THE MOLECULAR BIOLOGY OF PROSTATE CANCER

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INTRODUCTION

Prostate cancer is one of the important causes of death in men. The overall incidence of prostate cancer in Indonesia is not available, however a limited study done at Soetomo Hospital, Surabaya, on the number of patients admitted and treated for genito-urinary cancer during the year 1989 – 1992, revealed that prostate cancer is the second most frequent genito-urinary malignancy (35/218 = 16%), superseded only by the first ranking bladder cancer (104/218 = 48%) and exceeding the third ranking testicular cancer (23/218 = 11%) (Hardjowijoto et al 1992).

The prostate gland depends on testosterone, an androgen synthesized by Leydig cells of the testis, for its growth and maintenance. The maintenance of the gland's structural and functional integrity required the constant support of an adequate level of circulating testosterone, which in the prostate gland is converted to α-dihydrotestosterone (DHT), its active derivative, by a chemical reaction catalyzed by 5-α steroid reductase present in the gland.

The androgen dependence of the gland is best illustrated by the fact that castration causes involution of the gland, which can be reversed by supplying exogenous testosterone. Although testosterone by way of its active derivative DHT, is essential for the growth and maintenance of the prostate gland, DHT does not directly stimulate prostate growth. This is exemplified by the fact that in experiments using a normal canine prostate epithelial cell line (CAPE cell line), it is the epithelial growth factor (EGF) rather than DHT that stimulates cell proliferation. Three important conclusions can be drawn from these experiments regarding prostate growth and maintenance in- vivo:

1. Prostate growth is maintained by EGF supplied externally by other cells residing in the gland and not by the epithelial cells themselves.
2. EGF receptors (EGFR) is expressed constitutively by the epithelial cells.
3. DHT acting as a switch, stimulates the expression and secretion of EGF

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It was later shown that normal prostate epithelial cells do indeed express EGFR, whilst EGF and a related growth factor TGF-α (tumor growth factor α), both binding to the same receptor (EGFR), are mainly found in the stromal compartment (Leav et al. 1998).

THE GENESIS OF CANCER (CARCINOGENESIS)

Cancer arises from normal cells. Innocuous normal cells can be transformed into highly malignant cancer cells in a rather complicated, multi-step process involving many genes. Considering the growth and spread of tumor cells, one can distinguish four steps in the genesis of cancer: intra-epithelial, local, invasive and metastatizing cancer. These steps in cancer progression is often (not always) accompanied by morphological changes in which cancer cells deviate more and more from that shown by their normal counterpart. Although there are more than a hundred types and sub types of cancer all (or almost all) went through those 4 steps. Furthermore, the molecular events giving rise to cancer are essentially the same.

In the early days, it was thought that cancer is the result of mutations in 2 sets of genes, whose products antagonize each other: gain of function in pro-proliferation genes called proto-oncogenes, giving rise to oncogenes (i.e tumor causing genes), and loss of function in anti-proliferation genes called tumor suppressor genes. It now appears that this simple concept of carcinogenesis is far from adequate. For instance, the classification of genes involved in carcinogenesis as being either pro- or anti-proliferation genes is far too simple. Proto-oncogenes for example, now includes not only pro-proliferation genes, but also anti-apoptotic genes. Tumor suppressor genes now includes genes whose products antagonize the action of proteins encoded by proto-oncogenes, genes encoding DNA repair enzymes and pro-apoptotic genes. There are also genes involved in the later stages of tumor progression which can not be classified as either proto-oncogenes or tumor suppressor genes as is the case of invasion or metastasis promoting genes. Furthermore, it is now known that cancer is not only the result of mutations, which are genetic events, but that in certain tumors, epigenetic events can be involved (see below).

Thus it is more appropriate to describe the molecular events collectively leading to malignant growth as

alterations of normal cell physiology resulting in the acquirement of different characteristics not previously found in normal cells, although in its original meaning as tumor inducing and tumor inhibiting genes the terms "oncogenes" and "tumor suppressor genes" remains valid. These acquired characteristics are:

1. the loss of ability to repair mutation
2. the ability to evade apoptosis
3. the ability to proliferate in the absence of externally produced growth factors (GFs)
4. the ability to evade growth inhibitory, anti-proliferation signals
5. the ability for sustained cell replication
6. the ability for sustained angiogenesis
7. the ability for tissue invasion and metastasis.

