European **Rev**iew for **Med**ical and **Pharmacol**ogical **Sci**ences 2008; 12: 167-175

Genetic and microenvironmental implications in prostate cancer progression and metastasis

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Abstract. – **Prometastatic gene expression events occur during the early phases of prostate oncogenesis, even though overlaping with genes that induce primary cancer growth. Cytogenetic and genomic profiling analyses have identified many cancer-associated chromosomal abnormalities consisting mainly in losses in the early phases of sporadic primary prostate carcinoma. Metastatic genes are those in which gains in oncogene functional activity or lack of tumor suppressor genes enable cancer cells to detach, escape into the circulation, penetrate and colonize distant organs. In metastatic prostate carcinoma some genes, such as MTA1 and MYBL2, are differentially upregulated in comparison to primary site, while IGFBP, DAN1, FAT and RAB5A appear to be downregulated. Epigenetic alterations, such as histone deacetylation/hypermethylation, are also involved in the metastasis promotion. Nevertheless, during oncogenesis and cancer progression, prostate cancer cells may regain pluripotent stem cell-like properties or, as an alternative, may be, them selves, malignant stem cell clones, equipped with self-renewal mechanisms. Pleiotropic contributions to cancer progression and metastatic spread are also brought up from a variety of tumor microenvironment-associated factors. Moreover, inflammatory processes can partecipate in prostate tumorigenesis and cancer progression through several mechanisms, such as generation of both oxygen and nitrogen reactive species, induction of cyclooxygenase-2 and production of growth factors and cytokines by neutrophils and macrophages of host microenvironment. The knowledge of both genetic and microenvironmental cancer aggressiveness factors is necessary to define timing and suitability of therapeutical strategies.**

Key Words:

Prostate carcinoma, Tumor progression, Microenvironment, Metastatic genes, Inflammation.

Introduction

Prostate carcinoma is the most commonly diagnosed non-skin male malignancy in Western world and, despite recent favorable decline in specific mortality, it remains today the second leading cause of cancer-related death in men in United States[1]. It's thought to evolve from the precursor HGPIN, *high grade prostate intraepithelial neoplasia*, to localized tumor lesions and, then, to metastatic disease. Whereas the prostate gland-confined carcinoma may be effectively treated by surgery and/or radiotherapy, metastatic disease cannot. Androgen deprivation can control cancer progression for a while but the malignancy relentlessly is relapsing in the course of few years.

Oncogenesis may be viewed approximately as a "Darwinian-evolutionary process", casual *gene changes* producing variant cell populations among which, by natural selection, a neoplastic cell line can emerge with following development of the *primary tumor*[2,3]. HGPIN and early phases of sporadic primary prostate cancer mainly include loss of tumor suppressor genes (6q, 8p, 10q, 13q, 16q, 18q) compared with rare gain of 8q oncogene[4,5]; particularly, the loss of 10q23.3, PTEN, *phosphatase and tensin homologue*, that negatively modulates the phosphatidylinositol-3-kinase/Akt pathway, is present in 23 per cent of HGPIN and 69 per cent of primary carcinomas[4,6]. Nevertheless, recent findings have also shown a complete-homozygous PTEN deletion in prostate cancer progression, thereby PTEN functional activity lack, at an early phase of prostate carcinogenesis, could be assumed as a clue of molecular pathway which leads to more aggressive cell behaviour[4,6]. Subsequently, as far as the above-mentioned concept of tumor Darwinian evolution is concerned, among the neoplastic cells of primary

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tumor a more aggressive cell sub-set, because of selective advantage of additional genetic abnormalities, acquires the ability to metastazise[2,3]. While the development of the primary tumor may be related to deletion of tumor suppressor genes, *progression and metastasis* are also accompanied by activation or amplification of several oncogenes, such as gain in the chromosomes 8q (PSCA, *prostate stem cell antigen*, IF3-p40 gene, Myc), 7 (caveolin), Xq11-q13 (AR, *androgen receptor*)[7,8].

However, a part from cancer cell genetic and epigenetic implications, an important pleiotropic contribution to cancer progression and metastatic spread is also brought up from a variety of *tumor environment factors*[9,10].

