

REVIEW

Genomics of Prostate Cancer: Is There Anything to „Translate”?

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This review provides an up-dated collection of data concerning the genetic and epigenetic changes during development, growth and progression of prostate cancer. Hereditary and susceptibility factors have a long list, similarly to the expression of single genes connected to various cell functions. It was a hope that covering a large set of genes, array technologies would clarify very rapidly the role of genetics in malignant diseases, offering targets for molecular diagnostics and therapy. The power of high-throughput techniques for the detection and global analysis of gene expression is unquestionable, interesting, astonishing as well as puzzling data have already been obtained. However, the standardization of the procedures is still missing

and the reproducibility is rather low in many instances. Moreover, the different array methods can select different gene expression profiles, which makes the decision rather difficult. Another important question is, coming again from the array technologies, how far the genotype (the gene profiles or fingerprints) can reflect the actual phenotype in a highly complex and readily changing disease as cancer. Proteomics will provide a closer look to this seemingly unanswerable problem. We are at the beginning of the exploration of the behavior of cancer cells in order to apply a more effective therapy based on a more reliable set of diagnostic and prognostic informations. (Pathology Oncology Research Vol 11, No 4, 197–203)

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Introduction

Prostate cancer (PRCA) is a heterogeneous disease ranging from asymptomatic to rapidly fatal systemic malignancy. The prevalence of PRCA is so high that it could be considered a normal age-related phenomenon. PRCA is a leading cause of cancer death in the Western countries (especially in the African-American population in the US), in spite of the fact that the incidence of the latent form of the disease is similar in different regions of the world and in racial groups. Understanding of the factors influencing the progression from latent to clinical cancer is essential to identify who is at high risk, and the targets to prevent and control this disease. It is a hot question today how far the molecular studies can help to extend our knowledge clarifying the mechanism of prostate carcinogenesis.

Prostate is divided into peripheral, central and transitional zones with ducts and acini lined by an epithelial sheet. Epithelium consists of a bi-layer of basal cells beneath the secretory, luminal cells, and is interspersed with neuroendocrine cells. Basal cells are mainly AR-negative stem cells which can differentiate into AR-positive luminal cells (via AR-negative transit amplifying cells) and into AR-negative neuroendocrine cells. Epithelium is surrounded by a fibromuscular stroma containing AR-positive smooth muscle cells, fibroblasts and other cells.

Development of PRCA is a multi-step process through a series of morphologically distinct lesions initiated by genetic and epigenetic changes. Regardless of the cell of origin, high-grade prostatic intraepithelial neoplasia (HGPIN) is the precursor of PRCA. PIN is present in more than 85% of PRCAs. Recently, proliferative inflammatory atrophy (PIA) has been proposed as a pre-runner of PIN. PIA is characterized with high proliferative activity, and some chromosomal and genetic changes that are present in PIN and invasive PRCA.

PRCA can be originated either from AR-negative stem cells or transit amplifying cells, or from AR-positive

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luminal cells. There may well be – at later stages – multiple subtypes of androgen-independent PRCA cells. It is important to elucidate the signaling mechanism of these androgen-independent cells in order to design effective therapeutic strategies.⁵⁹

Molecular mechanisms in PRCA genesis

In general, similarly to other cancer types, both hereditary and environmental factors can contribute to the development of PRCA.

Hereditary and predisposing factors

Although PRCA is not involved in cancer syndromes, the hereditary factors could be important in PRCA genesis (a study went up to 42% of cases). The respective genes can show high penetrance (with rare mutations) or polymorphisms with low penetrance (which could be less important, but much more frequent at population level). Linkage analysis picked some chromosomal sites carrying genes with high penetrance (none of them proved): 1p36 (CABP), 1q24q25 (HPRCA1, where RNASEL is the candidate allele), 1q42.4-q43 (PRCAAP), 8p22-23, 16q23, 17p12-13, 19q13, 20q13 (HPRCA20), Xq27-28 (HPRCAX). The most plausible candidate genes responsible for the familiar accumulation of PRCA are HPRCA2/ELAC2, RNASEL, MSR1, CAPZB, CHECK2, D-vitamin receptor and paraoxonase 1 (PON1).^{9,44}

It is believed that among the hereditary factors the germline-mutations in BRCA1 or BRCA2 can increase cancer risks, including the risk of PRCA, in male carriers.³⁵ However, another review claims that according to epidemiological and sibling studies BRCA2 is the only high-risk gene, at a relatively young age (<55 yr), and is responsible for about 5% of PRCA in this age group.¹⁴ The „treatment” of PRCA relatives is controversial. It seems to be acceptable that prostate-specific antigen (PSA) is used for screening in certain target groups (in case of early onset: <65 yr, or in familiar accumulation), but not before 35-40 yr. It should be kept in mind that PSA could be false negative, and the clinical outcome of PIN is also uncertain.

