events underlying the development of metastatic disease allows gene therapy approaches to be developed that specifically target these molecular events.

Specific genes that are predominantly expressed or exclusively expressed in prostate cells, prostate cancer cells, and prostate metastasis cells at the level of DNA, RNA and protein products are the targets of several new approaches to prostate cancer therapy (49). As an example, a prostate apoptosis response (Par-4) gene was recently identified, which exclusively induces apoptosis in cancer cells and not normal cells, and constitutes a prospective molecule for therapy of the disease (50).

The greatest challenge in the treatment of advanced prostate cancer is to access and eliminate

metastatic cells. Therefore, effective prostate cancer therapy will require novel strategies to target cancer cells both at the site of the primary tumor and at distant metastasis. To achieve these aims, one of the strategies is the development of specific gene promoter regulatory sequences, with the possibility to express genes in the desired target cells. A research group focused on developing prostate tumor-specific promoters, such as osteocalcin (OC) promoter based on therapy that specifically targets osseous metastases, the most lethal form of the disease (51). New approaches using specific promoter chimeric constructs with the heretologously expressed TRLP (Ca²⁺ permeable transient receptor potential-like channels) protein leads to a reduction in prostate cell survival due, in part, to the induction of apoptosis. This finding suggests a new approach to modify the growth of prostate cancer cells that fail to undergo apoptosis following androgen ablation therapy (52).

As cited above, the development of better delivery systems are one of the critical steps for the effective use of gene therapy for PCa. The vectors used to transfect the genetic material in PCa clinical gene transfer protocols have advantages and disadvantages as couriers of genetic information. For example, the adenovirus can transfer a large amount of genetic information with high efficiency, regardless of cell cycle considerations and without toxicity to the cellular genome (genotoxicity). Unfortunately, this virus results in only transient expression of the genetic material and most individuals will have innate immunity due to prior exposure to the adenovirus, which limits multiple dosing or systemic administration. Several new vectors are being tested in pre-clinical models including oncolytic herpes viral vectors and leti virus vectors containing prostate specific regulatory elements (e.g. PSA promoters). Another major advance relates to the creation or designing vectors that are genetically engineered to exert their DNA transfer to the target cell only. The combination of the abilities to manipulate the viral genomes and the information about cancer cells allow researches to design vectors that will specifically target and destroy prostate cancer cells with precision.

Another question relating PCa gene therapy is what DNA must be transferred. The answer relates

to the objective of the therapy. For example, the genetic material from the herpes simplex virus (HSV) encoding the thymidine kinase (TK) enzyme has been utilized in a number of gene transfer protocols for PCa. The viral form of TK enzyme can convert a number of well-tolerated clinically approved pro-drugs to a potent intracellular toxin, which interferes with DNA replication. Due to the activated pro-drug effect on dividing DNA, this form of suicide gene therapy will effectively kill a cell when it attempts to proliferate or divide, but it is limited to the cells infected by the virus and those immediately surrounding the infected cell ("bystander effect") (51).

Another concept being developed relates to targeting hypoxia-response system of prostate tumor cells as a means to suppress prostate tumor progression and metastasis or perhaps as a means for eliminating prostate tumors in advanced prostate cancer patients (53).

Finally, a new approach is combining gene therapy strategy with more conventional therapy such as radiation or chemotherapy. There have been several elegant pre-clinical studies that demonstrated the ability of combining chemo-gene therapy and radio-gene therapy that have led to the proposed clinical trials (51). The use of RNAi (RNA interference) is an approach to target signaling/repair proteins (ATM, ATR) and DNA-dependent protein kinase catalytic subunit [DNA-PK (cs)] as targets to confer enhanced radio and chemosensitivity to tumor cells. RNAi targeting ATM and DNA-PK (cs) increased radio and chemosensitivity of PCa, providing evidence for the potential use of RNAi as a novel radiation/chemotherapy-sensitizing agent (54).

CONCLUSIONS

In summary, prostate cancer molecular data, especially the information concerning the relevant mechanisms involved in oncogenesis and progression of this neoplastic disease, are the result of a number of genomic studies. These findings have important implications for defining future directions of research in the diagnosis and prognosis of PCa, mainly looking for new more sensitive and discriminatory biomarkers. Also, these data emerge as an important source of

putative targets for prostate cancer therapies. As an important perspective, gene therapy comes as a powerful approach to specifically treat advanced prostate cancer.

