Molecular mechanisms of prostate cancer

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Introduction

In recent years, our insight into the molecular biology of the cell has increased substantially. This knowledge has accumulated in the elucidation of the sequence of the almost complete human genome of approximately 3.2×10^9 bp [1,2]. This large genome is expected to contain a surprisingly small number of genes: 30 to 40 thousand. Prior to, and in parallel with, the elucidation of the human genome sequence, technology has been developed, which enables the detailed study of the expression of individual genes and the properties of their protein products. More recently, it has become feasible to study the gene expression patterns of large numbers of genes simultaneously (transcription profiling and protein profiling). The insight into the molecular biology of the normal cell, and the interaction with its environment, combined with the available technology, is currently being explored for the detailed molecular characterization of cancer cells. It is expected that, not only the major molecular pathways of cancer development will be elucidated, but also that cancer diagnosis will be improved and that novel therapeutic targets will become available in the near future.

According to generally accepted concepts, a cancer cell exhibits a number of unique properties, which distinguishes it from its normal counterpart. Major differences are an increased growth rate, loss of differentiation capability, escape from programmed cell death (apoptosis) and escape from senescence. Differences between tumour cells and normal cells are the results of genomic instability of the tumour cell. Genomic instability is characterized by chromosomal aberrations, including chromosome deletions, amplifications and translocations. Importantly, mutations in specific genes involved in cell growth and cell survival are frequently detected in tumour cells. The accumulation of multiple genetic alterations drives the progressive transformation of the normal cell into a highly malignant derivative.

Historically, two classes of tumour genes have been discriminated, i.e., oncogenes, and tumour suppressor genes. Alterations in genes involved in the repair of DNA damage seem to be very important in the development of a mutator phenotype in tumour cells [3,4]. More recently, a picture is emerging indicating that not only functional alterations in genes, but also modulation of gene expression induced by a broad variety of molecular mechanisms can contribute to the development of a cancer cell. In addition, it has been recognized that the microenvironment can affect gene expression patterns in the tumour cell, and, as a consequence, can influence its biological properties (see for a review Ref. [5]). An overall molecular model for tumorigenesis can be postulated, which combines specific irreversible activating or inactivating mutations in a limited number of genes with, to a certain extent, reversible patterns of altered expression of a large number of genes.

General aspects of prostate cancer

Prostate cancer is the most frequently diagnosed cancer in men in Northern and Western Europe, Northern America and Australia, and the second leading cause of male cancer deaths in these parts of the world. Because the population in Western countries is ageing and because other countries tend to adopt a Western life style, prostate cancer is an increasing medical problem. In both Europe and the United States, over 200,000 new cases of prostate cancer are diagnosed each year, and approximately 40,000 men die of prostate cancer [6].

Prostate cancer is often detected at an advanced stage, because of its location at an inaccessible site. Therefore, its natural progression pattern has been difficult to monitor. However, several steps in its morphological development have been identified, which might give insight into the path of progression. The vast majority of prostate cancers are adenocarcinomas. A now clearly recognized histological precursor of prostate cancer is the prostate intra-epithelial neoplastic (PIN) lesion, which is characterized by a multilayer of luminal epithelial cells and a disappear-

ing basal epithelial cell layer [7]. It is believed that, most, if not all, prostate cancers develop from such PIN lesions. At the cellular level, it is thought that prostate cancer cells result from aberrant differentiation of a putative prostate stem cell into the luminal epithelial cells. Prostate cancer lesions show a remarkable heterogeneous and multifocal appearance. This makes it difficult to obtain sufficient amounts of homogeneous tumour tissue for molecular studies.

Currently, the diagnosis prostate cancer is based upon an elevated level of the serum marker Prostate Specific Antigen (PSA), rectal examination, echoscopy and histological examination of a biopsy specimen. If the tumour is confined to the prostate, surgical intervention based on complete excision of the prostate can be performed. Because growth of the majority of prostate cancers depends on continuous androgenic stimulation, therapy of metastatic disease is generally based upon androgen ablation or blockade of androgen receptor function by anti-androgens. Unfortunately, although the majority of metastatic tumours initially respond to endocrine manipulation, inevitably, within a few years a hormone-refractory, and now lethal, tumour continues to grow.

