

Review

Pathological factors evaluating prostate cancer

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Summary. Prostate cancer is one of the most prevalent malignancies for men world wide. However, only a small fraction of prostate cancer cases are metastasizing and life-threatening. Even though the detection rate of prostate cancer has been steadily increased in the last two decades due to implementation of PSA screening, it is still not clear what factors govern its clinical outcomes. In this review, we will discuss several recent pathological advances that might contribute to the progression of prostate cancer. In addition, this review will cover a brief overview on conventional morphological evaluation of prostate cancer differentiation.

Key words: Gleason grading, Hepsin, AMACR, NKX3.1, Androgen receptor, p27kip1, E-cadherin

Introduction

Prostate cancer is a disease with considerable heterogeneity in biological aggressiveness and prognosis (Carducci et al., 1996; Freedland et al., 2005). Since the implementation of serum PSA screening program, the clinical detection rate of prostate cancers has been increased substantially due to uncovering otherwise unnoticed micro-adenocarcinomas of the prostate gland. However, most of these prostate cancers grow extremely slowly. Only a small fraction of prostate cancer cases eventually develop metastasis and cancer related fatality. Most of the prostate cancers occur in advanced ages. A slow developing prostate cancer may not be clinically significant, since patients likely die from other age related illnesses. Yet, close to 30,000 patients die of prostate cancer in the U.S. annually. Currently, the most popular therapeutic options for prostate cancer treatments are surgical removal, radiation therapy and watchful waiting, based on the projected likely clinical

outcomes of the disease. In order to determine what is the best treatment option for a prostate cancer patient, there is a need for developing pathological criteria to accurately predict the behavior of the disease.

In the last several decades, much information emerges that several factors, either alone, or in combination, predict the clinical or chemical relapse of prostate cancer. Traditionally, combined Gleason scores (tumor differentiation), pathological staging (extent of tumor involvement), and PSA level have been the mainstay of criteria to predict the behavior of prostate cancer. Several new marker studies and microarray analyses recently suggest that other new factors may contain significant prognostic value in predicting the course of the disease. The goal of this review is to highlight and discuss these developments in prostate cancer research.

Gleason grading to predict prostate cancer relapse

Grading is an equivalence of artificial ranking of degree of tissue differentiation. It provides an estimation of the relatedness of a prostate cancer sample to normal prostate glands microscopically (Gleason, 1966). Although several grading systems were developed in the last half century, Gleason's grading system has been the most commonly used grading system in the US and world wide today due to its ease in grading and reproducibility. This system was first proposed by Dr. Donald Gleason in 1966 (Gleason, 1966). The principle of this grading system is to rank the differentiation of prostate cancer in a sample based on tissue architecture, with minimal consideration of tumor cell morphology. The system also takes into consideration of heterogeneity of a tumor by allowing two grading per tumor sample. A dominant architectural pattern combining with a minor architectural pattern generates a combined Gleason's score. If the second architectural pattern accounts for less than 1% of the tumor, or there is no secondary architectural pattern, the primary pattern will be counted twice to generate a combined score.

The Gleason's score of a tumor sample ranges from

2-10. A tumor sample with combined Gleason's score between 2-4 is considered well differentiated, 5-6 moderately differentiated, and 7-10 poorly differentiated. The Gleason grading system, like other histological grading system, is not immune to inherent subjectivity. Intraobserver and interobserver variation do exist. Confusion in grading may occur when there are more than two Gleason's patterns in a tumor sample. Since development of primary prostate cancer is closely related to its surrounding stroma, metastatic prostate cancer samples have different stromal milieu. As a result, metastatic prostate cancer may have so distinct differentiation pattern that Gleason's grading is no longer applicable. Similarly, therapies that potentially alter the differentiation and survival of prostate cancer cell would have profound impact on the Gleason's pattern. Grading on those samples that have undergone radiation or hormonal therapies has to be extremely cautious.