Genetic Basis for Cancer Progression and Metastasis

A complex multi-step process, involving changes in several genes and transcriptional programs, is necessary to cancer cells detach from the primary site, penetrate vessels (intravasation) by which reach target organs and, after extravasation, colonize them. Refering to their level of involvement in the metastatic pathway, three groups of *metastasis genes* may be recognized[2,11,12]:

- metastasis initiation genes, that, in primary tumor, allow the malignant cell escape into the circulation, by inducing tumor cell detachment, motility and invasion, together with promoting the neoplastic angiogenesis;
- metastasis progression genes, that have dual functions in mediating, on the one hand, the above-mentioned metastasis initiation and, on the other hand, metastatic colonization, together with fulfiling some functions that contribute to primary tumor growth;
- metastasis virulence genes, that are exclusively involved in colonization of target organs, by providing selective growth advantages in metastatic sites but not in the primary tumor.

For instance, regarding the breast cancer, EREG gene, that encodes *EGF receptor ligand epiregulin*, Cox2 gene, that encodes *cyclooxygenase 2*, MMP-1 and -2 genes, which encodes *matrix metalloproteinase-1 and -2*, collectively "gang of four", are lung metastatis promoting genes, by constituting, taken together, a vascular remodeling program that is coopted by neoplastic cells to promote tumor angiogenesis, intravasation and, then, lung extravasation[2,12,13]. MMP_S, by them-selves, are capable of digesting extracellular matrix (ECM) and basement membrane although, in physiological conditions, they are regulated by TIMP, *tissue inhibitors of metalloproteinases*[14].

In prostate cancer metastases, some genes, such as MTA1, MYBL2, are differentially upregulated in comparison to primary site whereas IGFBP5, DAN1, FAT and RAB5A appear to be downregulated, suggesting that the development of prostate cancer metastasis is related to many gene expression changes and that metastatic prostate carcinoma is molecularly distinct from the primary tumor, thus also deserving of different clinical approaches[15]. Progression from hormone-sensitive to hormone-refractory prostate cancer growth involves many genetic alterations, reflected by up-regulation of oncogenes, such as c-myc, clusterin/TRPM (testosterone-repressed prostate message), bcl-2, AR, FAS (fatty acid synthase), EZH2 (enhancer of zeste homolog, *Drosophila*), and/or down-regulation of tumor suppressor genes, such as p53, p27, pRB, PTEN, E-cadherin. AR gene mutations, racing from its activation by un-proper agents such as some growth factors (EGF1, EGF, KGF) to atypical signaling pathways, are responsible for androgen-insensitivity. *Epigenetic alterations*, such as histone deacetylation/DNA hypermethylation, are also involved in the metastasis promotion. As fas as prostate cancer cells is concerned, DNA methylation induces the silencing of expression of several suppressor genes and, consequently, it allows the development of metastatic phenotype[2,16,17]. In fact, in epithelial cells, epigenetically-mediated switching from typical expression of E-cadherin to inappropriate expression of mesenchymal cell-related N-cadherin, is associated with EMT, *epithelial-to-mesenchymal transdifferentiation*, that characterizes the invasive behavior of cancer cells[18].

Nevertheless, during tumorigenesis and tumor progression, prostate cancer cells may regain, through a dedifferentiation process, *pluripotent stem cell-like properties* such as osteomimicry, EMT, myofibroblastic transdifferentiation by switching their gene expression profiles, or, as an alternative, may be, them selves, *malignant stem cell clones*, because of the aberrant Wnt (Wingless gene, *Drosophila* + Int-1, *murine* protooncogene) signalling activation, that peculiarly is involved in their self-renewal and multidirectional differentiation[19-21]; in fact, just resulting from

variable aptitude of pluripotent tumor stem cells to differentiate, heterogeneous nature of prostate cancer cells has been recognized[22]. Interestingly, prostate cancer stem cell growth may be androgen-insensitive, hence primarily refractory to androgen deprivation.

Tumor Microenvironment

Coevolution of stromal mesemchyme with cancer epithelial cells during tumorigenesis and tumor progression has been identified[9,23-25]. Gene changes that occur in prostate cancer cells and in surrounding microenvironment are reciprocically interconnected, thus supporting a *coevolutionary dynamic process*.