Genetic polymorphism has been described, with conflicting data in almost all cases, in several genes that take part in the sex hormone (androgen) metabolism. These genes include, among others, androgen receptor (less and shorter CAG repeats in exon 1 of AR increase the risk for PRCA), PSA, 5 α reductase type II (SRD5A2), cytochromes CYP17, CYP3A4, and ELAC2. Such genes could influence the individual sensitivity to PRCA.¹⁹ A recent study showed that the association of CYP3A4*1B, steroid hydroxylase, with prostate cancer risk is highly complex in relation to age, family history and clinical factors, suggesting the role of interactions with other endogenous factors. Consequently, the activity of CYP3A4*1B is probably not an independent risk factor.²⁶

Single gene studies

It is generally believed that cancer is resulted from the continuous accumulation and dissemination of transformed cells due to the failures of key regulatory gene programs such as cell proliferation, cell death, and of the interactions between cells and their environment. Alterations of several members of these programs have been identified in different types of cancers including PRCA. However, in the past 10-15 years only few genes were recognized that play an important role in the development of PRCA, and lesions of which are present in the majority of prostate cancers: GSTP1, PTEN, TP53, AR.⁴⁴ Many others were described as potentially non-random changes.

Chromosomal changes. Deletions are more frequent than amplifications; the former appear, presumably, at the early stage of PRCA, while the latter during progression, mainly at the hormone-refractory stage. Most common deleted regions are 8p and 13q. Potentially involved genes at 8p21 and p22 are NKX3.1, N33, FEZ1, PRTLS, while at 13q14, q21-22 and q33: RB1 (although RB mutation is rare in PRCA) and EDNRB. Most common amplified regions at 8q - 8q are MYC, elongin C, EIF3S3, KIAA1196, RAD21, PSCA (prostate stem cell antigen) and TRPS1.

Other gene lesions. GSTP1, which detoxifies environmental electrophilic carcinogens and oxidants, has the most frequent gene lesion in PRCA: methylation of the promoter region leads to inactivation. For NKX3.1, deletion is frequent, but the mutation of the remaining allele is not, resulting in haploinsufficiency where inactivation of one allele can lead to loss of function. In the case of PTEN and TP53, deletion or mutation is rare at the early stages, and somewhat more common in the advanced form. For AR, mutation is rare in non-treated PRCA, and more frequent after anti-androgens (e.g. flutamide). It is a paradox that AR can be activated by an anti-androgen. Amplification of AR can sensitize the tumor cells and the second-line androgen-blockade can be effective. In the androgen-refractory PRCA the AR could be overproduced without amplification with unknown mechanism. Decreased expression of E-cadherin, activating mutation of β -catenin and loss or mutation of KLF6 (Kruppel-like factor) are common characteristics of PRCA.

Several attempts tried to determine the cascade of genetic changes throughout the development of PRCA, and described changes from normal prostatic cells to localized prostate cancer,²⁴ as well as changes from localized to metastatic prostate cancer (*Table 1*). It is possible that activation of certain signal transducers, AKT and MAPKs (ERK, p38, JNK) support androgen-independence, while the bad prognosis is the result of overproduction of survival factors.⁵⁸

Among the epigenetic factors, the hypermethylation of the promoter region of various genes can contribute to altered cell functions. Hypermethylation can inhibit the expression of glutathione-S-transferase pi class (GSTP1) gene at an early stage of PRCA development. Studies on samples from core biopsies or paraffin-embedded samples from normal prostate (cells or tissues) found no hypermethylation of CpG islands of GSTP1, but it was present in 70% of PIN and 90% of PRCA. Hypermethylation was also identified in the blood, in the urine and in the ejaculates.⁴¹ Besides GSTP1, hypermethylation can occur in all stages of PRCA development, growth and progression involving several genes with diverse functions (e.g. suppressor genes p16, PTEN, steroid receptor family members AR and RARb2, adhesion molecules, E-cadherin, CD44, and others like RASSF1A, APRCA, PMP24).^{8,33}

If somebody tries to reach a conclusion, the outcome is rather discouraging: despite all of these efforts the results on the individual gene activities are still insufficient to explain the complexity of PRCA and to design a more effective therapy.