Studies in 80' and 90' indicate that Gleason's grade is a significant predictor of clinical outcome (Bostwick and Foster, 1999; Cheng et al., 1998; Epstein et al., 1993; Ornstein et al., 1998; Zagars et al., 1995, 1997). This includes clinical stage, response to different therapies, PSA failure free survival or physical evidences of the presence of metastases. Gleason's grade from needle biopsy samples, along with age, the extent of cancer involvement, is one of the major factors in determining the type of therapies a patient will receive. Men with advanced age and low Gleason's grade are more likely to elect for watchful waiting as cancer management, while higher Gleason's grading and large tumor volume would warrant more aggressive therapies

such as radiation or radical prostatectomy. Gleason's grade obtained from specimens of radical prostatectomy is one of the most useful predictor of PSA failure after surgery (Bostwick and Foster, 1999). Gleason's grade above 8 poses the greatest risk of recurrence, independent of serum PSA level, pathological stages and the tumor volume. Some study suggests that even a tertiary minor high grade Gleason's score would associate a higher risk of tumor recurrence. Even though high Gleason's grading is associated with risk of extracapsular invasion of the tumor, and to a less extent, pelvic lymph node involvement, it is not certain that high Gleason's grade is a predictor of distant metastasis.

Molecular markers for prostate cancer

Molecular markers are increasingly becoming an important tool in assisting and supplementing morphological diagnosis of prostate cancer. Many markers were found to be independent predictor of PSA recurrence or prostate cancer related mortalities. In the last several years, the number of molecular markers related to prostate cancer has been dramatically increased due to genome wide mRNA analysis by expression microarray. This review will focus several promising molecules (Table 1).

Hepsin

Hepsin is a transmembrane serine protease originally isolated from normal hepatocytes (Leytus et al., 1988). However, it was found later that hepsin was expressed in most of the human tissues (Tsuji et al., 1991). Structural

Table 1. Summary of genome abnormalities in prostate cancers.

Locus	Chromosomal abnormality	Genes identified	Gene abnormality
1p33		TNFRSF6	Expression suppression
4q24-25		Myopodin	Deletion
8p21-8p23	Deletion	N33	Expression suppression
		FEZ1	Mutation
		Dermatin	Deletion
		NKx3.1	Expression suppression
		CSR1	Methylation
7q31	Amplification	MCM7	Overexpression
8q	Amplification	PSCA, MYC and eIF3	Over-expression in prostate cancers
9q21	Deletion	p16	Hypermethylation and deletion
10p		KLF6	Mutation
10q23		PTEN/MMAC1	Mutation
10q21		ANX7	LOH
11q13		GSTpi	Hypermethylation
11p11.2-13		KAI1	Hypermethylation
11p13		CD44	Hypermethylation
12q12-13	Deletion	p27	Expression suppression
13q21-22	LOH	PCD1	Over expression in cancer lines
		Endothelin b receptor	Hypermethylation
15q21-22		Annexin II	Expression suppression in prostate cancers
16q22.1	Deletion	E-cadherin or deletion)	Hypermethylation
17q21	LOH	BRCA1	Mutation?
21q22.	Fusion	TMPRSS2/ERG	Fusion

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analysis indicates that hepsin forms a transmembrane protein with a small cytoplasmic domain, a transmembrane domain and 373 residue C-terminus domain in the extracellular surface. Recent study indicates that hepsin activates pro-urokinase type plasminogen activator by cleaving on its lysine158-isoleucine159 peptide bond (Moran et al., 2006). Over-expression of hepsin does not result in higher level of cell growth and proliferation (Srikantan et al., 2002). Instead, hepsin expression disrupts basement membrane formation and promotes invasion and metastasis (Klezovitch et al., 2004).

Several microarray analyses indicated over-expression of hepsin in prostate cancer samples (Dhanasekaran et al., 2001; Luo et al., 2001, 2002b). However, hepsin overexpression in prostatic intraepithelial neoplasia (PIN) was also identified. Increased expression of hepsin is also seen in morphological benign tissues adjacent to prostate cancer. Thus, hepsin is quite likely one of the field effect genes and its expression alteration occurs in the early stages of carcinoma. It is not certain what role hepsin plays in clinical metastasis of prostate cancer, even though its biological function appears to relate to cancer invasion.