Inability to repair mutations

Most cancers are the result of mutations. Mutations can be repaired by the action of a set of enzymes called DNA repair enzymes. Loss of function mutation of these enzymes will thus increase the probability of acquiring cancer.

Ability to evade apoptosis

Apoptosis or also called: programmed cell death (PCD) is a way of our body to dispose of senescent, badly damaged or otherwise unusable cells. In the context carcinogenesis, when mutations are irreparable, the damaged cells are triggered to kill themselves, thus preventing the detrimental effect of mutation. The ability to evade apoptosis is achieved by up-regulation or gain of function mutations of anti-apoptotic genes, or alternatively, loss of function mutations of pro-apoptotic genes. Apoptosis and DNA repair are two ways by which our body ensure genomic stability. If mutations occur, our body first attempts to repair the damaged DNA, and if this attempt fails, the cell will then commit suicide. Thus in the context of maintaining genomic stability, the DNA repair genes are called "care-taker genes" and the apoptotic genes are called "gate-keeper genes"

Ability to proliferate in the absence of external GF

Normal cells can only proliferate in the presence of GFs expressed and secreted by other cell . Normal prostate cells e.g. proliferate only on stimulation of GFs secreted by stromal cells (see above).The ability to proliferate in the absence of external GF can be achieved by at least 3 ways;

a. by ectopic expression of GFs toward which the cell is responsive
b. by up-regulating the GFRs so that the cell become hyper-responsive to low ambient level of GFs, or in other cases, gross over-expression itself can cause ligand independent signaling
c. gain of function mutations of growth signal transducing genes.

Ability to evade growth inhibiting anti-proliferative signals

This characteristic is the result of loss of function mutations in genes regulating the initiation or progression of the cell proliferation cycle. The loss of function of these genes, effectively dispose of the brakes which normally prevents inappropriate cell proliferation

Ability for sustained cell replication

The ability for GF independent proliferation and evade anti-proliferation signals does not necessarily ensure sustained replication. Studies performed during the past 30 years revealed that somatic cells can not be cultured indefinitely. After a certain number of replicative doubling, somatic cells cease to replicate and die by apoptosis (Hayflick phenomenon). On the other hand, the so-called embryonal stem cells (E.S. cells) i.e. cells taken from blastocysts can be cultured indefinitely. Further studies revealed that the inability of somatic cells to replicate indefinitely has something to do with the length of telomeres. (the two ends of chromosomes). It was found that in somatic cells, telomeres shorten after each round of replication. When cell telomeres shorten to a certain critical length the cell stop replicating and dies by apoptosis. ES cells on the other hand, maintain their telomere length after each round, so they are able to replicate indefinitely, i.e. they are immortal. It is now known that shortening of telomeres are prevented by an enzyme called telomerase. Telomerase is expressed in early embryonic life , but is no longer expressed in somatic cells. Cancer cells re-expressed telomerase, and thus like ES cells replicate indefinitely.

Ability to sustain angiogenesis

Cancer cells can only grow to a limited size unless they are able to induce formation of new blood vessels. Angiogenesis is induced by vascular endothelial growth factor (VEGF) and inhibited by thrombospondin. The ability of cancer cells to induce angiogenesis is acquired by up-regulating angiogenic genes or down- regulating anti-angiogenic genes or both. One of the potent inducer of angiogenesis is hypoxia. When cancer cell grows, some of its cell are located more than 100 μm away from blood vessel (the diffusion limit for oxygen) and thus become hypoxic. Hypoxia will activate hypoxia inducible transcription factors (HIF-1α and HIF-1ß)

which after forming a heterodimer will then induce expression of several angiogenic factors such as VEGF and Ang (angiopoetin) (Carmellette P, Jain RK 2000). VEGF is also known to be regulated by the ras proto-oncogene, one of the growth signal transducing genes. Gain of function mutation of ras, a common event in many tumor types result in up-regulation of VEGF expression. The anti-angiogenic protein thrombospondin is known to be regulated by the pro-apoptotic tumor suppressor gene p53, consequently loss of p53 function which also occurs in many cancer types can cause a fall of thrombospondin level.