REFERENCES

1. Li PE, Nelson PS: Prostate cancer genomics. *Curr Urol Rep.* 2001; 2: 70-8.
2. Karan D, Lin MF, Johnsson SL, Batra SK: Current status of the molecular genetics of human prostatic adenocarcinomas. *Int J Cancer.* 2003; 103: 285-93.
3. Hegarty NJ, Fitzpatrick JM, Richie JP, Scardino PT, Devere White RW, Schroder FH, et al.: Future prospects in prostate cancer. *Prostate.* 1999; 40: 261-8.
4. Harding MA, Theodorescu D: Prostate tumor progression and prognosis: interplay of tumor and host factors. *Urol Oncol.* 2000; 5: 258-64.
5. Verhagen PCa, Tilanus MG, de Werger RA, van Moorselaar RJ, van d JG, Boon TA: Prognostic factors in localized prostate cancer with emphasis application of molecular techniques. *Eur Urol.* 2002; 41: 363-71.
6. Brooks JD: Microarray analysis in prostate cancer research. *Curr Opin Urol.* 2002; 12: 395-9.
7. Lukes M, Uebam M, Zalesky M, Zachoval R, Heroucek J, Zdarsky E: Prostate-specific antigen: current status. *Folia Biol.* 2001; 47: 41-9.
8. Balk SP, Ko YJ, Bubley GJ: Biology of prostate-specific antigen. *J Clin Oncol.* 2003; 21: 383-91.
9. Karazanashvili G, Abrahamsson PA: Prostate specific antigen and human glandular kallikrein 2 in the detection of prostate cancer. *J Urol.* 2003; 169: 445-57.
10. Grouse LH, Munson PJ, Nelson PS: Sequence databases and microarrays as tools for identifying prostate cancer biomarkers. *Urology.* 2001; 57 (4 Suppl 1): 154-9.
11. Ho SM, Lau KM: DNA microarrays in prostate cancer. *Curr Urol Rep.* 2002; 3: 53-60.
12. Asmann YW, Kosari F, Wang K, Cheville JC, Vasmataz G: Identification of differentially expressed genes in normal and malignant prostate by electronic profiling of expressed sequence tags. *Cancer Res.* 2002; 62: 3308-14.
13. Dhahasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, et al.: Delineation of prognostic biomarkers in prostate cancer. *Nature.* 2001; 412: 822-6.
14. LaTulippe E, Satagopan J, Smith A, Scher H, Scardino