Many bioactive factors, that are produced and secreted in stromal cells (growth factors, cytokines), together with ECM products, are responsible for genetic alterations that are able to induce non-metastatic prostate cancer cells to acquire a metastatic phenotype.

Increased release of TGF-β, *transforming growth factor-β*, from bone stroma cells, promotes, in the prostate cancer cells, the expression of the gene encoding PTHrp, *parathyroid hormone-related peptide*, that, in turn, not only induces osteoclast activation but also can protect prostate cancer cells from apoptosis [26-29]. Moreover, TGF-β stimulates the angiogenesis, thereby contributing to cancer cell growth and intravasation, and induces EMT via Smad activated transcriptional pathway, that is directed to expression of mesenchymal genes and associated proteins (N-cadherin, α-smooth muscle actin, vimentin, filopodia-fascin) in epithelial tumor cells[8,26,30]; like this, EMT confers invasive properties on prostate cancer cells through activation of their pseudopodial protrusions[30,31]. Reciprocally, neoplastic cells, by releasing TGF-β in the tumor microenvironment, are able to induce the transdifferentiation of the stromal fibroblastic phenotype in that myofibroblastic, also called "reactive stroma", which results from activated expression of vimentin, calponin, α-smooth muscle actin in stromal fibroblasts[25]. Reactive stroma, in turn, exerts a proinvasive signal on epithelial cancer cells, by promoting, through release of ECM products, their motility and metastatic spread[32]. Even more, TGF-β is the most potent immunosuppressive growth factor known to-date by acting on cytotoxic T lymphocytes to block the expression of cytolytic gene products (granzyme-A and -B, Fas ligand, perforin and γ-interferon), that are, all together, responsible for cytotoxic T lymphocyte-mediated cancer cell killing[2,11,13]. On the contrary, TGF-β family and its receptors can inhibit the proliferation of prostate cancer cells; TGF-β cytostatic program is based on both the transcriptional activation of several genes, among which that encoding p21Cip1 (target of Smad-Fox0 complex), and the repression of others, among which that encoding c-Myc (target of Smad-E2F4/5 and Smad-ATF3), a growth-associated transcriptional promoter[11-13].

IGF-1 and -2, *insulin growth factor-1 and -2*, are potent microenvironment-produced mitogens towards the tumor cells; reciprocally, prostate cancer cells can enhance IGF_S levels through proteolytic degradation of IGF-BP, *IGF-binding proteins*[19]. IGF-1, in mouse model, is able to induce neoplastic transformation of prostate epithelial cells, while antisense mucleotides to IGF-receptors inhibit prostate cancer growth and invasion phenotype[9].

At the beginning of their development, the prostate primary tumor isn't angiogenic, while, as a tumor mass grows, a cohort of cells assume angiogenic activity[11,14]. VEGF, *vascular endothelial growth factor*, that is released by neoplastic cells and several cell types of tumor microenvironment, binds to its tyrosine-kinase receptors on endothelial cells, thus promoting cancer angiogenesis[2,33]. Nevertheless, the newly developed microvessels allow a poor blood flow, while the neoplastic cells undergo adaptive genetic changes to hypoxia, resulting in their raised aggressiveness[34]. HIF-1, *hypoxia inducible factor-1*, by binding to the promoter of hypoxia-modulated genes, further enhances the VEGF production and, therefore, cancer cell growth and invasion. Moreover, in the prostatic carcinoma, VEGF increases the osteoblastic activity[35,36].

PDGF, *platelet-derived growth factor*, plays a supportant role in the tumor angiogenesis by stimulating pericytes and vascular muscle cells to grow[14,37].

HGF/SF, *hepatocyte growth factor/scatter factor*, can increase prostate cancer growth by raising the interleuchin-6 (IL-6) expression and its negative effects on tumor cell androgen-sensitivity; furthermore, interactions of HGF/SH with its specific tyrosine-kinase receptor, that is expressed by hormone-refractory prostate cancer cells, increases their invasive behavior. However, in cancer cell metastatic spread, Met-receptor is mainly activated by semaphorin ligand[38,39].

