REVIEW

Recent advances on multiple tumorigenic cascades involved in prostate cancer progression and targeting therapies

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Recent advances on differently-expressed gene products and their functions during the progression from localized androgen-dependent states into androgen-independent and metastatic forms of prostate cancer are reported. The expression levels of numerous oncogenes and tumor suppressor genes in distinct prostate cancer epithelial cell lines and tissues relative to normal prostate cells are described. This is carried out to identify the signaling elements that are altered during the initiation, progression and metastatic process of prostate cancer. Additional information on the interactions between certain deregulated signaling pathways such as androgen receptor (AR), estrogen receptors, epidermal growth factor receptor (EGFR), hedgehog and Wnt/β-catenin cascades in controlling the proliferation, survival and invasion of tumor prostate epithelial cells during the disease progression is described. The emphasis is on the critical functions of the AR and EGF–EGFR systems at all stages during prostate carcinogenesis. Of therapeutic interest, new strategies for the diagnosis and treatment of localized and metastatic forms of prostate cancer by targeting multiple tumorigenic signaling elements are also reported.

Introduction

Prostate cancer (PC) is the most common malignancy diagnosed in men and the metastatic PC forms represent the second cause of mortality (1,2). The causes of PC remain poorly understood. Many gene products show deregulated functions. Numerous growth factors and their receptors are also overexpressed during the progression of this hyperproliferative disease (3–18). These specific changes of gene expression in epithelial and stromal tumor cells during the different developmental stages of PC notably contribute in enhancing the tumor cell growth, survival, migration and invasiveness. In particular, the activation of multiple developmental signaling cascades including androgen receptor (AR), estrogen receptor (ER), epidermal growth factor receptor (EGFR), HER-2, hedgehog and Wnt/β-catenin signaling pathways may confer to them the aggressive phenotypes that are observed in high prostatic intraepithelial neoplasia (PIN) grades of malignancy and adenocarcinomas (Figures 1–3) (3,4,11,14,18–22). Moreover, the down-regulation of several apoptotic signaling cascade elements in metastatic PC cells, such as the ceramides and caspases, combined with the enhanced expression of anti-apoptotic factors, such as Bcl-2, may also contribute to the survival of tumor epithelial cells (3,23–25). More specifically, the enhanced expression of enzymes involved in the ceramide catabolism and/or down-regulation of caspase cascades appears to be responsible, in part, for the resistance of certain metastatic PC cells to cytotoxic responses induced by diverse chemotherapeutic drugs (3,23,24,26–28).

The current treatments for PC, consisting of malignant prostate ablation by radical prostatectomy (RP), radiotherapy, hormonal therapy and/or neo-adjuvant chemotherapy, are generally curative for the majority of patients diagnosed with localized androgen-dependent PC forms; however, progression to androgen-independent and metastatic disease states is often accompanied by a recurrence of PC (29–31). The available chemotherapeutic treatment options for patients with hormone-refractory PC (HRPC) are rather palliative and remain mostly ineffective with a poor prognosis. The prognosis is associated with a median survival rate of ~12 months after diagnosis (31–34). Therefore, the development of a novel treatment that is more effective against antiandrogen- and chemotherapy-refractory PC forms is highly desirable. One new approach is the molecular targeting of distinct deregulated signaling elements in PC cells whose tumorigenic products are involved in the development of resistance to conventional cytotoxic agents used in the therapy.

Prostate carcinogenesis

PC initiation

Although the events associated with the initiation of PC are not precisely known, some recent lines of evidence suggest that PC could be derived from precancerous lesions occurring during prostate tissue injury, such as chronic proliferative inflammatory atrophy (11,35–39). As a matter of fact, a pool of prostate specific stem cells, which are implicated in cell renewal during the prostate regeneration process, has been proposed to represent the minority of epithelial cells that

Abbreviations: AR, androgen receptor; CTCs, circulating tumor cells; E2, 17β-estradiol; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FGF, fibroblast growth factor; GSK3β, glycogen synthase kinase 3β; HGF, hepatocyte growth factor; HRPC, hormone-refractory prostate cancer; 17β-hydroxysteroid dehydrogenase; IGF, insulin-like growth factor; IL-6, interleukin-6; ILK, integrin-linked kinase; MAPK, mitogen-activated protein kinase; MIC-1, macrophage inhibitor cytokine-1; NE, neuroendocrine; NF-κB, nuclear factor kappa-β; PC, prostate cancer; PI3K, phosphatidylinositol 3′-kinase; PIN, prostatic intraepithelial neoplasia; PKA, protein kinase A; PKCβ, protein kinase C-β; PNI, prostatic perineural invasion; PSA, prostate specific antigen; PTEN, tensin homologue deleted on chromosome 10; RP, radical prostatectomy; siRNA, small interfering RNA; SHH, sonic hedgehog; TCF, T-cell factor; TGF-α, transforming growth factor-α; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

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could provide the PC progenitor cells following the sustained activation of different growth factor signaling cascades (Figure 1) (11,35–37). In support of this model, certain prostate progenitor cells have recently been isolated from proximal regions of prostatic ducts (37,40–42). Prostate progenitor cells have some of the properties associated with stem cells due to their striking plasticity. These properties include the ability to show unlimited growth in a specific microenvironment and to generate multiple, more differentiated prostate cells. In fact, the niche of prostatic stem cells, which represents ~1–2% of basal epithelial cells, appears to be localized at the basement membrane of the prostatic gland (37,43–46). More specifically, the prostatic adult stem cells are characterized by specific markers such as $\alpha_2\beta_1^{(ha)}$-integrin, CD133, stem cell antigen-1 (Sca-1), prostate stem cell antigen (PSCA) and cytokeratin 6a (K6a). The prostatic adult stem cells are also characterized by the basal cell-like phenotypes including their androgen-independence due to the lack of AR and significant expression levels of K5, K14, p63, antiapoptotic Bcl-2 protein and telomerase (35–37,41,42,44,47). The multipotent adult prostatic stem cells at the basal layer may replenish themselves occasionally, including during prostatic regeneration after tissue injury, to reconstitute the normal prostatic epithelium. In fact, the basal stem cell may generate the transit-amplifying/intermediate cells that, in turn, undergo terminal differentiation and give rise to the more differentiated cells, including neuroendocrine (NE) cells and luminal secretory epithelial cells (Figure 1) (35–37,41,48). The NE cells are characterized by significant expression levels of the typical NE markers including chromogranin-A and enolase, while the secretory epithelial cells express significant levels of AR, prostate specific antigen (PSA), K8 and K18. The establishment of xenografts derived from human prostatic stem cells in nude mice reconstituted prostatic epithelium layers, including intermediate cells and more differentiated secretory epithelial cells in response to androgens and the NE cell lineage in response to androgen deprivation (42,44,45).