Ability for tissue invasion and metastasis

The ability for tissue invasion and to spread to distant tissues is what makes cancer deadly. As long as tumors remain localized, it usually can be removed by surgery. This characteristic is acquired in late tumor progression, however the propensity of spreading differs among various tumors. Some tumors are known to remain localized for a long time, while others rapidly spread to distant tissues in a relatively short time.

Tissue invasion is a complicated process. To proceed to the invasive stage, cancer cells must be able to breach the basement membrane, detach from neighboring cells, able to survive while remaining unattached, and invade the extra-cellular matrix (ECM). The ability of cancer cells to breach the basement membrane and invade the ECM is due to their ability to secrete special tissue degrading enzymes called matrix metallo-proteinases .(MMPs). How this feat is accomplished is not completely known since most normal cells except leucocytes and macrophages, do not secrete these enzymes.

In normal cells MMP activity is modulated through interaction with their natural inhibitor, the tissue specific inhibitor of MMP (TIMPs). Subsequently MMP expression has been shown to be modulated by certain oncogenes and other growth factors which presumably overcome the inhibitory factor TIMPs (McCawley LJ, Matrisian LM, 2000). Cell attach to each other by a special molecule called E-cadherin. Another class of molecules called: integrins attach cells to basement membrane or ECM.E-cadherin is not expressed or its function is inactivated in most cancer cell especially those derived from epithelial tissues.

When the basement membrane is degraded by MMPs, the cells lose contact to the basement membrane. Unattached normal cells are unable to replicate and finally die. Normal cells are thus anchorage dependent for survival. Cancer cells are able to survive and replicate unattached: they are anchorage independent. How this characteristic is achieved is again not completely known, but it seems that it has something to do with integrin signaling. Soon the invading cancer cells will encounter and breach lymphatic and blood vessels walls. Entering the circulatory system cancer cells must overcome tumor killing cells: cytotoxic T cells (CTLs) and natural killer (NK) cells. These cells kill cancer cells by recognizing and binding to certain molecules displayed on the surface of the tumor cells, where upon they then kill the target cells by secreting cytotoxic compounds. Only a few, probably one out of 10,000 cancer cells are able to evade the action of the tumor killing immune cells.

Cancer cells evade this immune-surveillance actions by CTLs and NK cells using several strategies: (1) down regulating the surface molecules recognized by CTLs and NK cells (2) masking their surface molecules under a thick glycocalyx and (3) by secreting TGF-ß (Tumor growth factor ß), a cytokine known to suppress immune responses. These surviving cancer cells finally arrive at distant tissues, again by breaching vessel's wall, and if conditions are right, they will then grow in this new environment forming metastatic colonies.

The Role of Epigenetic Events on the Genesis of Cancer

Activation and inactivation of genes can not only be the result of mutations, but can also be due to covalent modification of their promoters. DNA can be methylated on special cytosine residues called CpG islands present in gene promoters. Promoter methylation prevents gene expression, a phenomenon called "gene silencing", whereas demethylation of previously methylated promoter reactivate the gene. Such changes in gene expression by methylation-demethylation of these promters are called: "epigenetic" because there are no genetic changes in the involved gene itself.

In the context of carcinogenesis, promoter demethylation occurs in ectopic expression of GFs by cells responsive to the said GF making them independent of externally produced GF. Examples are: ectopic expression of PDGF (platelet derived GF) by glioblastomas and TGF-ß by sarcomas (Hanahan D, Weinberg RA 2000). Promoter methylation of previously active gene can inactivate the genes causing loss of function of many genes, involved in the genesis of many tumors. Examples are: loss of function in pRb, p53, p16, and E-cadherin (Schmutte C, Jones PA 1998). Another epigenetic event that can modulate gene expression involves covalent modification of core histone tails (Zang Y, Reinberg D. 2001). Histones are DNA binding protein component of nucleosomes, the basic repeating unit of chromatin.