S100A4 gene (also called *metastatin-1, calvasculin, fibroblast-specific protein-1*) expression in host stroma fibroblasts can contribute to prostate cancer progression and metastasis, through its myosin IIA-mediated effects on cancer cell motility[40].

IL-6, that is secreted by stromal fibroblasts as well as cancer cells, can enhance the effects of PTHrp on the osteoclasts[19,26,41]. Moreover, IL-6, by disregulating androgen-receptor activation and facilitating transdifferentiation of prostate cancer cells to neuroendocrine phenotype, contributes to promote the androgen-indipendent development and progression of prostate carcinoma; neuroendocrine cells, in turn, are involved in the prostate tumor progression by secreting several growth factors, such as neurotensin and bombesin, that are able to stimulate, *in vitro*, the growth of some prostate cancer cell lines[9,36,42]. Intriguingly, in the prostate carcinoma, neoplastic cells expressing Bcl-2, a potent antiapoptotic factor, are closely neighbouring with neuroendocrine cells, that also produce survivin, another apoptosis inhibitor[43,44].

Endothelin-1 (ET-1), production of which by metastatic prostate cancer cells as well as endothelial cells is upregulated under influence of many growth factors (TGF-α and -β, IGF-1 and -2, PDGF, bFGF) and interleukins (IL-1β and -6), interacts with ET_A and ET_B receptors of osteoblasts, thus enhancing the expression of bone matrix proteins (BMP), osteopontin and osteocalcin, together with the bone mineralization[45]. These effects are counteracted by atrasentan, a highly selective ET_A receptor antagonist.

Receptor activator of Nuclear Factor-kB ligand, RANKL, that is expressed by osteoblasts and bone marrow stromal cells, is able to bind RANK receptor on osteoclast precursors, thus inducing activated osteoclast formation; this effect is inhibited by osteoprotegerin, a soluble protein that acts as decoy RANK receptor[19].

Prostate specific antigen, PSA, as kallicrein-related protease, besides the physiological functions (hydrolysis of seminogelin-I and -II, resulting in seminal clot liquefaction), can promote, in prostate cancer bone metastases, osteoclast generation and degrade IGF-BG proteins, thereby enhancing the bioavailability of IGF-1[46].

ECM and BMP components (fibronectin, laminin, heparan-sulfate polysaccaride, type-I and -IV collagen, vitronectin, osteopontin, osteonectin, etc) and their degradative products, also affect prostate cancer cell behavior by increasing, through interactions with cell surface integrin and nonintegrin receptors, cell motility; furthermore, they are able to induce EMT, thus promoting cell-cell scattering and migration[47]. Osteonectin, by itself, is a potent bone chemoatractant for prostate cancer cells; such aptitude is clearly shown, in mouse model, by homing of intravenous injected prostate neoplastic cells to a subcutaneously implanted osmotic pump secreting this protein[48].

Inflammation and Prostate Carcinoma

For a long time it has been suspected that chronic inflammation can promote carcinogenesis and cancer growth. Several potential inflammatory mechanisms could be involved in the carcinogenesis and malignant progression[49,50]:

- production of *growth factors and cytokines* (particularly TNF-α, *tumor necrosis factor-α*) by monocyte-macrophages of inflammatory microenvironment. TNF-α may play an ambivalent role because, in high amounts, it could have an antitumor activity, whereas, when chronically produced in low amounts, it can act as a cancer promoter and enhancer through activation of *NF-kB* (*Nuclear Factor-kB*). In the *LNCaP*, prostate cancer cell line, TNF-α is specifically able to suppress androgen receptor expression, thus contributing to their hormone-insensitivity[49-55];
- induction of *cyclooxygenase-2* (Cox-2) in the microenvironment macrophages as well as in epithelial cells by a variety of growth factors and cytokines (e.g., VEGF, IL-1 and -8, TNF-α). Cox-2, that catalyzes the conversion of arachidonic acid to eicosanoids and prostaglandins, can promote carcinogenesis by means of these products, particularly of prostaglandin-E_2 (PGE_2), that is able to block the apoptosis and enhance VEGF-mediated angiogenesis[49-56]. Moreover, PGE_2 can also induce an increase of β-catenin, through which various Wnt genes are activated[20,55]. On the contrary, Cox-2 inhibition has proapoptotic effects by shunting arachidonic acid metabolism towards the synthesis of ceramide, a cell death mediator [49, 57];
- generation, by leucocytes and macrophages, of both *reactive oxygen species* (ROS, either free radical superoxide anion or nonradical hydrogen peroxide), through activation of NADPH, *nicotinamide adenine dinucleotide phosphate-H*, and *reactive nitrogen species* (RNS, nitro-