There are recent advances in the identification of putative prostatic stem cells; however, the molecular and cellular mechanisms of the oncogenic transformation of prostate progenitor epithelial cells and the changes in stromal–epithelial cell interactions mediating initiative events are still not precisely known. Among the models of PC initiation, there is the possibility that prostate dysplastic lesions may derive from deregulated mitogenic signaling cascades in either basal multipotent stem cells and/or transit-amplifying/intermediate cells. Prostate dysplastic lesions, in turn, may subsequently give rise to a heterogeneous population of cancer epithelial cells showing aberrant differentiation, unlimited division and a decreased rate of apoptotic cell death (Figure 1) (11,35–37,48). In this matter, the conversion of androgens into estrogens in the prostate compartment may notably constitute a very early event of the ethiopathogenesis of PC (20). Indeed, it has been reported that 17$\beta$-estradiol (E2) may induce the up-regulation of the expression of a catalytic subunit of human telomerase (hTERT) and telomerase activity in human prostate epithelial
cell lines. This increase of telomerase activity constitutes an event that is generally associated with unlimited cell proliferation (49). In addition, some in vivo distinct animal model studies on different stages of prostate carcinogenesis have also provided direct evidence of hormones inducing the dysfunctions that lead to PC (50,51). More specifically, it has been observed that high doses of E2 plus testosterone induced the apparition of dysplasia in dorsolateral lobes of Noble rats after only 2 months followed by the development of carcinoma in situ at 4 months, and adenocarcinoma at 7 months in dorsolateral and ventral lobes (50). Interestingly, it has also been reported that inducing dorsolateral lesions was inhibited by the selective antiestrogen ICI 182780 and associated with the overexpression of TGF-\(\alpha\) in dorsolateral lobes; however, the expression of this factor was negative in ventral lobes (50,51). This suggests that the carcinogenic effect induced by the combined use of estrogens and testosterone in rodent animal models may be mediated, in part, via ER and inducing an EGFR signaling cascade through the up-regulation of TGF-\(\alpha\). Additionally, E2 and androgens have also been reported to activate WOX1 in PC cells. This activation is correlated with the progression of malignant transformation from normal prostate into hyperplasia and cancerous and metastatic stages in vivo (52).

More recently, the activation of distinct developmental signaling pathways, including hedgehog, Wnt/\(\beta\)-catenin and EGFR cascades, and the inactivation of the transforming growth factor-\(\beta\) (TGF-\(\beta\)) signaling cascade in prostatic adult stem cells have been proposed to represent potential events that may also contribute to the initiation of primary lesions leading to PC development (Figure 1). For instance, SHH-GLI developmental signaling, which may be re-activated in prostatic stem cells during the regenerating tissue process, also appears to be able to induce PC initiation and development (11,21,53). Indeed, the overexpression of the hedgehog signaling element, GLI-1, in human normal prostate progenitor epithelial cells, hPrEC, has been observed to result in unlimited cell growth in vitro and the formation of an aggressive tumor in vivo (11). Similarly, it has also been reported that the stabilization of \(\beta\)-catenin was sufficient for initiating PIN-like lesions that resembled early human PC states in mice as early as 10 weeks of age (54). More specifically, \(\beta\)-catenin-induced prostate lesions were associated with increasing c-Myc and AR expression levels and an increasing rate of cell proliferation. In this matter, it has also been reported that a single genetic event, the c-Myc oncogene overexpression in hPrEC, was able to induce the immortalization of these cells by up-regulating telomerase expression and accumulating cell-cycle inhibitor proteins including p16\(^{Ink4a}\) (55). Importantly, the sustained activation of the AKT survival cascade in the Sca-1-enriched fractions of murine prostate-regenerating cells by infection with lentivirus containing a constitutively active form of AKT1 also resulted in inducing mouse PIN lesions at low moi of AKT1 lentivirus and fully developed carcinoma

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**Fig. 2.** Scheme showing the possible mitogenic and antiapoptotic cascades induced through the AR, ER, EGFR family members, IL-6 and PKA signaling pathways. The possible stimulatory effect of growth factor signaling elements on the MAPK and/or PI3K/Akt which may be involved in androgen-dependent and androgen-independent activation of AR in certain PC cells are shown. The enhanced expression levels of AR- and ER-target genes which can contribute to an increase in the tumorigenicity of PC cells are indicated.

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Deregulated gene products and targeting therapies for prostate cancer
in situ at high moi in β-actin GFP transgenic mice (42). Similarly, the in vivo characterization of PTEN-mutant mice expressing decreased PTEN (tensin homologue deleted on chromosome 10) tumor suppressor gene levels revealed that the up-regulation of Akt activity also resulted in tumor initiation and progression (56). More recently, the BMI-1 oncogene pathway, which is also involved in normal stem cell renewal, has been reported to be activated in transformed cells from numerous cancers including PC. The BMI-1 oncogene pathway also contributes to tumor progression (57). Additionally, alterations in stromal–epithelial interactions and/or genetic changes leading to a decreased expression of the TGF-β/TGF-βR system in the proximal region of prostatic ducts and/or the down-regulated expression levels of the negative cell cycle-regulators p27kip1 and p63 and the antiapoptotic factor Bcl-2 in quiescent stem cells may also lead to an enhanced rate of stem cell division and excessive prostatic epithelial growth. In certain cases, excessive prostatic epithelial growth may trigger prostatic neoplasia (40,43,58–60). On the other hand, the enhanced motility and migratory properties of prostatic adult progenitor cells, which is observed during the regeneration of normal prostate epithelium after tissue injury, also represents an important event in initiating dysplastic lesions leading to PC. In this matter, EGF and the alterations in cell–surface receptors such as integrins seem to assume a critical role in the regulation of the migration of normal and transformed prostatic epithelial cells. More particularly, it has been observed that EGF-induced α6β1-integrin expression in non-tumorigenic and non-invasive prostate RWPE-1 cells was accompanied by a reduction of their ability to undergo normal acinar morphogenesis through the alterations of interactions between these cells and the extracellular matrix (ECM) (61). This oncogenic effect of EGF also conferred a more malignant and invasive phenotype to RWPE-1 cells.

Altogether, these observations suggest that the sustained activation of androgens, estrogens and distinct growth factor signaling cascades in prostate progenitor epithelial cells may lead to the generation of a heterogeneous population of cancer progenitor cells showing uncontrolled growth and altered differentiation. These cancer progenitor cells, in turn, may induce the formation of PIN-like lesions and, ultimately, PC development.

**PC development and metastasis**

Almost all PCs initially develop from secretory epithelial cells of the prostate gland and generally grow slowly within the gland. When the tumor cells penetrate the outside of the prostate gland they may spread to tissues near the prostate, first to the pelvic lymph nodes and eventually to distant lymph nodes, bones and organs such as the brain, liver and lungs (Figure 1) (16,62–64). The in vitro and in vivo characterization of the behavior of numerous human PC cell lines as compared with the normal prostatic epithelial cells has notably indicated that several oncogenic signaling cascades
are involved in regulating the progression from localized and androgen-dependent PC forms into aggressive and androgen-independent states (3,11,12,14,19,21). In addition, the genetic changes in stromal–epithelial cells may also alter prostate homeostasis in adults, which is maintained via the reciprocal mesenchymal–epithelial interactions. This leads to the differentiation of prostatic smooth muscle and an enhanced proliferation of vascular endothelial and epithelial cells during the transition of low- to high-grade PINs and PC development (16,40,43,62,63). In fact, the enhanced expression of a variety of growth factors, including EGF, TGF-α, fibroblast growth factor (FGF), hepatocyte growth factor (HGF), nerve growth factor, insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) and their cognate receptors concomitant with the alterations in TGF-β signaling elements, appears to cause a critical role in inducing changes in stromal–epithelial cell interactions during PC development (3,16,22,43,60). More specifically, the enhanced VEGF expression induced by several growth factors in tumor epithelial cells seems to contribute to the angiogenesis process of paracrine fashion during the early stages of PC. The subsequent expression of the VEGF receptor (VEGFR) on the tumor epithelial cells at late stages may participate in the autocrine and paracrine regulation of the invasiveness of tumor cells.