Hereditary prostate cancer

Most cancers can be genetically subdivided into hereditary and sporadic forms. In hereditary cancers, at least one genetic defect is already present in the germ line. Therefore, it is not surprising that hereditary cancers are associated with an early onset of the disease. Hereditary prostate cancer accounts for 10% or less of all patients [8]. Linkage analysis indicates a complex pattern of different hereditary prostate cancer loci on chromosomes 1q24–25, 1p36, 1q42–43, 20q13 and Xq27–28, respectively. Although an extensive search for the tumour genes in these loci has been carried out, they have not been identified, as yet. Recently, Tavtigian reported the *ELAC2* gene with an unknown function on chromosome arm 17p as a candidate prostate cancer gene. However, the relevance of this gene remains to be confirmed [9].

Chromosomal aberrations in sporadic prostate cancer

Several different techniques are available to study consistent chromosomal aberrations in tumour cells. These include comparative genomic hybridization (CGH), allelotyping with highly polymorphic microsatellite markers, and in situ hybridization on cell nuclei with chromosome centromere or gene-specific hybridization probes. Most recently, array-CGH has been added to this panel of experimental approaches [10]. According to classical concepts, oncogenes can be mapped in amplified chromosomal regions, and recessive tumour suppressor genes should be located in regions with frequent chromosomal loss. In prostate cancer, several characteristic, amplified or deleted chromosomal regions have been identified by CGH and whole genome allelotyping [11–13]. Most frequent deletions have been found for chromosome arms 8p, 13q and 16q; 6q, 10q and 17p are less frequently deleted. Chromosome arm 8q is amplified in a high percentage of late stage prostate cancers. Part of the chromosomal defects (e.g. deletions) can already be recognized in PIN lesions, however, chromosomal alterations are most frequent in tumour metastases. In a subset of endocrine therapy-refractory prostate cancers, amplification of a specific part of the X chromosome has been found [14,15]. An example of a CGH analysis of a prostate cancer DNA is shown in Fig. 1. In this DNA some of the most common chromosomal alterations in prostate cancer are present, including amplification of 7 and 8q (bars at the right side of the chromosome), and deletion of 6q, 8p and 13q (bars at the left side of the chromosome). Other chromosomal alterations are specific for this individual tumour.

Oncogenes and tumour suppressor genes in prostate cancer

As far as it is known at this time, out of the many known classical oncogenes and tumour suppressor genes, only mutations in the *TP53* gene and in the *PTEN* gene contribute substantially to prostate cancer.

TP53 maps at chromosome 17p13. It is the most commonly mutated gene in human cancer. *TP53* mutation leads to the accumulation of an aberrant p53 protein in the nucleus, allowing its immunohistochemical detection. P53 plays a pivotal role in the cell cycle as a G1 checkpoint, regulating the transition into S-phase. Under conditions that promote DNA damage, p53 will either induce growth arrest or commit the cell to a programmed cell death pathway. In Li Fraumeni syndrome, germline *TP53* mutations cause a predisposition to various tumour types. In organ-confined prostate cancer, *TP53* mutations can be found in less than 10% of all tumours. At metastatic sites, *TP53* mutations might be found in 50% [16,17]. However, there is no consensus about the prognostic importance of *TP53* mutations [18,19].