The protein component of nucleosomes consists of one copy of histone H1 and two copies each of the four core histones: H2A, H2B, H3 and H4. Each core histone consists of a structured triple-helix domain and two unstructured tails on each end of the molecule (N and C terminal tails). These tails are susceptible of covalent modification, which includes: acethylation, methylation and phosphorylation. The significance of these covalent modifications are just beginning to be elucidated, one of which is its role to modulate gene transcription . How covalent modification of histone tails can result in gene transcription modulation is a rather complicated process., but to make complicated things simple, covalent modification of histone tails can modulate gene transcription by at least 2 ways: (1) by facilitating or denying access of proteins necessary for gene transcription to gene promoters and (2) by facilitating DNA methylation . It remains however to be seen whether such epigenetic modulation is involved in the genesis of tumor.

Hereditary Cancer

Some cancers run in families. Such hereditary cancers always involved in loss of function mutations in tumor suppressor genes. Well-known examples are: hereditary retinoblastoma, involving the anti-proliferation gene pRb and the Li-Fraumeni syndrome, involving the pro-apoptotic gene p53. Loss of function mutations involving tumor suppressor genes are recessive, i.e. the tumor phenotype will only appear when the mutation is homozygous (both homologous genes are affected). Heterozygous mutations (i.e. only one gene affected) will have no effect, since the product of the other still healthy gene is present.

A hypothetical but probable scenario on how hereditary cancer is acquired will be as follows: a male person accidentally acquire a heterozygous mutation in a tumor suppressor gene located not in somatic cells but in a diploid germ-line cell, a precursor of haploid sperm cells called spermatogonium. Since only one gene is disabled, the diploid germ cell remains normal. After several mitotic divisions, a meiotic division follows giving rise finally to haploid sperm cells. Since the mutation in the diploid germ-line cell is heterozygous, half of the progeny sperm cells will contain the defective gene while the other half will inherit the healthy one. Suppose, he then marries a healthy woman , then half of their children will inherit the defective gene, and this unfortunate child will have the defective gene in all her/his cells (including germ-line cells). As long as the second gene remains normal, all will be well. But if a second mutation occurs, the cancer phenotype will appear. This event is called: loss of heterozygosity, abbreviated: LOH, because the heterozygous mutation become homozygous. At this stage, one could not be sure whether one is dealing with a sporadic case or a case of hereditary cancer. If then cancer arise in one or more sibs of the patient, then hereditary cancer is suspected, but the diagnosis will be clinched when the same kind of cancer arise in more than one generation.

THE GENESIS OF PROSTATE CANCER

Prostate cancer is thought to arise from prostate stem cells. Although our knowledge regarding the molecular events leading to prostate cancer is far from complete, prostate cancer follows the general course of carcinogenesis as mentioned above. Also, all the mayor players and events are represented including proto-oncogenes, tumor suppressor genes, sustained angiogenesis etc.

Some chromosomal loci have been found by linkage studies to be involved in hereditary prostate cancer, although so far, no genes has been identified, let alone their role in the genesis of prostate cancer. Examples of such loci, often called "candidate genes" are HPC-1 located at chromosome 1: 1q 24-25 and HPX located at the X- chromosome: Xq 27 -28 (Reiter RE,Dekernion JB , 2002).

In other cases the gene is known, but its exact contribution to the genesis of prostate cancer remains to be elucidated. Examples are: the NKx 3.1 gene, an androgen regulated specific homeobox gene (Xu LL 2000), ANX7 a gene encoding annexin-7, (Srivastava M et al. 2001) and genes involved in fatty acid or cholesterol synthesis (Eder IE et al., 2004). How the products of all these genes relates to the genesis of prostate cancer is far from clear.

Many of these genes are detected by "gene profiling" using DNA micro-arrays (Eder IE et al 2004), a method which can detect gene expression in tissues, both in normal and in pathological conditions. By comparing gene expression in normal and cancer tissues, one can then detect which genes are up-regulated or down-regulated in prostate cancer. Gene profiling is a powerful method to detect abnormal expressions in various pathological conditions, however it does not reveal how the abnormal expressions relates to the pathological condition.

This article is not meant to be a complete and exhaustive discourse on the molecular biology of prostate cancer, but rather to provide the reader with an idea on the complexity of molecular interactions occurring in the genesis of prostate cancer, by high-

lighting a number of genes whose involvement and mechanism are known. One remarkable feature of prostate cancer is the fact that prostate cancer cells remain androgen dependent until late in their progression, at which time they will become androgen independent.