In this matter, a model has been proposed to explain the high frequency of bone metastasis of PC cells (62,63). According to this model, the molecular mechanism at the basis of osteotropicism of PC metastasis could implicate an enhanced expression of VEGF and VEGFR-2 on the PC cells. This could subsequently result, through an autocrine loop, in activating αvβ3- and αvβ5-integrins at the surface of PC cells. Hence, the occurrence of these specific changes in the PC cells could preferentially lead to their migration and adhesion at a component of bone matrix, the SPARC protein (Figure 1). In addition, since the transfection of parathyroid hormone-related oncoprotein has been observed to transform the non-invasive PC cell line into one showing a greater skeletal tumor progression, it appears that this hormone may also contribute to the preferential bone migration of PC cells (64). In this matter, it has also been reported that the activation of the developmental Notch signaling pathway in the osteoblastic C4-2B PC cell line derived from LNCaP cells may confer the osteoblastic properties to these cells by inducing the expression of specific genes, such as osteocalcin, in the bone microenvironment (65).

On the other hand, the up-regulation of telomerase activity induced by E2 in LNCaP, DU145 and PC3 cells through the binding of ER-β at telomerase promoter sequence, may also give a more aggressive phenotype to these metastatic PC cells (49). Hence, the oncogenic changes in the tumor stromal–epithelial cells, which may be induced by the activation of distinct growth factor signaling cascades, may confer a more malignant behavior to cancer progenitor cells during the progression from localized PC forms into metastatic states.

**Differently-expressed genes**

Several approaches have been developed to establish the gene expression changes occurring in tumor epithelial and stromal cells during the pathological processes associated with PC progression. Many genetic alterations are associated with the different stages leading to the malignant transformation of the normal prostate glandular epithelium (5–13,15,16,66,67). In fact, the deregulated expression of some genes in stromal–epithelial cells from neoplastic lesions appears to result in low- to high-grade PINs, corresponding initially to localized androgen-dependent disease states. These PINs subsequently progress to androgen-independent carcinomas and adenocarcinomas followed by the formation of metastatic lesions, resulting ultimately in the invasive forms of PC (Figure 1). Phenotype changes associated with PC cell behavior are, in part, due to an enhanced expression of numerous oncogenes and/or a decreased expression of tumor suppressor genes induced through the gene amplification and chromosomal deviation or deletion, respectively (Table I).

**Prostatic intraepithelial neoplastic and prostatic cancer tissues**

Numerous microarray, immunohistochemical and real-time PCR analyses of tumor tissue samples from patients at different stages of PC have identified specific patterns of gene expression which are associated with PC progression. In particular, PC cells overexpress several growth factors and their receptors and show enhanced expression and/or activity of a variety of antiapoptotic gene products (Table I). More specifically, the enhanced expression of cell survival products, such as EGF (Erbb1), Erbb2 (Her-2/neu), cl-2, p53, syndecan-1 and clusterin, are among the most frequent genetic alterations observed in metastatic HRPC (7,13,17,28). Among them, syndecan-1 can notably provide pericellular matrix heparan sulfate chains to FGF and, thereby, form a FGF-binding complex which may also contribute to PC progression induced through FGF receptor-1 (FGFR-1) (68). In this matter, the down-regulation of the Sprouty gene family in the epithelial cells during PC development, which are negative regulators of FGF signaling, may further contribute to potentiate the carcinogenic effect induced through the FGF–FGFR system (69). Moreover, the stress-associated gene product, clusterin, which is highly expressed in HRPC cells after post-hormone treated RP while its expression is very low or undetectable in untreated specimens, may also confer a PC cell survival advantage by decreasing the rate of apoptotic cell death (28). In addition, the up-regulated expression of diverse markers, such as macrophage inhibitory cytokine-1 (M1C-1), Myc, caveolin-1, integrin-linked kinase (ILK), mucin 1 (MUC1), mucin 18 (MUC18) and histone deacetylase 1 (HDAC1), has also been associated with PC progression to more advanced pathological stages (Table I) (66,70–76).

On the other hand, inactivating mutations in several tumor suppressor genes, such as phosphatase PTEN/MMAC1 and p53,
as well as silencing by hypermethylation of distinct invasion-suppressor genes, including differentiation related gene-1 (Drg-1), E-cadherin and membrane-anchored serine prostatasin, have also been associated with high-grade PINs and a more malignant phenotype of PC cells (Table I) (5,13,77–79).

Prostatic cancer cells

The analyses of differently-expressed genes in tumor primary cells and different PC cell lines have identified multiple gene products which may be involved in the transition from androgen-dependent into androgen-independent forms of PC. For instance, the microarray analyses of genes expressed in human CWR22 xenograft models have revealed that the expression levels of HGF, cyclin D1, insulin-like growth factor-binding protein 2 (IGFBP2) and 27 kDa heat-shock protein (HSP27) were up-regulated in the CWR22R cell variants showing less dependence on androgens as compared with the parental hormone-sensitive CWR22 cells (80,81). Similarly, the microarray analyses of differently-expressed genes in early passage androgen-sensitive LNCaP-C33 cells and late passage androgen-independent LNCaP-C81 cells have revealed that several genes, including guanine nucleotide-binding protein Gi, α-1 subunit, cyclin B1, cyclin-dependent kinase-2 (CDK-2), c-Myc, c-Myc purine-binding transcription factor, macrophage migration inhibitory factor (MIF) and MIC-1, are up-regulated in androgen-independent LNCaP cells (82). Importantly, the analyses of five generations of androgen-independent tumors derived from primary LNCaP cells established orthotopically in male nude mice that are surgically castrated have also revealed that the changes in expression levels of p53, p21waf and Bcl-2 occurred in these cells as compared with parental androgen-sensitive LNCaP cells (83). In this matter, the results from microarray analyses of the expression profiling in androgen-dependent LNCaP and androgen-independent LNCaP-LNO cells have also revealed that androgens and EGF may induce the expression of several similar genes, and certain gene products were also constitutively activated in androgen-independent LNCaP-LNO cells (84). Moreover, the cDNA microarray analyses of alterations in gene expression profiles in a series of PC cell lines isolated at different stages of tumorigenesis from C3(1)/Tag transgenic mice have also indicated that significant changes occur in the expression of selenoprotein-P, L1-cell adhesion molecule, metastasis-associated gene (MTA-2), Rab-25 and tumor-associated signal transducer-2 (Trop-2) (85). Additionally, a RT-PCR analysis indicated the higher expression levels of four HOX cluster genes (HOXC4, HOXC5, HOXC6 and HOXC8) in tumor primary cells and cell lines derived from lymph node metastases as compared with those detected in benign cells and cell lines from other tumor sites. This suggests that these gene products could be implicated in the process leading to the metastasis of PC cells to lymph nodes (Figure 1) (86). In this context, the analyses of gene expression in prostatic perineural invasion (PNI) cells performed by cDNA microarray, real-time quantitative PCR and immunofluorescence have also revealed that the NF-κB survival signaling element is up-regulated in the PNI cells (87).

Tumor stromal cells

The migration and invasion of PC cells and the angiogenesis process are important for tumor growth and metastasis. They are also controlled by the changes of gene expression in tumor stromal cells (16). In this matter, the results of immunohistostaining combined with in vivo experiments performed on LNCaP xenograft tumor tissues indicated that the expression of sonic hedgehog ligand, SHH, in tumor epithelial cells may lead, in paracrine fashion, to the up-regulated expression of the GLI-1 transcription factor in stromal cells and the activation of an SHH signaling cascade in these cells (88). Moreover, the immunohistochemical studies carried out on the Noble rat animal model revealed that the smooth muscle cells (SMCs) subjacent to dysplastic lesions and carcinomas usually exhibited a preferential loss of myosin, desmin and laminin, suggesting that a dedifferentiation of surrounding SMCs may occur during the development of PC (89). Importantly, a quantitative analysis of the specific stroma markers expressed on tumor tissues from patients performed by tissue microarrays has revealed that a reduction of the desmin and smooth muscle α-actin levels, which occur in cancer-associated reactive stroma relative to normal fibromuscular stroma, represents significant and independent predictors of a recurrence-free survival of patients (87). Conversely, the prostate stromal cells also express a variety of paracrine factors that may influence the differentiation and proliferation of tumor epithelial cells (87,90,91). Therefore, these observations underline the importance of additional studies on the gene expression changes in prostatic tumor stromal cells to establish a more complete profile of gene products involved in stromal–epithelial cell interactions during PC progression.