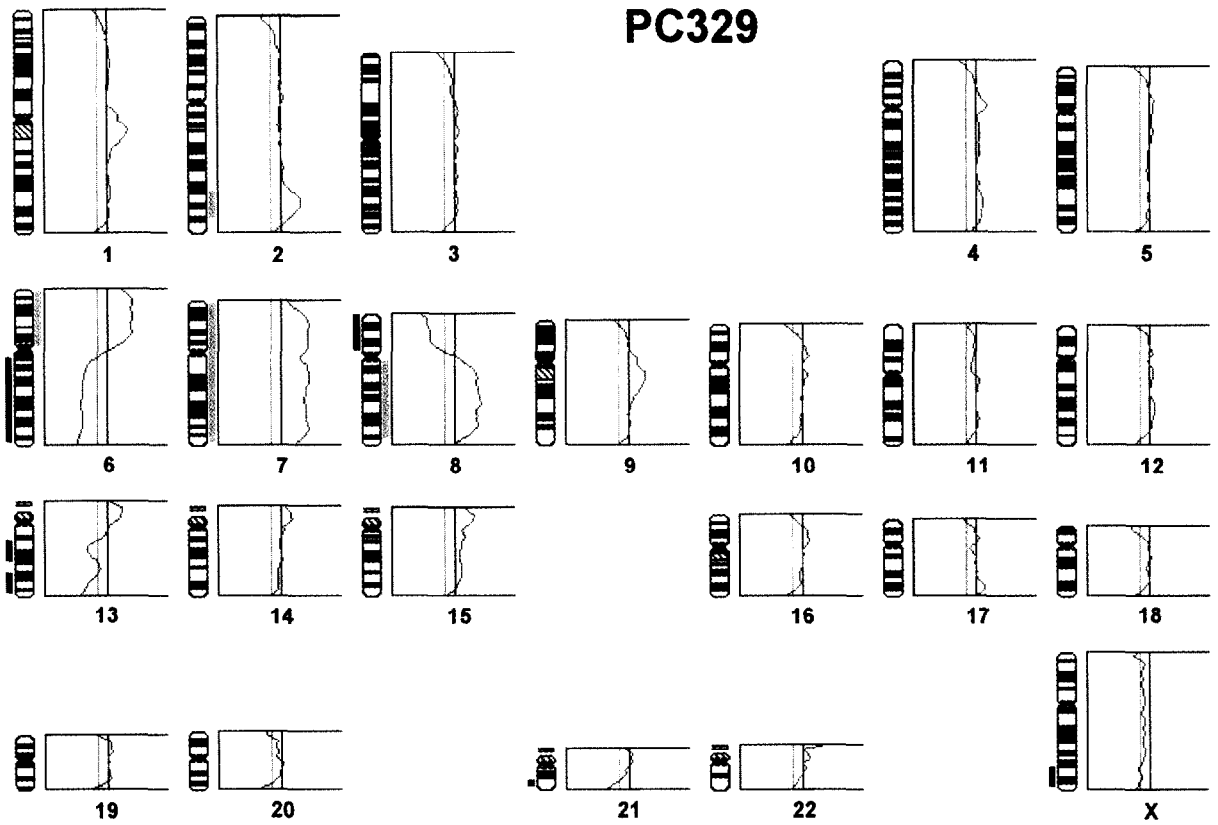

Fig. 1. Comparative genomic hybridization of prostate cancer xenograft PC329. Deletions (bar at the left side of each chromosome): 6q, 8p, 13q, 21q, Xq. Amplifications (bars at the right site of each chromosome): 2q, 6p, 7, 8q.

The *PTEN* tumour suppressor gene, also known as *MMAC1* or *TEP1*, is located at chromosomal band 10q23.3. PTEN is able to dephosphorylate the phospholipid PI(3,4,5)P3, which mediates intracellular signal transduction pathways through the protein kinase Akt/Protein Kinase B (PKB) [20,21]. Constitutive activation of Akt, due to absence of a functional PTEN leads to inhibition of apoptosis and stimulation of the cell cycle. Amongst the Akt target proteins are proteins directed or indirectly involved in cell cycle regulation and apoptosis, like GSK3, BAD and several members of the forkhead family of transcription factors. Germ line mutations in *PTEN* are the cause of Cowden's disease, which is characterized by hamartomas and a predisposition to several cancers. *PTEN* mutations are very frequent in prostate cancer cell lines and prostate cancer xenografts propagated on nude mice [22]. *PTEN* defects have been found in 5% of primary prostate tumours and in 30–60% of local and distal metastases [23,24]. More recent data indicate that PTEN is not only inactivated by mutation, but also by downregulation of expression [25].