gen oxide and its reactive intermediates, among which peroxynitrites) through activation of the inducible nitric oxide sinthase (iNOS)[50-52,55]. *Cell oxidative stress* results from an imbalance between ROS generation and antioxidant availability. At high concentrations, ROS are important mediators of damage to cell structures, including lipid-membranes, cytoskeleton, nuclear matrix proteins and DNA; furthermore, they induce the dissociation of NF-kB from IkB, *inhiting proteins of NF-kB*, thus allowing its translocation in the nucleus, hence activation of DNA and mRNA transcriptional processes that, in turn, drive the synthesis of several potentially carcinogenetic cytokines and growth factors[49,52,54,55]. In addition to mitochondria, a major source of ROS, extramitochondrial generators of ROS, NADPH oxidase (Nox) systems, are also involved in the prostate cancer cell mitogenic signaling. ROS can affect cell-cell adhesion by modulating integrin expression and suppress anoikis, a cell-EMC detachment-induced apoptotic mechanism. Moreover, ROS and RNS are able to degrade ECM by increasing expression of MMP_S and heparanase (enzyme that cleaves the ECM heparan sulfate), together with inactivating $TIMP_S$[55]. Nitric oxide overproduction, by itself, induces activation of proto-oncogene p21 and generation of carcinogenic p53 mutations[49,54,55]. ROS production in inflammatory processes as well as in tumor microenvironment may be shown by detection of chemoluminescence by means of its specific enhancers for hydrogen peroxide and anion superoxide[58,59]. Interestingly, in human prostate cancer cell lines, mitochondrial activity and, therefore, oxidative stress are peculiarly enhanced by androgens[60].

NF-kB plays a crucial role between inflammation and cancer promotion and progression: on the one hand, it results from inflammatory cytokine-chemokine-ROS pathways and, on the other hand, it mediates the expression of a variety of genes which are involved in cancer development and progression[61,62]. Besides its antiapoptotic effects, NF-kB also inhibits the "autophagy", an alternative tumor cell death process towards the lack of apoptosis, thus facilitating cancer growth[55]. In a murine model of prostate cancer metastasis to the liver, activation of NF-kB pathway induces the transcriptional repression of maspin, a metastasis suppressor gene[63].

The inflammatory implications in prostate cancer pathogenesis are also suggested by epidemiological positive correlation between long term use of non-steroidal anti-inflammatory drugs (e.g., aspirin) and reduction of the prostate cancer onset[61-63].

Chronic inflammations, caused by persistent low-grade sexually transmitted *infections*, could play a role in development of prostate tumors, in same way by which other infections can promote specific malignancies (e.g., *Helicobacter pylori*-associated gastric cancer or intestinal stromal tumor; Hepatitis-B and C virus-associated hepatocellular carcinoma; Human papilloma virus-related cervical and penile tumor; Schistosomiasis-induced bladder cancer)[55,64,65]. Even *prostate gland atherosclerosis*, as a vascular inflammatory process, by producing local oxidative stress with DNA damage, may be assumed as a risk of prostate carcinogenesis[66].

Implications for Current and Future Research Activities

The knowledge of both genetic and microenvironmental cancer pro-metastatic factors is necessary to define timing and suitability of therapeutic measures.