Microarray studies

The recent development of different array methods on gene and protein expressions promised more relevant insights into the alterations of PRCA geno- and phenotype. As in other malignancies, microarray studies identified several novel genes with potential importance in PRCA.

A comparison between normal and PIN/PRCA samples (23,040-gene array) identified 21 up-regulated and 63 down-regulated genes (e.g. OR51E2, RODH, SMS), while comparing PIN to PRCA, 41 up-regulated and 98 down-regulated genes were documented (e.g. CDKN2C, EPHA4, APOD, FASN, TIMP1).²

A selected list of genes changing during the development of PRCA is given by Calvo et al.⁵ Upregulated genes were hepsin, RabGTPase-activating protein (PRC17), calcium-binding protein (S100-P), polycomb group protein enhancer of zeste homolog 2 (EZH2), alpha-methylacyl-CoA racemase (AMACR), Wnt signaling (Wnt5A), elongin-C, prostate A-regulated transcript-1 (PART-1), claudin-8, specific granule protein-28 (SGP28), while selenoprotein-P (SePP) and Gro-2 were down-regulated. These results are puzzling from several aspects: (i) the microarray platforms recognized hardly any individual genes that were considered as important regulators in the growth of PRCA (see section Simple gene changes); (ii) the functions of these novel genes are diverse, and many of them have very little relevance to the main cellular programs according to our recent knowledge.

Hughes et al.,²¹ reviewing microarray studies, found that the following gene changes are the most important in PRCA: overexpressed – hepsin (membrane bound serine

Table 1. Genetic alterations during prostate carcinogenesis

Changes	From normal to localized cancer	From localized to metastatic cancer
Predisposing alleles	RNASEL R462Q	
Loss	8p	16q, 13q (RB1)
Inactivation		P53 GSTP1
Hypermethylation	various suppressor genes	
Decreased expression		E-cadherin
Decreased activity	vitamin D receptor	
Mutation	CAPB HPRCA1 PRCAAP MSR1 KLF6 ELAC2 HPRCA20 HPRCAX	
Increased activity	SRD5A2	

protease), AMACR (α -methylacyl coenzyme A racemase), PIM1 (protein kinase), MTA1, EZH2; underexpressed – interferons, annexins.

It is a special task to connect genetic profiles to certain tumor functions, e.g. metastatization or response to therapy. These can be called as predictive or prognostic profiles. Using disease-free survival after therapy as endpoint, molecular signatures defined a poor- and a good-prognosis subgroup. Activation of Wnt signaling pathways and decreased expression of FKL6 (COPEB) seem to be critical genes in the poor-outcome group.¹⁶

Pharmacogenomic studies suggested that the apoptotic effect of genistein is dependent on the down-regulation of NF- κ B and AKT signaling pathway, as well as uPA, MMP-9, VEGF and TGF- β .³⁴ The selenium-mediated growth inhibition is regulated, probably, by GAAD153, CHK2, p21WAF1 and cyclinA.¹²

Tissue microarray (TMA) is used mainly for protein expression studies applying immunohistochemistry, and have already produced some valuable informations, including the re-evaluation of the expression of traditional markers. A TMA study found an inverse correlation of SKP2 protein with p27^{KIP} and PTEN, and a positive correlation of SKP2 expression with pre-surgical PSA level and Gleason score. It also called the attention to the decreased expression of many annexins (I, II, IV, VII, XI).⁶²

Under the umbrella of proteomics, several sample sources gained interest in the past year both for biomarker discovery and understanding the pathobiology of PRCA. These approaches include proteomic profiling of serum, prostate cancer cell lines and tissue samples. The integration of proteomics with immunology also yields promising findings that may translate into clinically relevant bioassays.³