Alpha-methylacyl-CoA racemase

AMACR is responsible for conversion of pristanoyl-CoA and acyl-CoA to their (s)-stereoisomers, and allows the subsequent degradation of these substrates through β -oxidation (Schmitz et al., 1995). Reduction of AMACR level in prostate cancer cell line LAPC-4 inhibits androgen independent cell growth and induced cell cycle arrest (Zha et al., 2003). Several microarray analyses at mRNA level identified that AMACR encoding mRNA were elevated in prostate cancer (Luo et al., 2002a; Rubin et al., 2002). Subsequent large-scale tissue array analysis indicated that up to 88% of prostate cancer samples had elevated AMACR immunostaining levels (Rubin et al., 2002). Strong immunostaining of AMACR was also found elevated in high grade PIN. A recent study suggested that AMACR expression was elevated in several other human malignancies, including hepatocellular carcinoma, papillary renal cell carcinoma and colorectal cancer (Nassar et al., 2005).

Utility of AMACR in diagnosing and prognosticating prostate cancer had been extensively evaluated. Most of the analyses demonstrate some value of AMACR immunostaining in prostate biopsies in improving positive identification of prostate cancer in small focal areas. It appears that AMACR staining in combination of high molecular weight cytokeratin have substantial higher accuracy of identifying prostate cancer than either staining alone (Martens and Keller, 2006). Increased protein level of AMACR appears to elicit antibody generation against this enzyme. As a result, quantifying the prostate cancer patient's anti-AMACR antibodies has been proposed to be used as a sera marker for detection of primary prostate cancer (Sreekumar et al., 2004). Little information was obtained thus far for

relationship between prostate cancer behavior and AMACR expression, even though some indicate that lower level expression of AMACR occurs in metastatic prostate cancer samples. Lower AMACR expression in tumor tissues signals higher level of chemical failure.

NKX3.1

NKX3.1 was originally identified as a homologue of *Drosophila* NK homeobox gene. It is highly expressed in prostate tissue but little elsewhere (Bieberich et al., 1996). It attracts some interest because NKX3.1 was located in 8p21, a locus frequently deleted in aggressive prostate cancer. NKX3.1 appears negatively regulate androgen receptor (AR) expression (Jiang et al., 2004; Simmons and Horowitz, 2006). Overexpression of NKX3.1 induces p53 transcription through increased acetylation of its associated histones. Thus, it suppresses androgen mediated cell growth and induces arrest of cell cycle (Lei et al., 2006). The abnormality of NKX3.1 level in prostate cancer is mostly related to its loss of heterozygosity (LOH) in prostate cancer cells and hypermethylation of its promoter region (Voeller et al., 1997; Asatiani et al., 2005). As a result, expression of NKX3.1 is suppressed. Several studies indicate a significant weakening of NKX3.1 immunostaining in cancer cells in comparison with adjacent normal acinar cells (Xu et al., 2000; Gelmann et al., 2003; Aslan et al., 2006). Some study suggested that translational regulation or protein degradation also contributed to the lower level of NKX3.1 protein in prostate cancer cells since the correlation of mRNA and protein levels were marginal.

Androgen receptor

Experiments in 80's and early 90' indicated that growth and development of normal prostate gland was dependent on androgen (Rennie et al., 1988). This was demonstrated by induction of rapid prostate gland atrophy after animal castration. The castration induced prostate gland atrophy could be reversed by administration of exogenous testosterone (English et al., 1989; Furuya and Isaacs, 1993). Majority of prostate cancers are also androgen dependent. Intervening androgen receptor signaling or androgen ablation has been the paradigm of prostate cancer therapy for the last 40 years. Most of the studies have been focusing on androgen mediated transcription activity that induces differentiation of prostate epithelial cells. One study suggested that activation of AR induced prostate cancer cell proliferation by enhancing the translation of cyclin D1 (Burd et al., 2005). Another study suggested that AR was a DNA replication licensing factor in prostate cancer cells based on the fact of phase-specific degradation of AR in these cells (Litvinov et al., 2006). Mutations of androgen receptor were found in both primary and metastatic prostate cancer samples (Suzuki et al., 1993). Some of these mutations, however, were associated with hormonal treatment. The hormonal naïve