Prostate carcinoma-associated aggressiveness and metastatic behavior may represent a newly acquired variant due to interactive mechanisms that interplay between cancer cells and host microenvironment rather than a selection of a preexisting gene abnormalities in cancer cell clones[9,23]. *Tumor surrounding microenvironment*, through a variety of growth factors and cytokines produced by host stromal cells and tumor-associated macrophages, is able to affect nuclear function of cells cancer by inducing the expression of a set of metastatic oncogenes and/or, on the contrary, the loss of tumor-suppressor genes. Even the stem-cell aptitudes of malignant cells are sustained by the surrounding microenvironment. Several polymorphisms in innate or adaptive immune genes – such as MSR1, *macrophage scavenger receptor1*, MIC-1, *macrophage inhibitory cytokine-1*, RNASEL, *ribonuclease L*, besides VEGF and IL-8 and IL-10 – that can affect immune response pathways so inducing a persistent prostatic inflammatory process, have been recently recognized to be linked with prostate carcinoma risk[67,68]. Even either G allele- or GG-ACT (α1 antichymotrypsin) genotype appears to be associated with such risk[69]. Tumor angiogenesis plays a well established critical role in the pro-

gression of prostate carcinoma, as well as of any solid malignancy, and an increase in expression of *endoglin*, a vascular endothelial cell surface glycoprotein-receptor for TGF-β1 and TGF-β3, may contribute to tumor progression and lymph node/bone metastasis, regarding which it means as useful marker; counteractively, anti-endoglin monoclonal antibodies are able to suppress angiogenesis and tumor growth[70]. As well as it has been recently recognized about tumor metastasis to the lung[71], TGF-β, through angiopoietin-like 4 factor, could also play a role in prostate cancer cell seeding to the bone.

Therefore, modulation of inflammatory- and tumor microenvironment represent an intriguing target of prostate cancer treatment strategies[14,23,49,61,71-74]. Besides the various well-known antiangiogenetic agents (anti-VEGF monoclonal antibodies such as bevacizumab; multitargeted tyrosine kinase inhibitors such as sunitinib and sorafenib), gambogic acid, a polyprenylated xanthone compound of *Gamboge hanburyi*, appears to suppress VEGF receptor-2 and its signaling, thus leading to the inhibition of angiogenesis and prostate cancer growth[73]. Otherwise, as far as oxidative stress in prostate cancer cells is concerned, diphenyliodonium, a specific Nox inhibitor and ROS production blocker, is able to induce a loss of both mitochondrial potential and MMP activity, plausible reasons for an increased apoptosis and a decreased invasive phenotype of prostate cancer cells[74].

On the other hand, just considering that familial aggregation studies and segregation analyzes provide strong evidence that Mendelian genetic inheritance of prostate carcinoma – genetic determinism and predisposition – exists in about 5 per cent of cases and according to stem cell biology-related paradigm by which cancer cells may be, them selves, malignant stem cell clones, therapeutic measures, such as post-transcriptional gene inactivation by *siRNA*-interference and/or epigenetic modulation by DNA methyltransferase/histone deacetylase inhibitors should be directed to interfere with aberrant gene expression. Much intriguing work is been carried out in the field of gene therapy for prostate carcinoma. In addition to the large variety of gene delivery vehicles – either viral or non-viral vectors such as liposomes, polymers and in future nanoparticles-nanospiders, quantum dots – gene oncotherapy includes several cytoreductive-antiproliferative strategies, rancing from prodrug suicide gene therapy (transduction of gene encoding a nontoxic prodrug-activating enzyme to obtain a toxic metabolite) or antisense gene therapy (inactivation of oncogenes through antisense oligonucleotides) to immunotherapeutic gene techniques (delivery of genes encoding for cytokines) or corrective replacement of tumor suppressor genes. Further developments are directed to the use of PSA promoter-driven gene therapy approaches such as suicide gene strategy[75-85].

The immunotherapy, like PSA-, PSMA (prostate specific membrane antigen)-, PAP (prostatic acid phosphatase)-, PSCA-vaccine, could represent a suitable treatment strategy to suppress tumor progression and metastasis, unless a immune-refractory subset of malignant cells is selected by Darwinian evolutionary processes together with the achievement of immune tolerance through $MDSC_S$, *myeloid-derived suppressor cells*, unlucky events that represent the "Achilles' heel" of current cancer immunotherapeutic approaches[77,86,87].

Further studies are needed to closely investigate the use of such above-mentioned strategies. Looking to the future, the time will show us whether these therapeutic perspectives will remain as a sheer unrealizable dream or not.

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