Inability to Repair Mutations

Gene mutations can be repaired by a set of enzymes, collectively called: DNA repair enzymes. These enzymes are organized into several pathways depending on the nature of the DNA damage and the action of the enzymes, these are: BER (base excision repair), NER (nucleotide excision repair), MMR (mismatch repair), RR (recombination repair) and DSBR (double stranded break repair). Inactivation of one MMR gene, called MLH 1 has been implicated in prostate carcinoma (Boyer et al, 1995) .

Ability to evade apoptosis

Up-regulation of Bcl2 and clusterin , two anti-apoptotic genes, inactivation of PTEN (phosphatase and tensin homolog deleted in chromasome ten) and p53, two apoptotic genes have been found in prostate cancer (Eder IE et al, 2004). Dysregulation of these 4 genes result in the ability to evade apoptosis.

Ability to proliferate in the absence of externally produced GFs (Rattan HL et al 2003)

Normal prostate epithelial cells express EGFR constitutively while the growth stimulating factors EGF and TGF-α are produced by stromal cells. The ability of prostate epithelial cells to proliferate independently from externally produced GF is due to:

a. ectopic expression of EGF and TGF-α by prostate epithelial cells (mainly the latter)
b. expression of a mutant EGFR constitutively active in the absence of external GF.
c. Gross over-expression of EGFR. When EGF binds to EGFR , the receptors forms homodimers, a prerequisite for further growth signal transduction. Gross overexpression of EGFR stimulates homo-dimer formation, which leads to growth signaling in the absence of EGF.

Prolonged signaling also contributes to the higher proliferation rate of prostate cancer cells as compared to their normal counterpart. There are at least 2 ways in which this trait is acquired:

a. switching off the dominant GF from the externally produced EGF to the ectopically expressed TGF-α.
b. ectopically expressing Erb-2 receptor, a receptor related to EGFR

TGF-α is the dominant ligand in prostate cancer, while in the normal gland, EGF is the dominant one. The EGF-EGFR complex remain stable in the endosome after endocytosis and are rapidly targeted to lysosomes to be degraded. On the other hand the TGF-α- EGFR complex is less stable, so that it rapidly dissociates in endosome, leading to recycling of the receptor instead of degradation. Thus a switch of GF production from EGF to TGF-α would result in prolonged signaling resulting in a higher proliferation rate.

Ectopic expression of Erb-2 leads to formation of EGFR-Erb2 heterodimer. This heterodimer is less stable than the EGFR homodimer, which again leads to recycling instead of degradation. Other gene dysregulation may support the higher proliferation rate in prostate cancerous cell. One is a gain of function mutation in the SRD5A2 gene encoding the 5-α steroid reductase enzyme, which converts testosterone to dihydrotesto-sterone (Makridakis et al. 2000). The CAG repeat polymorphism in the AR (androgen receptor) gene may influence the activity of the receptor. A shorter length of the CAG repeat encoding a poly-glutamine chain the length of which is inversely correlated to the transcrip-tional activity of the receptor (Giofanucci et al. 1997). The proto-oncogene c-myc which encodes a transcription factor Myc, is amplified in many tumors including prostate cancer (Eder IE et al, 2004).

Terminally differentiating cells stop replicating. Under normal conditions after replicating several times, the cells finally enter terminal differentiation. Terminal differentiation is induced by a heterodimer of 2 transciption factors Mad and Max. When Myc is up-regulated as happens in many tumors, Myc replaces Mad forming a Myc-Mad heterodimer which inhibits differentiation and assuring continuous replication. (Hanahan D, Weinberg RA, 2000).

Akt is another proto-oncogene which is activated in androgen independent prostate cancer. It encodes a protein kinase called protein kinase B (PKB) which forms a part of the so called PI.3K (phosphatidyl inositol 3 kinase)-Akt pathway. PKB shows 2 synergistic activities: it induces cell proliferation by inducing expression of cyclin D1 and inhibiting the cell cycle inhibitor gene $p27^{kip1}$, while at the same time suppressing the pro-apoptotic functions of BAD thus preventing apoptosis (Graff JR et al, 2000).