mutations, nonetheless, appeared to alter AR binding activity with its ligand (Sluysers, 1994). No definitive association of androgen receptor mutations and prostate cancer relapse is found. Amplification of AR was found in up to 30% recurrent prostate cancer after androgen ablation (Visakorpi et al., 1995), raising the possibility that amplification of AR as a result of selective growth of poorly differentiated and unstable cancer cells resistant to the therapy. Over-expression of androgen receptor has been correlated with proliferative activity of cancer cells, and to a less extent, with poor recurrence-free survival (Henshall et al., 2001). High level of androgen receptor heterogeneity in terms of expression levels is also associated with aggressiveness of prostate cancer.

p27kip1

p27kip1 is a member of cyclin-dependent kinase inhibitor. It binds to cyclin D, E and A dependent kinase complex and inhibits their activity (Polyak et al., 1994). Expression of *p27kip1* induces cell cycle arrest. *p27kip1* is generally expressed in quiescent prostate epithelial cells but is inhibited in prostate stem cells.

p27kip1 is probably the most informative marker of prostate cancer behavior among cell cycle regulating genes. Even though there is little evidence of mutations of *p27* in human malignancies, it was generally accepted that *p27* contains tumor suppressor activity. Deficiency of *p27* propels the transformation of high grade PIN to outright cancer in mice. Many studies have correlated the loss of expression of *p27kip1* with increased invasiveness and clinical relapse of prostate cancer (Yang et al., 1998; Ribal et al., 2003). Lower levels of *p27kip1* expression are generally associated with higher tumor grades and more advanced pathology stages (Tsihlias et al., 1998). Loss of expression of *p27kip1* in prostate cancer is thought to result from increased proteolytic degradation of this protein rather than reduced level of transcription. The expression of *Skp2*, a protease that recognizes phosphorylated *p27kip1* and degrades it through ubiquitination, is inversely correlated that of *p27kip1* in some prostate cancer samples (Yang et al., 2002; Ben-Izhak et al., 2003), suggesting that *Skp2* might play a role in *p27kip1* down-regulation in prostate cancer. Androgen stimulation decreases the expression of *p27kip1*, while castration or using androgen antagonists increase its expression level. However, some studies indicated that up to 50% metastatic prostate cancer samples contained hemizygous or homozygous deletion of *p27kip1* (Kibel et al., 2000; Dong, 2001), suggesting genome alterations were the basis of its loss of expression for aggressive types of cancer.

E-cadherin

E-cadherin is one of the adhesive molecules involving in epithelial cell-cell interaction in normal

prostate gland. E-cadherin is complexed with actin-cytokeratin through cytoplasmic catenins to maintain epithelial differentiation. The tumor suppressor activity of E-cadherin is thought to be mediated its signaling through β -catenin, independent of its adhesive activity. Reduction of E-cadherin releases β -catenin from the complex, and results in increasing translocation of β -catenin into nucleus and activation of oncogenic and pro-growth genes such as C-MYC. The tumor suppressor activity of E-cadherin was demonstrated both in vitro cell culture system through standard colony formation and soft agar growth (Quinn et al., 2005). Knock-out of E-cadherin propelled pancreatic adenoma into adenocarcinoma in rotten model (Perl et al., 1998). Mutations of E-cadherin in several human malignancies were reported (Isaacs et al., 1994; Chesire et al., 2000; Graziano et al., 2003). Most of the down-regulation of E-cadherin is a result of transcription suppression or methylation in the CpG island of its promoter area, rather than a result of chromosomal deletion (Li et al., 2001; Woodson et al., 2003). Down-regulation of E-cadherin is thought to relate to higher aggressiveness of cancers.

Altered expression of E-cadherin has been extensively studied. Decreased expression of E-cadherin is associated with poor differentiation and advanced pathological stages of prostate cancer. Loss of membranous expression of E-cadherin in prostate cancer signals higher level of prostate cancer relapse after prostatectomy (Li et al., 2001; Woodson et al., 2003). However, expression of E-cadherin in prostate cancer is quite heterogeneous. This makes it difficult to evaluate its potential as a marker for clinical prognosis simply based on immunostaining.