- P, Reuter V, et al.: Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease. *Cancer Res.* 2002; 62: 4499-506.
15. Ernst T, Hergenhahn M, Kenzelmann M, Cohen CD, Bonrouhi M, Weninger A, et al.: Decrease and gain of gene expression are equally discriminatory markers for prostate carcinoma: a gene expression analysis on total and microdissected prostate tissue. *Am J Pathol.* 2002; 160: 2169-80.
 16. Singh D, Febbo PG, Ross K, Jackson DG, Manola J, Ladd C, et al.: Gene expression correlates of clinical prostate behavior. *Cancer Cell.* 2002; 1: 203-9.
 17. Srikantan V, Valladares M, Rhim JS, Moul JW, Srivastava S: HEPsin inhibits cell growth/invasion in prostate cancer cells. *Cancer Res.* 2002; 62: 6812-6.
 18. Magee JA, Araki T, Patil S, Shrig T, True L, Humphrey PA, et al.: Expression profile reveals hepsin overexpression in prostate cancer. *Cancer Research.* 2001; 61: 5692-6.
 19. McGarvey TW, Nguyen T, Puthiyaveetil R, Tomaszewski JE, Malkowicz SB: TEREI, a novel gene affecting growth regulation in prostate cancer. *Prostate.* 2003; 54: 144-55.
 20. Xia W, Unger P, Miller L, Nelson J, Gelman IH: The Src-suppressed C kinase substrate, SseCKS, is a potential metastasis inhibitor in prostate cancer. *Cancer Res.* 2001; 61: 5644-51.
 21. Nanni S, Narduci M, Pietra LD, Moretti F, Grasselli A, De Carli O, et al.: Siganling through estrogen receptors modulates telomerase activity in human prostate cancer. *J Clin Invest.* 2002; 11: 219-27.
 22. Akalin A, Elmore LW, Forsythe HL, Amaker BA, McCollum ED, Nelson PS, et al.: A novel mechanism for Chaperone-mediated telomerase regulation during prostate cancer progression. *Cancer Research.* 2001; 61: 4791-6.
 23. Garcia-Fernandez MO, Solano RM, Sanchez-Chapado M, Ruiz-Villaespesa A, Prieto JC, Carmena MJ: Low expression of Galpha protein subunits in human prostate cancer. *J Urol.* 2001; 166: 2512-7.
 24. Igawa T, Lin FF, Rao P, Lin MF: Suppression of LNCaP prostate cancer xenograft tumors by a prostate-specific protein tyrosine phosphatase, prostatic acid phosphatase. *Prostate.* 2003; 55: 247-58.
 25. Lin MF, Lee MS, Zhou XW, Andressen JC, Meng TC, Johansson SL, et al.: Decreased expression of cellular prostatic acid phosphatase increases tumorigenicity of human prostate cancer cells. *J Urol.* 2001; 166: 1943-50.
 26. Hering FL, Lipay MA, Rodrigues PR, Nesralah LJ, Srougi M: Comparison of positivity frequency of bcl-2 expression in prostate adenocarcinoma with low and high Gleason score. *São Paulo Med J.* 2001; 119: 138-41.
 27. Nuppomen NN, Carpten JD: Prostate cancer susceptibility genes: many studies, many results, no answers. *Cancer Metastasis.* 2001; 20: 155-64.
 28. Bratt O: Hereditary prostate cancer: clinical aspects. *J Urol.* 2002; 168: 906-13.
 29. Kim HL, Steinberg GD: New insights and candidate genes and their implications for care of patients with hereditary prostate cancer. *Curr Urol Rep.* 2000; 1: 9-14.
 30. Tavtigian SV, Simard J, Teng DHF, Abtin V, Baumgard M, Beck A, et al.: A candidate prostate cancer susceptibility gene at chromosome 17p. *Nature Genetics.* 2001; 27: 172-80.
 31. De la Taille A, Olsson CA, Katz AE: Molecular staging of prostate cancer: dream or reality? *Oncology.* 1999; 13: 187-94.
 32. Daher R, Beaini M: Prostate-specific antigen and new related markers for prostate cancer. *Clin Chem Lab Med.* 1998; 36: 671-81.
 33. Miller, JC, Zhou H, Kweke J, Cavallo R, Burke J, Butler EB, et al.: Antibody microarray profiling of human prostate cancer sera: Antibody screening and identification of potential biomarkers. *Proteomics.* 2003; 3: 56-63.
 34. Paweletz CP, Liotta LA, Petricoin III EF: New technologies for biomarker analysis of prostate cancer progression: laser capture microdissection and tissue proteomics. *Urology.* 2001; 57 (Suppl 4A): 160-3.
 35. Unwin RD, Knowles MA, Selby PJ, Banks RE: Urological malignancies and the proteomic-genomic interface. *Electrophoresis.* 1999; 20: 3629-37.
 36. Nelson PS, Han D, Rochon Y, Corthals GL, Lin B, Monson A, et al.: Comprehensive analyses of prostate gene expression: convergence of expressed sequence tag databases, transcript profiling and proteomics. *Electrophoresis.* 2000; 21: 1823-31.
 37. Meehan KL, Holland JW, Dawkins HJ: Proteomic analysis of normal and malignant prostate tissue to identify novel proteins lost in cancer. *Prostate.* 2002; 50: 54-6.
 38. Adam BL, Qu Y, Davis JW, Ward MD, Clements MA, Cazares LH, et al.: Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. *Cancer Res.* 2002; 62: 3609-4.

39. Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG, et al.: JAMA. 2002; 287: 1662-70.
40. Scheiermacher G, Delattre O: Détection des micrométastases et des cellules tumorales circulantes par les techniques de biologie moléculaire dans les tumeurs solides. Bull Cancer. 2001; 88: 61-70.
41. Freedland SJ, Seligson DB, Liu AY, Pantuck AJ, Paik SH, Horvath S, et al.: Loss of CD10 (neutral endopeptidase) is a frequent and early event in human prostate cancer. Prostate. 2003; 55: 71-80.
42. Zellweger T, Ninck C, Mirlacher M, Annefeld M, Glass AG, Gasser TC, et al.: Tissue microarray analysis reveals prognostic significance of syndecan-1 expression in prostate cancer. Prostate. 2003; 55: 20-9.
43. Srivastava M, Bubendorf L, Srikantan V, Fossom L, Nolan L, Glasman M, et al.: ANX7, a candidate tumor suppressor gene for prostate cancer. Proc Natl Acad Sci. USA. 2001; 98: 4575-80.
44. Xin W, Rhodes DR, Ingold C, Chinnaiyan AM, Rubin MA: Dysregulation of the annexin family protein family is associated with prostate cancer progression. Am J Pathol. 2003; 162: 255-61.
45. Shirakawa T, Gotoh A, Wada Y, Kamidono S, Ko Sc, Kao C, et al.: Tissue-specific promoters in gene therapy for the treatment of prostate cancer. Mol Urol. 2000; 4: 73-82.
46. Mabjesh NJ, Zhong H, Simons JNV: Gene therapy of prostate cancer: current and future directions. Endocr Relat Cancer. 2002; 9: 115-39.
47. Steiner MS, Gingrich JR, Chauhan RD: Prostate cancer gene therapy. Surg Oncol Clin N Am. 2002; 11: 607-20.
48. Kubo H, Gardner TA, Wada Y, Koeneman KS, Gotoh A, Yang L, et al.: Phase I dose escalation clinical trial of adenovirus vector carrying osteocalcin promoter-driven herpes simplex virus thymidine kinase in localized and metastatic hormone-refractory prostate cancer. Hum Gene Ther. 2003; 14: 227-41.
49. Ast G, Maul H: Drug-targeting strategies for prostate cancer. Curr Pharm Des. 2003; 9: 455-66.
50. Butler J, Rangnekar VM: Par-4 for molecular therapy of prostate cancer. Curr Drug Targets. 2003; 4: 223-30.
51. Gardner TA, Sloan J, Raikwar SP, Kao C: Prostate cancer gene therapy: past experiences and future promise. Cancer Metastasis Rev. 2002; 21: 137-45.
52. Zhang L, Brereton HM, Hahn M, Froschio M, Tilley WD, Brown MP, et al.: Expression of Drosophila Ca²⁺ permeable transient receptor potential-like channel protein in a prostate cancer cell line decreases cell survival. Cancer Gene Ther. 2003; 10: 611-25.
53. Anastasiadis AG, Bemis DL, Stisser BC, Salomon L, Ghafar MA, Buttyan R: Tumor cell hypoxia and the hypoxia-response signaling system as a target for prostate cancer therapy. Curr Drug Targets. 2003; 4: 191-6.
54. Collis SJ, Swartz MJ, Nelson WG, DeWeese TL: Enhanced radiation and chemotherapy-mediated cell killing of human cancer cells by small inhibitory RNA silencing of DNA repair factors. Cancer Res. 2003; 63: 1550-4.

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EDITORIAL COMMENT

The article entitled Molecular Aspects of Prostate Cancer: Implications for Future Directions serves as a review of recent advances in the investigation of the molecular basis of prostate cancer and how these discoveries impact on the way the disease is currently studied, how it can be diagnosed, how its progression can be predict and how it can treated genetically.

The article has adequate sections which aim to cover the topics of genomic studies, molecular mechanisms, susceptibility genes, molecular markers and gene therapy.

The article has touched on the recent subjects that are considered of high interest in the scientific field of cancer research such as AMACR and EZH2, two genes which have created quite a buzz in prostate cancer research. Also, the techniques employed for the discovery of these interesting genes have been tackled.

With regard to gene therapy, a truly controversial topic, it was appropriate the mention to the problems that present studies have encountered with this novel and developing therapeutic strategy.

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