Although the various specific chromosomal alterations in prostate cancer have been known for over three years, the identification of novel candidate tumour genes based on the most commonly deleted or amplified chromosomal regions has been disappointing (*PTEN* is the exception). Many reasons might be given to explain this failure. First of all, the search for novel tumour genes has been complicated by the fact that most deleted or amplified chromosomal regions in prostate cancer are large and encompass many hundreds of genes. In addition, inactivation of tumour suppressor genes may be mainly by point mutations, which are difficult to detect, and not by partial or complete deletion of a gene. Although several homozygous deletions, which may pinpoint the localization of a tumour suppressor gene have been reported on chromosome 8p, a bona fide cancer gene in these deleted regions has not as yet been described. Amplified oncogenes are difficult to identify, because high-level amplifications seem to be rare. The most promising candidate genes on 8q are *MYC*, prostate stem cell antigen (*PSCA*) and the translation initiation factor *eIF3–p40* [26,27].

Gene expression patterns in prostate cancer

The failure to detect novel tumour genes may lie in the fact that the concept of mutation, deletion or amplification of a limited number of genes involved in tumorigenesis is wrong. Presumably, it should be replaced by a modified concept, which takes also into account the overexpression and downregulation of expression of a large number of genes, due to duplication or deletion of large chromosomal areas, respectively, or by different mechanisms of regulation of gene expression. Other mechanisms of more global downregulation of gene expression can be promoter hypermethylation, for which an explosion of information is becoming available, or altered chromatin structure by histone modification [28,29]. A complicating factor in downregulation or upregulation of gene expression as a molecular mechanism of tumour growth, is the difficulty to prove that it is the cause, and not the result, of tumour development.

In prostate cancer, for many individual genes downregulation of expression by promoter hypermethylation has been documented. Well-known examples are decreased expression of *E-cadherin*, which is involved in cell–cell interaction, and glutathione-S-transferase (*GSTP1*), which plays a role in protection of the cell against carcinogens [30,31]. Another important gene that is downregulated in prostate cancer is the negative cell cycle regulator *p27Kip1* [32]. However, the molecular mechanism of *p27Kip1* downregulation is not completely understood.

During the last few years, methodology for high-throughput transcription profiling has been developed (cDNA- and oligonucleotide microarrays), and applied in comparisons of gene expression profiles in normal and cancer cells [33,34]. It can be expected that this technology will be of great help in the elucidation of the various signal transduction pathways involved in prostate cancer and, hopefully, in the improvement of prostate cancer diagnosis.

The androgen receptor

A protein that has been studied extensively in relationship with prostate cancer is the androgen receptor. The male sex hormones or androgens, testosterone, which is mainly produced in the testis, and the metabolite 5α-dihydrotestosterone, play a key role in prostate development and in maintenance of the structure and function of the adult prostate. The biological function of androgens is mediated by the androgen receptor.

Initially, the growth of most prostate tumours is androgen-dependent. Consequently, many different regimens of endocrine manipulation, aiming at testosterone depletion by chemical or surgical castration, or at blockade of the androgen receptor function by androgen antagonists (anti-androgens) have been applied as therapy for advanced disease. Although endocrine therapy is important as palliative treatment, ultimately all endocrine-manipulated tumours escape to a therapy-resistant stage. In order to explain the failure of current endocrine therapies and to be able to design improved therapeutic regimens, the molecular mechanisms of androgen receptor function in androgen-dependent tumours, and the molecular mechanisms of endocrine therapy-resistant prostate cancer should be understood.

The androgen receptor is a member of a large family of nuclear receptor transcription factors [35]. The protein has a modular structure with several molecular functions, a DNA-binding domain, a ligand binding domain, and one or two transactivation domains. Importantly, the *androgen receptor* gene is located on the X chromosome. Consequently, in males, alterations in the *androgen receptor* gene can dramatically affect its function in the cell.