Ability to evade growth inhibitory, anti-proliferation signals

The pro-apoptotic gene PTEN, is often inactivated by deletion, loss of function mutation and repressed by methylation of its promoter (an epigenetic event). PTEN is in fact an antagonist of Akt since it inhibits the action of Akt . Thus inactivation of PTEN will give the same result as activation of Akt (see above) (Whang JE et al 1998). The cell cycle inhibitor $p27^{kip1}$ located in chromosome 12 (p12-13) is often deleted in advanced cancer. It inhibits cyclin-E-cdk2 (Kibel et al 2000)

Ability for sustained cell replication and angiogenesis

Prostate cancer cells re-expresses telomerase and upregulate VEGF expression (Reiter RE, Dekernion JB, 2002)

Ability for invasion and metastasis

Loss of E-cadherin and increased MMP expression are present in prostate cancer (Reiter RE, Dekernion JB 2002). Another molecule: ezrin may play a part in prostate cancer invasion. Ezrin is a membrane-cytoskeleton linker molecule that can signal cell survival through the P1,3K/Akt pathway. It is down regulated after castration and up-regulated after androgen replacement (Pang ST et al. 2002) Ezrin. is up-regulated in human pancreatic adenocarcinoma cell-line with high metastatic potential (Akisawa N et al. 1999) and is involved in endometrial cancer cell invasion (Ohtani et al. 1999.). Ezrin signaling through the PI.3.K/Akt pathway may provide pancreatic adenocarcinoma and endometrial cancer cells the ability to survive when unattached. Whether it is involved in prostate cancer, remain to be seen.

Mucin 1 is a transmembrane protein highly expressed in more aggressive subtype of prostate cancer. High expression of its encoding gene MUC-1 was first found using DNA microarray gene expression profiling (Lapointe JA et al, 2004). It has been proposed that over-expression of MUC-1 increases the metastatic potential of cancer cells by reducing E-cadherin and integrin mediated cell adhesion. It is also possible involved in antigen masking, providing cancer cells with a way to evade recognition and killing by tumor killing CTLs and NK cells.

Loss of MHC proteins in cancer cells and inactivity of NK cells have been reported in prostate cancer. (Oikawa et al, 2003). Loss of MHC proteins gives cancer cells the ability to evade the attack of CTLs, however, loss of these proteins does not prevent the attack by NK cells. On the contrary, NK cells attack is induced by loss of MHC molecules. Thus the inactivity of NK cells in prostate cancer must be due to other causes. It has been found that in patients with prostate cancer, NK cells derived from PBMCs (peripheral blood mononuclear cells) is reduced more in patients with metastasis than those without or healthy controls. Moreover, it has been found that NK activity level correlated with the presence of tumor cells in the blood circulation. It is however not clear whether the presence of tumor cells in the blood is the cause or effect of NK cells inactivity.

Androgen independent (AI) prostate cancer

One remarkable feature of prostate cancer is that it remains androgen sensitive until late in their progression, at which time it will become androgen independent. This is an unfortunate fact, because androgen deprivation is the only effective systemic therapy available for metastatic prostate cancer. Androgen deprivation is associated with gradual transition of prostate cancer cells from androgen sensitivity to ultimately androgen independence, at which time androgen deprivation is no longer effective.

Androgen independence can be detected by monitoring the level of prostate specific antigen (PSA), a tissue specific, androgen dependent tumor marker routinely used by urologists (and oncologists) to monitor tumor responses, prognosis and progression in prostate cancer patients. In androgen dependent prostate cancer, androgen deprivation results in a sharp decline in PSA to normal level. Early onset of AI prostate cancer, manifests itself by initial decline in PSA level, usually still above normal, followed by a rising titre of serum PSA that may eventually surpass the pre-treatment value.

The presence of a high percentage of AR-positive cell, both in primary (hormone sensitive) and recurrent, hormone refractory tumors, and also the fact that AR can be detected within the nuclei of both, implies that AR is involved in the progression of prostate cancer to androgen independence (Sadar MD et al, 1999). That AR is detected within the cell nuclei also implies that it is activated in the absence of androgen, its normal ligand, since only activated AR enters the nucleus and interact with DNA.