Progression of prostate cancer

Development of hormone-refractory cancer

Unlike in the case of breast cancer, androgen receptor status of prostate cancer is not an obligatory step of the diagnosis and prognosis. The reason for this is that PRCA primarily is an AR-expressing tumor, which phenotype does not change during the progression of the disease. On the other hand, development of the hormone-refractory form of PRCA is the hallmark of the disease. One option is that resistance to hormonal therapy of PRCA is due to development of mutations in the AR gene, however, molecular analytic studies do not support this notion.^{29,55} Genomic comparison of PRCA tissue before and after hormone therapy demonstrated an overexpression of several tyrosine kinases (SRC, EGFR, etc), resulting in the activation of the MAPK and PI3K signaling pathways.¹³ Overexpression of HRAS, RAF1, MYC, MYB, MYBL3 and TERC are also characteristic of the tumor following hormone therapy. It is of note that MYC is one of the target transcription factors of AR.⁴ Meta-analysis of 4 clinical studies on the genetics of hormone-refractory PRCA revealed that HER2 is up-regulated along with EGFR.³¹ On the other hand, amplification or overexpression of EGFR in PRCA results in activation of the MAPK pathway, leading to the transactivation of the AR, which suggests the existence of an AR-EGFR autocrine loop.^{11,18,36} One target gene of AR is PSA itself, and it can be used to test the functionality of AR expression, similarly to PR detection in ER-expressing breast cancer.³⁹ It is of interest that, during the development of hormone resistance of PRCA, AR remains fully functional, therefore the raise in PSA level during hormone therapy can be used for the detection of the recurrence.^{27,30} It is also important that hormone-independence is followed by changes in tumor suppressors (loss of KAI-1 (11p), and PTEN mutation), as well as by overproduction of EZH2 polycomb protein and the antiapoptotic BCL-2. Death ligand-mediated apoptosis may also play a role in the development of hormone resistance of PRCA. FAS ligand levels have been found to be elevated in patients along the refractory phase of the disease when PRCA recurred following hormonal therapy,¹⁵ suggesting the development of apoptosis resistance in the hormone-refractory disease. Last but not least, it seems that, similarly to other cancer types, the Wnt-1/ β -catenin system is involved in the development

of the metastatic phenotype of PRCA when overexpression of both proteins is detected in hormone-resistant cancers.⁶

Genomics of progression

Since the introduction of DNA microarray technique, several papers have been published in the literature on the expression profile and prognostic aspects of PRCA. One of the first studies identified overexpression of MTA1 metastasis-associated gene, TIMP2, THBS1 and hepsin²⁸ as characteristic gene signature for PRCA, while it found the loss of PTEN, MYC, E-cadherin and fatty acid synthase.¹⁰ Further studies identified more genes such as LTB4 hydroxydehydrogenase, lipase-H, and an integrin-linked phosphatase as part of the PRCA-specific gene signature.²⁰ This latter study identified cation channel protein TRPM8 as characteristic of recurrence. In the largest study to date, 152 human PRCA tissue samples have been analyzed. This study confirmed the PRCA-specific genes hepsin and PSMA, but suggested BMP6 as well.⁶⁴ In the down-regulated gene set, beside E-cadherin, p27, KAI-1 and caveolin were defined, while in the over-expressed gene set CD44, GST as well as FOS/JUN oncogenes have been confirmed. This genomic study identified a 50-gene set marking high Gleason score and relapse. The majority of the genes have not been reported before, most of them are relatively unknown but some of them are well-known, such as endothelin A receptor, HSP40, TGF- β or tubulin- α .

Proteomics of progression markers

Cytokine milieu in PRCA may fundamentally affect the progression of the disease. Elevated serum level of IGF was found in PRCA compared to benign prostatic hyperplasia, and was further raised with progression.³⁸ Moreover, the IGF binding protein-3 level followed a similar trend and showed strong correlation with disease progression associated with TNM stages. Statistical analysis indicated that IGFBP3 is a strong predictor of poor prognosis. Similarly, elevated TGF- β levels have been detected in parallel to the development of extracapsular disease, while down-regulation of TGF- β R1/2 was documented,⁵⁰ suggesting the development of TGF- β -independent PRCA. DNA microarray studies identified the cytokine MIC-1, a TGF- β family member, in PRCA, and further studies on prostate cancer tissues at protein level found an increased expression with higher Gleason scores.⁴⁰

Decreased E-cadherin expression has been frequently reported in PRCA, analysis of the protein expression on a large series of cases (1200) indicated decreased levels associated with high Gleason score, elevated PSA and positive surgical margins, suggesting E-cadherin as a strong negative prognostic marker.⁴⁸ Transmembrane heparan

sulfate proteoglycans frequently play a negative role in tumor progression. Expression of syndecan-1, which used to be down-regulated in epithelial cancers during malignant transformation, was analyzed in PRCA, and was found to be increased in high-grade tumors, serving as marker of poor prognosis (recurrence and shortened survival).⁶⁵

The RAS-RAF-MEK-ERK signaling pathway may play a significant role during the progression of PRCA, supported by observations of two independent studies. MEK5 overexpression was detected in PRCA, and the expression level further increased in primary lesions producing bone metastases.³⁷ Recently a new member of the metastasis suppressor genes was identified in PRCA, RKIP, which serves as an inhibitor of RAF in the protein Kinase pathway.⁶¹ The expression of RKIP was decreased with advanced stage of the disease and with increased Gleason scores. One of the expression of NF- κ B targets of the ERK-signaling is the nuclear expression NF- κ B. The level of expression of NF- κ B correlated with higher grade and stages of the disease.⁴⁶