Other potential markers for prostate cancer

The advance of several DNA subtraction technologies allows rapid identification of genes that are deleted or amplified in prostate cancer. One example is pTEN, which was identified through combination of positional cloning and representational difference analysis. pTEN has dual tyrosine/phospholipid phosphatase activity, and is mutated in several types of tumors, including 10% of primary prostate cancer (Li et al., 1997; Steck et al., 1997). Disruption of pTEN expression in the mouse produced a dysplastic prostate phenotype (Di Cristofano et al., 1998; Kwabi-Addo et al., 2001; Park et al., 2002). Loss of pTEN expression in prostate cancer appeared correlated with poor differentiation of the tumour (Celebi et al., 2000). Myopodin, a gene involved in migration retardation and actin bundling, was identified through genomic differential subtraction chain and cDNA library screening (Lin et al., 2001a). It was found deleted in up to 50% aggressive type of prostate cancer. Loss of nuclear localization of myopodin is thought to associate metastasis in urothelial carcinoma. A recent immunohistochemistry screening of a large number of

prostate cancer samples suggested that complete loss of myopodin expression in prostate cancer samples predicted up to 83% of prostate cancer chemical relapse and was independent of Gleason's grading (Yu et al., 2006b). MCM7 is a DNA licensing protein. Overexpression of MCM7 was implicated in some microarray analysis of colon cancer. Over expression of MCM7 protein was also found in some samples of cervical cancer. Recently, using a modified oligonucleotide comparative genome hybridization array analysis, Ren et al found that MCM7 was amplified in up to 80% of prostate cancer cases that relapsed within 5 years. Over-expression of MCM7 in xenografted tumors induced dramatic higher rate of metastasis and cancer related mortality in mouse model (Ren et al., 2006). Using bioinformatics, DNA sequencing and FISH analysis, Tomlin et al. identified a fusion of 5' untranslated region of TMPRSS2, a multimeric protease to ERG, a DNA binding protein, in 23 of 29 prostate cancer samples (Tomlins et al., 2005). The fusion resulted in concomitant deletion of DNA sequence between these two genes (Perner et al., 2006). Characterizing the physiological role of this fusion gene might hold some promise for our understanding of prostate cancer development.

Altered methylation in prostate cancer

Altered methylation activity in malignancy in general was reported as early as 1971 (Pillinger and Wilkinson, 1971). The gene encoding GSTpi, an enzyme involved in detoxification and xenobiotic metabolism, was identified as one of the most widely methylated targets in prostate cancer. Methylation of cytidine nucleotides (CpG islands) in GSTpi regulatory sequences occurs in the majority of prostate cancer and its precursors and is responsible for downregulation of GSTpi expression in most prostate cancer cases (Brooks et al., 1998; Lee et al., 1994; Millar et al., 1999; Santourlidis et al., 1999; Lin et al., 2001b). However, GSTpi is not a tumour suppressor gene, since overexpression of GSTpi does not suppress tumour growth and no mutation has been identified in GSTpi sequence among prostate cancer samples (Lin et al., 2001b). Nevertheless, decreased expression of GSTpi probably plays a role in susceptibility to prostate cancer by exposing the genome to free oxidative radicals such that it results in irreversible genomic damage.

The gene encoding CD44, another potential marker for tumour progression and metastasis, was found to be hypermethylated in CD44-deficient cell lines (Verkaik et al., 1999). CpG islands of promoter sequence were methylated in a substantial number of samples of prostate cancer with low CD44 expression (Lou et al., 1999), and this appeared to correlate with progression and metastasis (Kito et al., 2001). However, other transcription regulation mechanisms were probably also involved since some of the CD44-negative samples were not methylated (Verkaik et al., 2000). CD44 is an

integral membrane protein involved in matrix adhesion. Downregulation of CD44 in metastatic prostate cancer might alter cell–matrix interaction in favor of invasion and metastasis.