Tumorigenic signaling cascades

Hormonal signaling cascades

The growth and homeostasis maintenance of the prostate gland is hormone dependent in that its functions require the supply of different circulating hormones. As a matter of fact, castration shortly leads to cell apoptosis in the rat prostate gland or human PC-82 PC xenografts in vivo (92). Among them, testosterone and dehydroepiandrosterone, which are produced abundantly by the testis and adrenal gland, are the major circulating androgens in males. In general, the testosterone levels found in serum decrease with advancing age while the estrogen levels, including E2, enhance locally in prostate fluid (20). In fact, the rise in the ratio of estrogens relative to androgens is an important factor that may contribute to the initiation of dysplasia and prostate carcinogenesis. Moreover, the enhanced expression and activity of AR have also been associated with high aggressive clinicopathologic features and decreased biochemical recurrence-free survival in patients treated by RP (15).

Androgenic signaling cascade

AR is a member of the nuclear receptor superfamily that functions as a ligand-activated transcription factor by inducing the expression of numerous mitotic gene products which are important signaling elements for the normal and neoplastic development of the prostate (93). Among the androgens, the active metabolic product, 5α-dihydrotestosterone (5α-DHT), which is produced from the transformation of testosterone catalyzed by the 5α-reductase, predominantly mediates its biological effects through binding to AR (Figure 2). In fact, the activation of AR signaling by the androgens may lead to the up-regulated expression of numerous genes, such as PSA, c-fos, Drg-1 and caveolin-1 (cav-1), and the stimulation of distinct intracellular pathways involved in the growth and survival of untransformed and prostatic tumor cells. More
particularly, AR activation induced by the treatment of LNCaP cells with androgens may result in the up-regulation of EGFR and caveolin-1 expression levels which, in turn, may be involved in the stimulation of the survival signals and metastatic activities in these cells (4,94). Moreover, the results from an analysis of c-Myc functions in LNCaP cells by using an AR inhibitor, bicalutamide (as known as casodex), or by RNA interference directed against AR or c-Myc have also indicated that c-Myc is required for androgen-dependent cell growth and acts downstream of AR by inducing an enhanced expression of several cell-cycle regulatory proteins (67). In this matter, it has also been observed that the overexpression of c-Myc in LNCaP cells conferred the more tumorigenic properties to cells which were then able to grow in an androgen-depleted medium. Additionally, the antia apoptotic effect of androgens also appears to be mediated, in part, by down-regulation of the ceramide accumulation in certain PC cells. Indeed, it has been reported that androgen deprivation was accompanied by a rise of the endogenous C16-ceramide level via the de novo pathway, a growth arrest in the G1 phase of the cell cycle followed by a progressive apoptosis in vitro in the androgen-sensitive LNCaP cells whose effects were inhibited in the presence of α-DHT or ceramide synthase inhibitor, fumonisin B1; however, androgen-independent PC3 and DU145 cells were unresponsive to this treatment (95). Similarly, the synthetic androgen R1881 also inhibited the apoptotic death of LNCaP cells induced by the bacterial sphingomyelinase, which acts by increasing the endogenous ceramide production, supporting the fact that the androgens may counteract a downstream signaling element in the ceramide-induced apoptotic cascade (96).

Multiple mechanisms by which PC progresses from androgen-sensitive into androgen-independent stages have been proposed. In general, the tumor epithelial cells appear able to adapt for growth and survival in a low-androgen environment as well as in the absence of androgens during the progression to more aggressive PC forms. In this context, the majority of androgen-independent prostatic tumors still express AR and the aberrant activation of the AR pathway may be due to AR mutation, amplification or deletion in PC cells (97–99). In fact, AR activation in the presence of low androgen levels may result from an enhanced expression of the AR protein, overexpression of AR co-activators or decreased co-repressor levels (15,99). In addition, the mutation in AR, as observed in the androgen-sensitive AR-T877A LNCaP and AR-HB74Y CWR22 cells, may also result in its activation by antiandrogens, other steroid types as estrogens and progesterone, and distinct signaling elements (97–99). Additionally, AR activity seems to be tightly regulated by the activation of distinct growth factor cascades which can induce the AR modifications, including phosphorylation and acetylation or changes in interactions of AR with other cofactors (98,100). Among them, EGF, IGF-1, KGF, interleukin-6 (IL-6), oncostatin M and ligands stimulating the cAMP-dependent protein kinase A (PKA) pathway may activate AR by phosphorylation in the absence of androgens either directly or indirectly via mitogen-activated protein kinase (MAPK) and/or phosphatidylinositol 3'-kinase (PI3K) cascades in certain PC cells and, thereby, contribute to AR-induced gene expression (Figure 2) (97–99,101). Hence, the activation of AR in the absence or presence of low androgen levels may contribute to androgen-independent growth and survival of certain metastatic PC cells as observed after antiandrogen therapy. Nevertheless, since the hypermethylation of the AR gene promoter may lead to the expression of undetectable AR levels in certain PC cells as those detected in DU145 cells (102), it appears that AR functions are not absolutely essential for the sustained growth and survival of certain highly metastatic and androgen-independent PC cells.

**Estrogenic signaling cascade**

Several lines of evidence from animal models and human epidemiologic studies indicated that the estrogens may assume an important function for the maintenance of adult prostate homeostasis as well as in initiation and PC development (20,49–51). In aging men, PC generally occurs in an estrogen-dominant environment concomitant with decreasing androgen levels (20). Moreover, ER-α is principally expressed by stromal cells in normal and malignant tissues, while the ER-β expression level decreases during PC development in the gland but reappears in lymph node, brain and bone metastases (103). The results from some studies revealed that metastatic LNCaP and DU145 cells express the ER-β receptor subtype while significant levels of ER-α and ER-β were detected in PC3 cells (103,104). In addition, the significant expression level and activity of aromatase, whose enzyme catalyzes the transformation of testosterone into E2, have been detected in LNCaP, DU145, PC3 cells and micro-dissected prostate epithelial tumor cells, while its enzyme was not detected in non-malignant prostate epithelial cells (Figure 2) (104,105). More recently, it has been reported that the nuclear expression of constitutively active estrogen-related receptors ERRα, ERRβ and ERRγ is reduced in human metastatic PC cells as compared with normal prostate epithelial hPrEC cells (104). Significant levels of ERRs detected on the metastatic PC cells suggest that these nuclear receptors in conjunction with ERs could also contribute to inducing ERR-transcriptional activation of certain tumorigenic genes during PC progression. This underlines the importance of further determining the precise functions assumed by ERRs in the normal prostate as well as during the initiation and progression of PC.