Lipid signaling is a significant contributor to the mitogenic and motogenic signaling. Metabolism of arachidonic acid is fundamentally altered in PRCA: while COX2 is overexpressed, 15-LOX-2 is down-regulated.^{49,54} As a consequence, PRCA is enriched in prostanoids but lack 15-HETE. Prostanoids are further metabolized by thromboxane A₂, which is up-regulated in less differentiated and invasive tumors.⁴² Other hand, other reports found that platelet-type 12-LOX is ectopically expressed in PRCA, resulting in the appearance of anti-apoptotic/motogenic 12-HETE in the tumor tissue.⁵⁶

Organ-selective metastasis: the bone

One of the hallmarks of the progression of PRCA is the selective targeting of the bones. Previously it was considered to be dependent solely on anatomical factors (connection of the periprostatic veins with the perivertebral ones). However, it is now evident that PRCAs exhibit a strong organ-specificity during progression.⁴⁵ Bone-metastatic PRCAs express several ECM proteins characteristic of the bone, such as osteocalcin, osteopontin or BSP. In parallel, a significant change can be detected in the expression of matrix receptors: while almost all integrins are down-regulated, α v β 3 and the platelet-type α IIb β 3 integrins are up-regulated.^{52,57} For successful homing to the bone, PRCA employs a wide range of proteases of the MMP family (MMP-2,-7,-9, MT1-MMP), cathepsin-B, -D and -K, uPA, as well as PSA (belonging to the kallikrein family). Bone-metastatic PRCAs express acid phosphatase 5B and TRAP, which serve as sensitive markers.²³ Accordingly, bone markers in the serum can be used to monitor the progression of the disease, where osteoprotegerin and RANK-

ligand proved to be as independent strong prognostic factors.²³ Organ selectivity of PRCA progression may depend on chemokines, and a study identified IL-8 expression as strongly correlating with the progression to the bone suggesting its involvement.³²

Angiogenesis

Microvessel density of PRCA as prognostic factor is a controversial issue. Early reports determined it by the CD31 marker, known as all-round endothelial marker nowadays (vascular and lymphatic). Later on, CD34 and FVIII were used to identify blood microvessels in PRCA and it was found that, although microvessel density may correlate with stage or Gleason score, it cannot be used reliably as prognosticator for survival.^{1,43,47} A rare form of microvascular aggregation, glomeruloid microvascular proliferation, however, was proved to be a strong predictor for survival of PRCA.⁵³ Microvascular density of PRCA was dependent on p53 loss,⁶³ COX2 expression⁶⁰ and VEGF,⁵¹ while bFGF expression was negatively correlated with it.¹⁷

Lymphatic microvessel density of PRCA may also play a role in the (lymphatic) progression of the disease, and new tools are now readily available to test this hypothesis. Immunohistochemical analysis of PRCA specimens for VEGF-C/D and VEGFR-3 indicated an elevated level of these proteins.^{22,25,66} These studies revealed that VEGFR-3+ lymphatic microvessel density is associated with the incidence of lymphatic metastasis of PRCA.⁶⁶ Another study found that early stage PRCAs are characterized by VEGF-A expression, while advanced stage disease is characterized by VEGF-D(C), together with activated VEGFR-1 in early stages and activated VEGFR-2-3 in advanced stage disease.^{22,25}

Conclusion

The power of high-throughput techniques for the detection and global analysis of gene expression is unquestionable; interesting, astonishing as well as puzzling data have already been obtained. However, the standardization of the procedures is still missing, and the reproducibility is rather low in many instances. Moreover, the different array methods can select different gene expression profiles, which makes the decision rather difficult. Chiorino et al⁷ provides a detailed analysis on the interpretation of the expression data obtained from different platforms and tissue sources using PRCA data as examples. They also claim that rapid translation into clinical application is desirable and will be available when protocols become standardized and sharable.

One of the most important questions is coming again from the array technologies: how far the genotype (the gene profiles or fingerprints) can reflect the actual pheno-

type in a highly complex and readily changing disease as cancer. Proteomics will provide a closer look to this seemingly unanswerable problem. We are at the beginning to explore the behavior of cancer cells in order to apply a more effective therapy based on a more reliable set of diagnostic and prognostic informations.

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