Recently, a gene called “cellular stress response 1” (CSR1) was isolated and analyzed. The function of this protein is still not entirely clear. Some suggest that it may induce apoptosis upon stress. Yu et al. found that CSR1 was hypermethylated in a subset of prostate cancer that were highly aggressive (Yu et al., 2006a). Immunostaining analysis of CSR1 revealed that CSR1 protein was located in plasma membrane and cytoplasm in normal prostate epithelial cells. There was a dramatic decrease of CSR1 expression in prostate cancer (Yu et al., 2006a). Cases with decreased CSR1 expression had poorer clinical outcome than those without. Only a fraction of prostate cancer samples whose CSR1 was down-regulated was methylated, suggesting other mechanisms might involve its expression suppression. Even though CSR1 is located in 8p21 region, deletion of CSR1 is rare in prostate cancer samples.

The promoter regions of several other genes have been suggested being hypermethylated in prostate cancer, including stathmin, neutral endopeptidase 24.11, p16, caveolin-1, tumour necrosis factor receptor superfamily 6 gene (TNFRSF6), annexin II and several Y chromosome genes (Prasad et al., 1999; Usmani et al., 2000; Chetcuti et al., 2001; Cui et al., 2001; Santourlidis et al., 2001; Dasari et al., 2002). Some of these methylation change might play important roles in developing prostate cancer (Chetcuti et al., 2001; Nakayama et al., 2001; Santourlidis et al., 2001; Tamada et al., 2001; Dasari et al., 2002). In a recent study of methylation profiling of ten genes in prostate cancer, RARbeta, RASSF1A and GSTPI were methylated in a third to over half of the prostate cancer cases (Maruyama et al., 2002). A methylation index was derived to reflect the methylation fraction of these genes. The methylation index appeared to correlate with differentiation states of these tumours, and might have prognostic values (Maruyama et al., 2002). When methylation profiling was expanded to include over 100 cancer related genes, a dramatic difference in methylation pattern between normal prostate tissues and prostate cancers was found (Yu et al., 2005).

High-throughput gene expression analysis

High-throughput gene expression profiling has increasingly become an important technology to detect patterns of gene expression alteration in cells. Since prostate cancer is highly heterogeneous, it is of interest to classify prostate cancer based on altered patterns of gene expression. Earlier microarray analyses used relatively small numbers of genes in the array analyses to compare normal versus prostate cancer samples, but were nevertheless able to identify new genes related to prostate cancer. Using a pre-selected gene microarray, one report suggested that expression of genes involving

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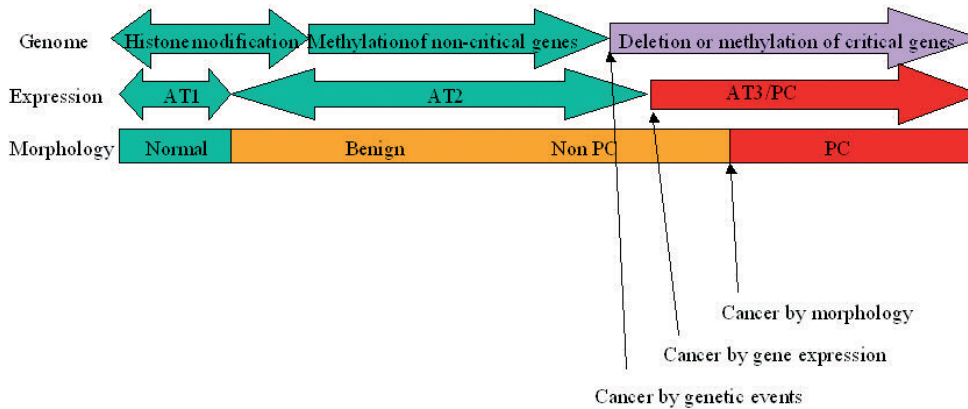


Fig. 1. Schema of development of prostate cancer and its underlying genomic and gene expression alteration. AT1-normal gene expression pattern; AT2-altered gene expression pattern but less than 90% overlapping with those of prostate cancer; AT3-altered gene expression pattern over 90% overlapping with those of prostate cancer.