Although the up-regulated expression of ER-β and local estrogen production may occur in metastatic PC cells, the molecular mechanism(s) associated with the estrogens are not yet established. In this matter, in vitro studies carried out on LNCaP cells have notably indicated that E2 induced the growth of these cells (20,106). The growth stimulatory effect induced by E2 in LNCaP cells, which appears to be mediated by activating ER-β, and mutated AR was inhibited by the addition of pure antiestrogen ICI 182780 or ER-β antisense as well as antiandrogen bicalutamide, respectively (106). In this context, it is noteworthy that the estrogenic effect mediated through AR appears to be related to mutations occurring in this receptor as detected in LNCaP cells and certain other tumor types. It has also been reported that the maintenance of LNCaP cells during several months in culture was accompanied by increasing expression and activity of reductive 17β-hydroxysteroid dehydrogenase (17HSD), the enzyme involved in the transformation of estrone (E1) into its more active estrogen metabolite, E2, while a decrease in activity of oxidative 17HSD was noted (Figure 2) (107,108). Moreover, it has also been observed that α-DHT was converted through the reaction catalyzed by reductive 17HSD into (5α-androstane-3β,17β-diol) 3α-DHT as well as 3βA-diol whose metabolite has been proposed to activate ER. These changes in late passage LNCaP cells were also accompanied by an enhanced
expression of ERE-targeted genes such as tissue plasminogen activator. In addition to the classical nuclear effect induced by estrogens through the transcriptional activity of nuclear receptors, it has recently been proposed that these hormones could also induce their mitotic and survival effects through the stimulation of extranuclear signaling elements. For instance, it has been reported that the activation of ER localized near the membrane within the caveolea by E2 could rapidly lead to stimulation of the Src-Raf-1-ERK2 cascade and enhanced proliferation of LNCaP cells (109). This suggests that the actions of estrogens and antiestrogens may be mediated, at least in part, via ER-β in LNCaP cells. Also, the estrogen-ER-β axis may confer a more malignant phenotype to these PC cells during the metastatic process. Although the effect of estrogens on the growth and survival of other PC cell lines have not been established, the antiestrogens, ICI 182,780 and tamoxifen, have been observed to induce antiproliferative and cytotoxic effects on DU145 and PC3 cells (103). Moreover, the cytotoxic effects induced by ICI 182,780 on DU145 cells were also inhibited by the pretreatment of cells with ER-β antisense construct, suggesting that the antiestrogens could mediate their anticarcinogenic effect, at least in part, via the ER-β subtype in these cells.

Altogether, these observations suggest that the estrogens may mediate their carcinogenic effects on tumor stromal cells of paracrine manner via ER-α during PC progression. They could also modulate the growth and/or survival of tumor epithelial cells at later metastatic stages by acting in an autocrine fashion through ER-β.

Growth factor signaling cascades
The complex events involved in the initiation and progression of PC are mediated by several growth factor-signaling cascades, including EGFR, hedgehog and Wnt/β-catenin pathways, that act in a cooperative fashion by inducing distinct tumorigenic cascades that regulate cell differentiation, proliferation, migration and survival (Figure 3) (3,11,14,18,19,21,22). The localization of different growth factor receptors and their intracellular signaling effectors in close proximity within specialized plasma membrane microdomains, termed caveolea and raft structures, may notably facilitate their interaction and, thereby, allow a more rapid integration and transduction of a variety extracellular signals in cellular responses (3,23). We report the specific oncogenic elements activated by distinct growth factors as well as multiple pathway interactions with the AR signaling pathway that may confer the more malignant phenotypes to neoplastic prostate cells as compared with their normal counterparts.

EGFR family member cascade
The enhanced expression of EGFR (ErbB1) and its ligands, EGF, TGF-α, HB-EGF and amphiregulin, has been reported to correlate with high grades of PC malignancies (3,6,7,13,17,18,22,110). The activation of EGFR by its ligands of autocrine and paracrine manner appears to contribute to the progression from localized PC to more metastatic states as well as the disease relapse. Activated EGFR may induce the stimulation of distinct mitotic cascades, including Shc, MAPK, PI3K/Akt, nuclear factor kappa-β (NF-κB) and phospholipase Cγ (PCγ) signaling pathways, which participate in the stimulation of proliferation, survival, motility and invasion of PC cells (Figure 3) (3,4,18,111). More specifically, it has been reported that EGF may enhance the invasive properties of androgen-independent DU145 cells via activation of the PCγ signaling pathway, which leads to up-regulation of urokinase type plasminogen activator (uPA) expression and, subsequently, to uPA secretion and its membrane uptake through the uPA receptor (112). Moreover, it has been proposed that EGF can induce the disruption of epithelial cell adhesion to the ECM through dephosphorylation and inactivation of the focal adhesion kinase signaling element. This leads to an enhanced motility and invasion of DU145 cells (113). It has also been observed that the treatment of DU145 with EGF in early phases caused the disruption of cell–cell adhesion junctions by caveolin-1-mediated E-cadherin endocytosis concomitant with the release of membrane-localized β-catenin into cytoplasm (114). In fact, this was followed by the translocation of cytosolic β-catenin to the nucleus where it promoted LEF-1/TCF transcriptional activity. In addition, it has also been noted that the reforming cell–cell junctions were prevented during late phases by EGF-induced down-regulation of caveolin-1. This event, in turn, leads to the up-regulation of sma-induced decreased E-cadherin expression. Hence, it appears that caveolin-1, which is a protein localized in the specialized plasma membrane microdomains, may assume multiple roles by regulating different signaling elements co-localized within caveolea that are implicated in the dynamic invasive process. As a matter of fact, it has been reported that caveolin-1 may stimulate PDK1, Akt and ERK activities by inhibiting serine/threonine protein phosphatases, PP1 and PP2A, and interact with AR to potentiate its transcriptional activity in PC cells (115). Moreover, the establishment of caveolin-1 (−/−) nude mice with a transgenic adenocarcinoma mouse prostate (TRAMP) model, which spontaneously develops advanced PC and metastatic diseases, has revealed that the down-regulation of caveolin-1 results in a decrease in the incidence of metastasis to regional lymph nodes and distant sites including the lungs (116). More specifically, the analyses of cell lines derived from TRAMP tumors have revealed a direct correlation between the expression of caveolin-1 and their ability to form tumors in vivo.

The enhanced expression of other EGFR family members, including the constitutively activated EGFRvIII mutant, ErbB2 and ErbB3 and the heregulin (HRG) ligand in certain PC types, which may lead to the formation of EGFR/ErbB2 and ErbB2/ErbB3 heterodimers, might also participate in the stimulation of proliferative and survival signaling cascades (Figure 2) (3,17,22,117–119). Several recent investigations also revealed that the EGFR–EGFR signaling elements could play a pivotal role during different stages of PC progression by modulating several other signaling pathways including AR, hedgehog and Wnt/β-catenin cascades (3,4,18).

Cross-talks between EGFR family members and other signaling pathways
Numerous works have indicated that bidirectional cross-talks exist between the EGFR–EGFR system and hormonal signaling during PC progression. EGFR may induce the activation of AR synergistically in the presence of low androgen levels or in the absence of androgens in a cell-type dependent manner (112,120–122). For instance, the addition of exogenous EGF has been observed to induce the stimulation of AR-mediated gene transcription in AR-transfected DU145 cells. The stimulatory effect was inhibited in the presence of the selective AR antagonist bicalutamide and the inhibitor of MAPK kinase cascade PD98059 (120). Moreover, it has also been reported
that EGF may induce the AR nuclear translocation and enhance the growth of the CWR22R 2152 cell subline. The stimulatory effects of EGF on this subline were significantly inhibited in the presence of bicalutamide and the PI3K inhibitor LY294002 (112). It has also been reported that cotreatment with EGF increased the α-DHT-induced AR transactivation through the activation of MAPK-induced phosphorylation of an AR co-activator, TIF2/GRIP1, in the relapsed CWR22R1 cells (122). The sustained activation of LNCaP cells by HB-EGF has also been reported to generate a LNCaP cell subline, LNCaP/sHB, expressing a secreted form of HB-EGF and characterized by decreased AR expression levels and sensitivity to bicalutamide (123). More specifically, the LNCaP/sHB subline showed a higher rate of growth in androgen-depleted conditions in vitro and formed larger tumors in nude mice in vivo as compared with parental LNCaP cells. This indicates that EGFR activation may also confer a more malignant phenotype to PC cells and, thereby, allow their sustained growth in the conditions of reduced androgen/AR levels. The overexpression of ErbB2 has also been observed to stimulate AR transactivation activity via the MAPK pathways in LNCaP cells, AR-transfected DU145 and PC3 cells, as well as the AR pathway in androgen-sensitive LAPC-4 cells in the absence of ligand or in synergy with low levels of androgens (124–126). Since the EGFR–ErbB2 and ErbB2–ErbB3 heterodimers appear to act in concert with the AR signaling cascades in certain PC cells (17,22, 119,124,125,127), it will be important to establish the specific role assumed by each heterodimer to estimate their implication at different stages leading to prostate carcinogenesis.