The molecular mechanism of ligand-independent activation is not fully known. Several possible mechanism has been detected or implicated, these are: (1) AR mutation (2) AR amplification and (3) interaction between AR and other signaling pathways involving GFs or other signaling molecules (Sadar MD et al 1999, Rattan HL et al 2003). These mechanisms do

not exclude each other, rather, they should be considered as possible alternatives. Mutation in the AR ligand domain, can alter the specificity of ligands bound to AR, so that activation of transcription can be induced not only by androgen but also by other steroid hormones, and surprisingly by anti-androgens used for therapy, such as bicalutamide (Reiter RE, Dekernion JB, 2002).

AR amplification has been reported by several investigators in recurrent , hormone refractory tumors. It is possible that an elevated AR copy number allow cancer cells to utilize low level of residual non-testicular androgen. Interaction with other signaling pathways has been reported, including the PKA (protein kinase A) pathway, the Ras-MAPK (mitogen activated protein kinase) pathway, the PKC (protein kinase C) pathway and the PI3K/Akt pathway. Among the GFs reported to be able to activate AR includes: IGF (insulin-like GF), KGF (keratinocyte GF) and EGF. But it is as yet not clear, which pathway is used by each GF since there are considerable "cross-talk" between these pathways. One of the more compelling evidence, involves the PKA pathway: activation of AR through up-regulation of the PKA pathway results in phosphorylation of the amino-terminus of AR (Sadar MD et al. 1999).

BENIGN PROSTATIC HYPERPLASIA (BPH)

Benign prostatic hyperplasia (BPH) is the most common benign tumor in men aged 60 years or more. The etiology of BPH is unknown. It must have something to do with a disturbed balance between growth promoting factors such EGF and TGF-α and growth inhibiting factors such as TGF-ß (Griffith K. 2000). BPH rarely, if ever, progresses to prostate cancer. Why is this so, may be related to the different origin of their precursor cells. (De Marzo AM et al, 1998).

Prostate epithelial cell can be divided into 2 compartments: basal cell and secretory cell compartments. Replicating epithelial cells in BPH maintain their normal restriction to the basal compartment, while the earliest precursor of malignant prostate cancer, the prostatic intra-epithelial neoplasma (PIN) always resides in the secretory compartment. The molecular implication of this different locatios is not immediately obvious, but may be related to the propensity of PIN precursor cells to undergo mutation finally leading to genomic instability and cancer.

Several genes are known to be differentially expressed between BPH and PIN cells.These are genes encoding cyclin-dependent kinase (CDK) inhibitor, $p27^{kip}$, telomerase, glutathione S transferase (GST) and hepsin, a transmembrane serine protease (De Marzo AM et al. 2001, Luo J. et al. 2001). Telomersase, $p27^{kip1}$ and hepsin are expressed in PIN cells but not in BPH cells, whereas GST is expressed in BPH cells, but not in PIN cells. Remarkably, GST is expressed in basal cells but not in secretory cells. The difference may be a clue for the different behavior between BPH and PIN cells. Since GST is an anti-oxidant enzyme, it could be that a population of still replicating secretory cells underwent mutations by oxidative damage due to inadequate anti-oxidant defense thus setting the stage for further genomic instability ultimately resulting in malignant cancer.

CONCLUDING REMARKS

Although our understanding of the molecular changes in prostate Ca has been much improved during these last 10 years, the molecular basis of androgen dependence is still not completely understood. This incomplete knowledge pose a problem for treating patients with metastatic prostate Ca, since in such cases, anti-androgen therapy the only therapy available is no longer effective. Since even in AI prostate carcinoma AR activation is still required albeit not by binding of androgen, its normal ligand, the most rational approach would be by inactivating the AR itself. Inactivation of AR could be difficult because of the intracellular location of the AR, so that any AR inactivating agent must first be able to penetrate the cell membrane to arrive in the cytosol. If this problem can be overcome, then perhaps we should think of molecules that can react and inactivate AR, molecules that binds to the DNA binding domain of AR or molecules that can bind to AREs (androgen responsive elements).

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