fatty acid synthesis was upregulated in prostate cancer (Swinnen et al., 2000). Larger-scale gene expression profiling on prostate cancer and non-tumour prostate samples were performed recently. These studies ranged from analyses of 6500 to over 40000 genes and expressed-sequence tags (ESTs) (Dhanasekaran et al., 2001; Luo et al., 2001, 2002a,b; Stamey et al., 2001; Welsh et al., 2001; Singh et al., 2002). The signature gene expression alteration patterns appeared to overlap significantly among these studies, even though the methodologies, sample selection and processing varied considerably. All of these studies suggested that hepsin, a transmembrane protease overexpressed in hepatomas, was an over-expression marker associated with prostate cancer. Downregulation of PIM1, a protein kinase that was overexpressed in organ-confined prostate cancer, was also similarly associated with a relapse of prostate cancer (Dhanasekaran et al., 2001). Another study identified 12 genes and ESTs whose alterations in expression were associated with aggressive tumor behavior (Luo et al., 2002a,b). Both these studies indicated that patterns of gene expression in metastatic and poorly differentiated tumors tended to cluster separately from organ-confined prostate cancer. Three studies suggest that pathological stages or clinical outcomes of prostate cancer can be predicted through identification of unique gene expression patterns for those samples. These studies were relatively large and outcome predictions were validated through vigorous statistical analysis (Singh et al., 2002; Glinsky et al., 2004; Yu et al., 2004). It should provide insight into the mechanisms of tumor metastasis and invasion. However, the gene lists that differentiate poor outcome from good one appear quite different among these studies, even though they all used Affymetrix array platform. Perhaps tumor heterogeneity and sample selection variation play roles in causing these discrepancies. Gene profile analysis of prostate cancer also yields a surprising finding: field effect. The field effect on the benign tissues adjacent to prostate cancer is so persuasive that practically up to 90% of benign prostate tissues adjacent

to prostate cancer contain gene expression alteration resembling prostate cancer. This appears to support the notion that genetic alteration precedes the development of prostate cancer. Genetic analysis, as a result, uncovers cancer that is not recognizable by morphology evaluation.

Conclusion

Prostate cancer is a highly heterogeneous disease. So far, 21 of the 23 pairs of human chromosomes have been identified as having abnormalities in subsets of prostate cancer and a plethora of information on candidate loci has exploded in this area. The changes include both loss of function and gain of function, resulting from gene loss, duplication, point mutation, fusion protein formation and gene silencing. There is a growing body of literatures suggesting that the cancer-causing genetic alterations occur in morphologically benign prostate tissues. As a result, prostate cancer might start with a set of common genetic events. The disease subsequently follows a period of reversible genomic alterations as a consequence of the interaction between the environment and genetic susceptibility (Fig. 1). When one or several critical genetic events occur, such as deletion or methylation of a critical tumor suppressor gene, or amplification of a proto-oncogene, or formation of a new oncogenic fusion protein, a malignancy cascade becomes irreversible, even though morphologically the cells may be "benign". When cells are totally off-balance in controlling their growth and metabolism, cancer phenotype would become obvious.

It will be intriguing to see whether the advent of high-throughput technologies for analyzing genomes and gene expression profiles in prostate cancer can help to sort through these abnormalities and produce a better understanding of prostate cancer. In order to generate a reasonable understanding of the genetic makeup of prostate cancer, the future direction of prostate cancer research will probably be directed towards applying the convertible and standardized high-throughput

technologies to analyze and pool large-scale prostate cancer data to connect the currently fragmented information. The advent of the high throughput gene expression technology capable of analyzing multiples genes simultaneously also makes single gene evaluation of prostate cancer unjustifiable, unless abnormality of one gene contains overriding power in predicting the prognosis of prostate cancer. The progress in molecular characterization of prostate cancer in recent years has become quite compelling and cannot be ignored for its potential usage in clinical setting. In the foreseeable future, prostate cancer can be subclassified and categorized based on the combination of genetic make-up and morphological evaluation of the disease. Since such classification reflects the pathogenic mechanisms, it may help development of gene-target-specific therapies for the treatment of the disease.

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