Conversely, the activation of AR by androgens can also up-regulate the EGFR expression and signaling in certain PC cells such as androgen-sensitive LNCaP and CWR22 cells and androgen-independent PC3 and DU145-AR cells (4,128,129). Hence, the integration of diverse external signals through the modulation of EGFR and AR activities as well as their interplay with the other oncogenic cascades underlines the importance of further analyzing the complex bidirectional cross-talks between these two cascades. Moreover, these works also suggest the potential clinical benefit to simultaneously targeting the EGFR and AR signaling cascades. This will help prevent the development of more malignant states by gaining androgen-independence of the PC cells which may be responsible for disease recurrence during antiandrogen therapies.

**Hedgehog signaling cascade**

The up-regulated expression of hedgehog signaling components also appears to occur in prostate tumor cells as compared with normal prostate tissue. In particular, the enhanced expression level of sonic hedgehog ligand, SHH, in PC cells may lead to the activation of the GLI-1 transcription factor. This results in the expression of numerous tumorigenic genes, including cyclin D1 and c-Myc, that participate in the sustained growth of PC cells (Figure 3) (10,11,19,88). Moreover, a negative regulator of hedgehog signaling, hSufu, which is produced from a gene localized at the chromosomal region 10q24–25, is also expressed in these cells. This suggests the potential benefit of targeting hSufu in prostate cancer treatment. In this regard, hedgehog signaling may play a role in the development of prostate cancer, and targeting hSufu may provide a new therapeutic strategy for the treatment of prostate cancer.

The up-regulated expression of hedgehog signaling components in prostate cancer cells may also contribute to the development of more aggressive and metastatic prostate cancer. The hedgehog signaling pathway has been shown to be activated in various types of prostate cancer, including PCa, PC3, and DU145 cells. The activation of hedgehog signaling may lead to the development of more aggressive and metastatic prostate cancer.

**Wnt signaling cascade**

The aberrant activation of the canonical Wnt/β-catenin signaling pathway also seems to contribute to prostate cancer progression at early stages. The Wnt/β-catenin signaling pathway is activated by the binding of Wnt proteins to Frizzled receptors, which then lead to the activation of β-catenin signaling in PC cells. This results in the activation of several downstream targets, including cyclin D1, c-Myc, and Akt. These targets are involved in the regulation of cell proliferation, survival, and metastasis. The activation of these downstream targets may contribute to the development of prostate cancer.

The deregulated expression of genes involved in the mesenchymal–epithelial transition may also contribute to the development of more aggressive and metastatic prostate cancer. The activation of hedgehog signaling may lead to the development of more aggressive and metastatic prostate cancer. The activation of hedgehog signaling may lead to the development of more aggressive and metastatic prostate cancer.
hormone-independent PC cells and high grades of prostatic tumors, it appears that distinct functions may be assumed by the different Wnt family members during PC progression.

Cross-talk between Wnt and other signaling pathways

The bidirectional interactions between the Wnt/β-catenin and AR signaling cascades also appear to be manifested in certain AR-expressing PC cells (134,135). It has been observed that Wnt3α induces AR transcriptional activity in the absence or presence of low concentrations of androgens, at least in part, through an increase of the cytosolic and nuclear β-catenin levels in AR-positive CWR22Rv1 and LNCaP cells. This was accompanied by an enhanced rate of cell growth (136). Similarly, the overexpression of β-catenin in LNCaP cells also induced the stimulation of T-cell factor (TCF)- and AR-mediated transactivation of genes (132,137). Additionally, it has also been reported that β-catenin or GSK3β can interact with AR and, thereby, enhance the ligand-dependent AR activity, androgen-stimulated gene expression and growth of PC cells in vitro (132,137,138). Therefore, this suggests that β-catenin may contribute to AR activation during the transition from androgen-dependent PC forms into androgen-independent and metastatic states. The androgen deprivation of LNCaP cells has also been reported to induce the expression of cytoplasmic protocadherin-PC (PCDH-PC) which, in turn, induced a rise of nuclear β-catenin levels and increased the expression of Wnt-targeted genes (139). Although the mechanism(s) of action of PCDH-PC have not been precisely established, it has been proposed that PCDH-PC can interact with the β-catenin and, thereby, impair its degradation. Moreover, the results from microarray analyses have also indicated that PCDH-PC may induce the up-regulation of distinct Wnt ligands, including Wnt-3, -7B, -10A and -11 (139).

Hence, these observations suggest that the activation of canonical Wnt signaling may represent an important event involved in PC initiation and progression by increasing the nuclear β-catenin levels. Increasing the nuclear β-catenin levels may, in turn, enhance CTR- and AR-mediated transcription of distinct tumorogenic genes in androgen-sensitive PC cells. The establishment of specific functions assumed by the different Wnt ligands in activating Wnt/β-catenin signaling relative to uncanonical Wnt pathways in later stages of androgen-independent PC requires further investigation.

Cytokine signaling cascades

The enhanced levels of several cytokines in the serum of patients with PC also appear to be associated with the development of more malignant forms of PC. For instance, the IL-6 levels are high in serum and tissues from patients with HRPC and this is associated with a poor prognosis (99,140). It has been reported that the PC cell lines, including LNCaP, CWR22Rv1, DU145 and PC3 cells, express the receptor IL-6R, showing a high affinity for IL-6 (99,141). Moreover, IL-6 is also secreted by highly metastatic CWR22Rv1, DU145 and PC3 cells, while LNCaP cells did not produce a significant IL-6 level. The treatment with the exogenous IL-6 of diverse PC cells has revealed that this cytokine may modulate AR activity. It has been reported that IL-6 may enhance AR activity in AR-transfected DU145 and PC3 cells as well as AR mutant LNCaP cells synergistically in the presence of low levels of androgen and/or in a ligand-independent manner (99,142). In fact, the stimulatory effect of IL-6 on AR activation was significantly inhibited by the AR antagonist bicalutamide and the specific inhibitor of MAPK or by blocking the Janus kinase/signal transducer and activator of the transcription-3 (STAT3) signaling pathway (142). Results from another study have also indicated that the activation of STAT3 signaling may inhibit the α-DHT-induced AR activity in LNCaP cells through the differential recruitment of cofactors to target genes. These observations suggest that the variation in experimental conditions may influence the modulatory effect of this pleiotropic cytokine on AR activity, which depends both on interplaying multiple intracellular signaling cascades and nuclear cofactors (143). In addition, IL-6 may also stimulate in vitro growth of CWR22Rv1, DU145 and PC3 cells via its receptor IL-6R by activating the PI3K/Akt and MAPK pathways, in autocrine and paracrine manners (Figure 2) (99,143,144). Similarly, it has also been reported that IL-6-type cytokine, oncostatin M, may induce in a paracrine fashion, the activation of AR and growth stimulation in DU145-AR and CWR22Rv1 cells (145). Of particular therapeutic interest, the use of the anti-IL-6 antibody has also been observed to induce an inhibitory effect on the PC-3 xenograft in vivo (99). In contrast, the effect of the exogenous IL-6 on the proliferation of LNCaP cells appears to depend on the number of passage and experimental conditions (99). As a matter of fact, the continued exposure of parental LNCaP cells to IL-6 resulted in LNCaP-IL-6+ that secreted IL-6 and showed enhanced in vivo growth compared with LNCaP-IL-6− cells (146). The stimulatory effect induced by conditioned medium collected from human osteoblasts containing high IL-6 levels on the AR-mediated PSA expression and proliferation in LNCaP, C4-2B and VCaP cell lines was also inhibited in the presence of anti-IL-6 antibody (147). This suggests that local IL-6 production in the bone microenvironment may assume an important role for the growth of PC cells at this metastatic site.