In the absence of androgens, the androgen receptor is mainly in the cytoplasm, but upon androgen stimulation the protein rapidly migrates to the nucleus. Androgen receptor homodimers bind to their cognate DNA binding sites in the regulatory regions of target genes, and upon interaction with coactivators, general transcription factors and RNA polymerase II a stable transcription initiation complex is formed. This allows tightly regulated expression of the target genes. The well-known serum marker *PSA* can be considered as the prototype of a tightly androgen-regulated gene [38,39]. Anti-androgens or antagonists compete with agonistic androgens for binding to the ligand-binding domain of the androgen receptor. There is accumulating evidence that antagonists induce an aberrant conformational change in the ligand-binding domain, which inhibits the transactivating function of the protein [36,37].

A key question in androgen receptor function concerns the characterization of its specific target genes. Because of the multiple functions and the complex expression pattern of the androgen receptor in the prostate, a simple answer to this question cannot be given. Direct androgen-regulated genes in cell cycle stimulation (cell proliferation), suppression of apoptosis (cell survival), and prostate function (differentiation) have been postulated. Candidate androgen target genes involved in cell cycle regulation are Cyclin Dependent Kinase (*CDK*)2,

CDK4, *p16INK4a*, *P27KIP1*, and *P21CIP1* [40–43]). *Bcl-2* is the most important candidate androgen-regulated gene involved in apoptosis [44]. However, the panel of genes to be investigated is far from complete and needs further evaluation. Similarly, the effect of androgens on the expression of growth factors, cytokines and their receptors, which all can indirectly affect cell proliferation, need to be studied in more detail. High-throughput technology for transcriptional profiling is rapidly increasing our knowledge of other important androgen target genes [45,46]. The genes, which are androgen-regulated in the normal prostate cells, will be at least partially identical to those stimulated during androgen-dependent prostate tumour cell proliferation.

The growth of endocrine therapy-resistant prostate tumours can essentially be explained by two molecular mechanisms (Fig. 2). First of all, it is possible that androgen-stimulated growth of a prostate tumour is bypassed by a mechanism that is regulated by a signal transduction pathway, which is independent of the androgen receptor. In these tumours, androgen receptor expression might be downregulated, or the androgen receptor might be inactivated. The endocrine therapy-resistant tumours can also result from an outgrowth of a tumour cell subpopulation that was already androgen-independent. Secondly, endocrine therapy-resistant prostate tumours might arise by adaptation of androgen receptor-dependent growth. Because the vast majority of locally progressive and metastatic endocrine therapy-resistant tumours show androgen receptor expression, this latter possibility is now widely accepted. Its predominantly nuclear localization in prostate tumours argues in favour of a functionally active conformation of the androgen receptor. Androgen receptor-dependent mechanisms of endocrine-therapy resistance might include androgen receptor overexpression, androgen receptor mutation, ligand-independent activation of the androgen receptor and modification of expression or properties of specific coactivators.

Androgen receptor overexpression can be the result of the amplification of the *androgen receptor* gene. Indeed, *in situ* hybridization experiments have shown *androgen receptor* gene amplification in approximately 30 percent of recurrent tumours following orchiectomy [14,15]. Importantly,*androgen receptor* gene amplification was not detected in tumours prior to endocrine therapy, but predominantly in tumours which relapsed relatively late during endocrine treatment. These findings strongly suggest that tumours with androgen receptor amplification responded primarily to endocrine therapy, e.g. androgen withdrawal, followed by an escape mechanism including *androgen receptor* gene amplification and androgen receptor overexpression.