Among other cytokines found in high levels in serum of patients with advanced PC forms, there are TGF-β family members which seem to assume a dual function during PC development (22,148,149). Indeed, TGF-β1 may inhibit the growth of normal prostate epithelial cells in culture and mediated programmed cell death after androgen withdrawal, more particularly when the survival growth factors including EGF are retrieved from culture medium (150). In advanced prostatic carcinomas, PC cells become generally insensitive to the growth inhibitory effect of TGF-β1 and TGF-β2. These cytokines appear to participate in conjunction with other growth factors in conferring the more malignant phenotypes to androgen-independent PC cells by altering the stromal–epithelial interactions and inducing genes involved in the survival, invasive and metastatic processes (60,89). This may be due, in part, to the silencing of either TGF-β type I receptor and/or TGF-β type II receptor expression by promoter methylation and down-regulated expression of downstream signaling effectors, including phosphorylated Smads in high-grade PINs and PC tissues as compared with benign tissues (151,152). As a matter of fact, it has been reported that TGF-β2 may stimulate the NF-κB survival pathway and IL-8 secretion in several prostate tumor cells (153). The down-regulation of TGF-β2 expression by using small interfering RNA (siRNA) technology has notably been observed to result in a decreased viability of PC3 cells in vitro (154). Similarly, the treatment of xenografted LNCaP cells in an animal model by TGF-β1 latency associated peptide, which acts as an inhibitor, has also been observed to enhance the rate of apoptotic cell death.
death (155). The inhibition of the p38 MAPK pathway by using a specific inhibitor, SB203580 or genistein, also blocked the induction of matrix metalloproteinase type 2 (MMP-2) and cell invasion induced by TGF-β in vitro (156). Altogether, these observations suggest that TGF-β1 and TGF-β2 may contribute to the enhancement of the proliferative, survival and metastatic properties of prostatic tumor cells. This is due to an attenuated activation of the Smad inhibitory signaling cascade concomitant with the induction of parallel mitotic signal pathways by these cytokines in late stages of PC.

Several recent works have also indicated that the serum levels of another cytokine of the TGF-β superfamily, the MIC-1 protein, are elevated in high grades of PC as compared with normal tissues. It markedly increases during the transition from androgen-dependent PC forms into androgen-independent states (70,73). Moreover, the expression of the MIC-1 gene and secreted mature MIC-1 protein was also elevated in androgen-sensitive LNCaP-C33 and androgen-independent LNCaP-C81 cells, while its expression was low in androgen-independent PC3 cells and undetectable in DU145 cells and normal prostatic PZ-HPV-7 cells (153,157–158). An analysis of the polymorphism in the MIC-1 gene has also revealed that a genetic change by the substitution of basic histidine to aspartic acid at position 6 in the mature MIC-1 protein was associated with an enhanced propensity for developing PC (159). The molecular mechanisms involved in up-regulating MIC-1 expression as well as the precise functions assumed by its secreted protein during PC development are not yet known. It has been reported that increasing androgen concentrations may result in the up-regulation of MIC-1 expression in LNCaP cells (160). Our recent works have also indicated that the up-regulation of MIC-1 expression in the metastatic and androgen-sensitive LNCaP-C33 cells and androgen-independent LNCaP-C81 and PC3 cells may be induced by multiple growth factor signaling elements including α-DHT, EGF and IL-6 (M.Murielle, S.K.Batra, unpublished data).

However, the establishment of précis functions of MIC-1 protein during PC progression requires further investigation. Hence, it appears that the transition to the metastatic and androgen-independent states may be accompanied by changes in the responsiveness of PC cells to numerous pleiotropic factors.

Neuropeptide signaling cascades

Several neuropeptides, including bombesin, neurotensin, serotonin, endothelin, calcitonin, bradykinin and lysophosphatidic acid (LPA) acting through the G-proteins-coupled receptors (GPCRs), also participate in the activation of multiple tumorigenic genes involved in NE differentiation, proliferation, migration and metastasis of PC cells (3,161–164). For instance, the activation of GPCRs by LPA and bradykinin and the type 1 neurotensine-receptor by neurotensin in PC3 cells may notably lead to stimulation of the EGFR signaling cascade by transactivating EGFR or inducing the processing of EGFR-like ligands in their mature and active secreted forms. Moreover, bombesin and calcitonin may induce the activation of the PKA cascade via GPCRs. The PKA cascade participates in conjunction with EGFR to stimulate the MAPK pathway (Figure 2) (3). Bombesin and neurotensine may also induce AR-mediated gene transcription in a ligand-independent manner or synergistically in the presence of low levels of α-DHT, suggesting that these neuropeptides can promote androgen-independent PC states during antiandrogen therapies (163,164). In this context, it has also been reported that the androgen depletion may induce a NE transdifferentiation of androgen-sensitive LNCaP-C33 cells through the enhanced expression and signaling of receptor-type protein-tyrosine phosphatase α via the activation of the MAPK cascade (165). Similarly, the up-regulation of PCDH-PC expression induced by the androgen deprivation of LNCaP cells, transfection of PCDH-PC or stabilized β-catenin has also been reported to induce their NE transdifferentiation by up-regulating neuron-specific enolase and chromogranin-A (139). This suggests that Wnt signaling may also assume an important role in the initiation of the transdifferentiation process of PC cells. Therefore, it appears that the blockade of certain GPCR signaling cascades could be the particular benefit during androgen deprivation therapy. For instance, the use of selective neuropeptidic antagonists, neutral endopeptidases, PKA inhibitors (8-Cl-cAMP) or inhibitors of Wnt signaling elements may counteract the transdifferentiation of PC cells and oncogenic effects induced by certain neuropeptides (Table II).

Early detection and prognostic markers of PC

Substantial improvement in the detection of PCs at early stages has been made by the combined use of several early detection tests. Testing includes digital rectal examination (DRE) and estimation of both serum total PSA (tPSA) and percentage of free PSA or complexed PSA levels in blood, followed by transrectal ultrasound-guided prostate biopsies; these tests allow diagnosis of previously undetected cases (166–168). More specifically, the establishment of the total serum PSA in patients with PC, which is a serine protease of the kallikrein superfamily, the secreted mature MIC-1 protein was also revealed that a genetic change by the substitution of basic histidine to aspartic acid at position 6 in the mature MIC-1 protein was associated with an enhanced propensity for developing PC (159). The molecular mechanisms involved in up-regulating MIC-1 expression as well as the precise functions assumed by its secreted protein during PC development are not yet known. It has been reported that increasing androgen concentrations may result in the up-regulation of MIC-1 expression in LNCaP cells (160). Our recent works have also indicated that the up-regulation of MIC-1 expression in the metastatic and androgen-sensitive LNCaP-C33 cells and androgen-independent LNCaP-C81 and PC3 cells may be induced by multiple growth factor signaling elements including α-DHT, EGF and IL-6 (M.Murielle, S.K.Batra, unpublished data).