A second mechanism that could explain a functionally active androgen receptor in endocrine therapy-resistant prostate cancer is the modification of androgen receptor functions by mutation. Some studies indicate that the length of a glutamine-stretch in the amino-terminal domain of the androgen receptor inversely correlates with the risk of prostate cancer [47]. Point mutations in the *androgen receptor* gene have been described with varying frequency in primary tumours and in metastatic tumours (see for an overview www.mcgill.ca/androgendb). The effect of most of these mutations on androgen receptor function has not been investigated in detail. Most information is available on amino acid substitutions in the ligand-binding domain. As the classic example, the *androgen receptor* gene in the LNCaP prostate cancer cell line possesses a threonine to alanine substitution at position 877 in the ligand-binding domain [48]. This modification has a dramatic effect on the ligand-specificity and transactivating

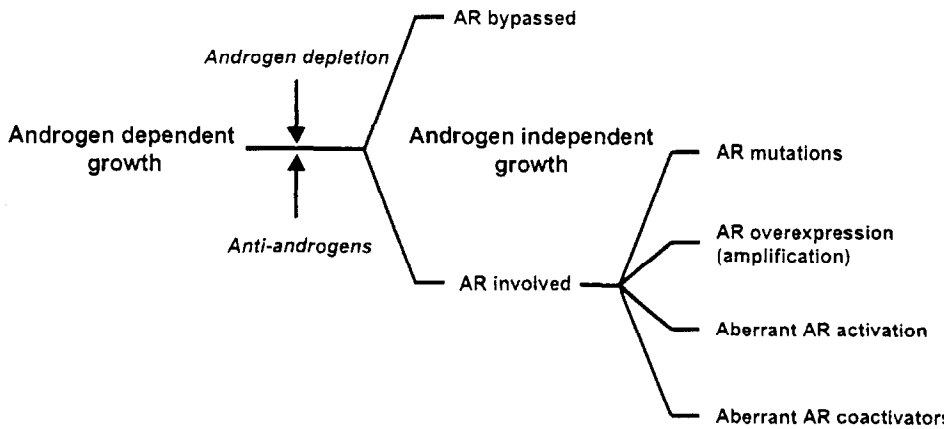

Fig. 2. Schematic representation of mechanisms of development of endocrine-therapy-resistant prostate cancer. AR: androgen receptor.

function of the receptor. The mutated androgen receptor is not only responsive to testosterone and 5α-dihydrotestosterone, but also to most anti-androgens, and to natural low affinity ligands, such as oestradiol and progesterone. The same mutation has also been found in tumours from prostate cancer patients [51]. Consequently, Thr877Ala substitution is a mutational hot spot in prostate cancer. Importantly, *androgen receptor* mutations were preferentially detected in endocrine-therapy-resistant tumours from patients under anti-androgen therapy. Recently, it has been found that a leucine to histidine substitution at position 701 sensitizes the androgen receptor to cortisol [50]. Because the three-dimensional structure of the ligand-binding domain has been elucidated, a reliable explanation of the effect of androgen receptor mutations on ligand-specificity can be given [51]. So far, only a limited number of endocrine-therapy-resistant patients have been studied. Therefore, the frequency of *androgen receptor* mutations remains to be established. Data published to date indicate that the mutation frequency in metastatic disease is 15 percent or less. Most likely *androgen receptor* mutations in late stage prostate cancer represent a mixture of functional mutations and random mutations, which might be the result of genetic instability.

A third mechanism of escape from endocrine therapy by prostate tumour cells might be by hormone-independent activation of the androgen receptor. In model systems, it has been shown that the androgen receptor can be activated by several different kinases, and by growth factors and cytokines [52–55]. However, it is still unknown whether hormone-independent activation of the androgen receptor is of importance for prostate tumour growth in the patient.

Concluding remarks

In this overview, it has been impossible to cover in detail all aspects of the molecular mechanisms involved in prostate cancer growth. Although we have rapidly increased our knowledge during the last decade, many questions remain. As expected, the questions that need to be addressed are much more complex than originally envisaged. Combination of detailed studies of the genes and their proteins, which are most important for prostate cancer growth, with more global investigations of patterns of gene expression, and protein expression and modification will further increase our insight into the molecular biological aspects of prostate cancer. The studies on the androgen receptor and its target genes, as well as investigations of P53 and PTEN signal transduction pathways in prostate cancer are good examples of such a combined approach.

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