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in ECM stores has been proposed to represent a predictor of PC relapse after RP (73). The restricted expression of cell-surface antigen PSCA in basal putative prostatic progenitor cells and its overexpression in the majority of metastatic and androgen-independent PCs indicate that it constitutes a promising molecular target for clinical diagnosis and prognosis as well as the treatment of patients with HRPC by peptide-based immunotherapy (170,171). Recently, it has also been reported that the number of circulating tumor cells (CTCs) in peripheral blood from PC patients appears to be related to the disease status and, therefore, the estimation of CTCs may provide significant information for a more rapid diagnosis and prognosis (172–174). Enhanced CTC levels in blood from patients with metastatic carcinomas have been associated with a shortened survival rate while their presence in bone narrow from patients diagnosed with PC was associated with poor prognosis. Moreover, the RT-PCR analyses of gene profiling of CTCs in blood samples from healthy donors and patients with HRPC indicated that PSA, prostate specific membrane antigen, AR, human glandular kallikrein 2 and EGFR were the most abundantly expressed genes in the CTCs (173). Additionally, the loss of the putative calcium channel protein, trp-p8, which occurs during the transition to the androgen-independent PC xenograft model and in patients treated preoperatively with antiandrogen therapy, has also been proposed as a potential molecular marker to predict the chance of PC relapse (175). Similarly, the assessment of change in expression of E-catherin and Drg-1 and enhanced levels of ILK and β-cadherin fragment in the serum, which inversely correlate with the overall survival rate of patients, also represent the events which may be predictive of metastases (72,79).

The use of a combination of distinct biomarkers also constitutes a promising approach for a more effective detection of PC and a better prognosis. It has been reported that the use of a combination of Drg-1 plus PTEN or c-Myc plus caveolin as biomarkers was a better predictor of PC patient survival than the individual markers (79,176). Moreover, the combined analysis of the expression levels of distinct biomarkers including PMSA, hepsin (a membrane-bound serine protease), DDA/PCA3 and UDP-N-acetyl-galactosamine transferase, which are overexpressed in PCs, has distinguished 100% of the PC samples from all benign prostate hyperplasia samples tested (177). On the other hand, the reduced expression of nonneopathial-reactive stroma elements, including desmin and smooth muscle α-actin expression in PC, may also represent the independent predictors of recurrence-free survival (87). Hence, these works have identified some new biomarkers that could be used for earlier detection and therapeutic intervention in the patients with locally advanced PC or HRPC. This would help to reduce the risk of progression to metastatic disease states.

PC therapies

Surgery and radiation therapy

The RP, which consists of removing the entire prostate gland and some surrounding tissue, represents the standard treatment for patients with localized PC (Table II). In general, patients treated by RP as monotherapy for localized PC with favorable preoperative characteristics, organ confined disease and negative surgical margins usually have a biochemical relapse-free survival rate of 5 (84%) and 9 (76%) years (178,179). Certain undetected PC lesions extended to the surgical margins may also lead to the recurrence of PC at distant sites following surgery. A PSA value >0.2 ng/ml detected after RP is usually considered as evidence of cancer recurrence (178). Radiation therapy by external beam radiation or internal radiation designated as brachytherapy may also be used as a treatment option for the localized PC at early-stage, low-grade and low-volume or when the PC has spread to near tissues (180). The outcome in men treated with permanent prostate brachytherapy presents a 12 year span before recurrence in patients with localized PC (181,182). In addition, surgery and radiotherapy are also often used in combination with hormone therapy and or chemotherapy.

Hormonal therapy

Hormone therapy or antiandrogen therapy, which blocks the effects of the androgens, is a treatment that is often used

### Table II. Possible therapeutic strategies against PC

<table>
<thead>
<tr>
<th>Type of therapy</th>
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<tbody>
<tr>
<td>Radiation therapy</td>
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<td>External beam radiation</td>
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<td>Brachytherapy</td>
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<td>Antiandrogen therapy</td>
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<td>CI-1033</td>
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<td>LOX-5 or LOX-12</td>
<td>MK886 or Baicalein</td>
</tr>
<tr>
<td>Caspase signaling activator</td>
<td>As-Bcl-2, As-clusterin, As-survivin</td>
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<tr>
<td>Ceramide signaling activator</td>
<td>Etoposide, Paclitaxel, tamoxifen, OE, B13, Ad-as-CLN3</td>
</tr>
<tr>
<td>Combination therapy</td>
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<tr>
<td>Estramustine plus etoposide</td>
<td>SU6668</td>
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<tr>
<td>PDGF, VEGF, FGF receptor inhibitor</td>
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[Downloaded from https://academic.oup.com/carcin/article-abstract/27/1/1/2390966 by guest on 06 April 2019]
in combination with surgery for patients with PC which has spread beyond the prostate or recurred after treatment (Table II) (183). Although patients with PC initially respond to hormone therapy for a few years, the development of androgen-independent states usually results in resistance to this type of treatment. It has been reported that the treatment of long-term deprived LNCaP- abl by AR antagonist bicalutamide leads to the stimulation of AR and cell proliferation of LNCaP cells (184). In addition, the treatment of androgen-sensitive LNCaP and CWR22 cells or AR-transfected DU145 cells with bicalutamide has also been observed to result in increasing EGFR expression levels on these cells indicating that androgen deprivation could favor the progression to androgen-independent PC states by up-regulating EGFR signaling in certain PC cells (185). Overall, these observations suggest that the simultaneous blockade of AR and EGFR signaling could be more appropriate for the treatment of PCs expressing high levels of these receptors and, thereby, could also counteract the recurrence of HRPC. In this matter, several new strategies have also been investigated for blocking AR signaling. More particularly, the down-regulation of the AR co-activators such as prostate-derived ETS transcription factor, which is highly expressed in PC as compared with normal tissue, may constitute an alternative strategy for PC expressing high AR levels (186). The selective inhibition of AR expression by using siRNA or antisense oligonucleotide (As) technology also represents a new therapeutic option that appears to be effective for metastatic androgen-dependent and androgen-independent PC forms (187).

Since estrogens in conjunction with androgens may also contribute to PC progression, certain antiestrogen strategies have been investigated (20,49–51). One is the use of selective ER modulators (SERMs). These drugs appear to act, in part, by blocking ER transcriptional activity. Moreover, the selective aromatase inhibitors, including anastrozole, letrozole and exemestane, may also be used for treating advanced PC (20,49,188,189). More specifically, certain SERMs, such as phytoestrogens, tamoxifen, 4-hydroxytamoxifen and raloxifene (LY156758), have been reported to inhibit the proliferation and/or induce apoptosis in metastatic and androgen-sensitive LNCaP cells and androgen-independent DU145 and PC3 cells (20,188,190,191). It has been reported that 4-hydrotamoxifen may induce the recruitment of ER-β on the hTERT promoter and, thereby, inhibit telomerase activity in LNCaP cells (49). This suggests that 4-hydrotamoxifen could represent an effective antitumor agent for the treatment of high-grade PC forms. Certain selective SERMs, including toremifene, raloxifene, LY117018 and ER-α selective antagonistic trioxifene (LY133314), and dietary phytoestrogens, such as genistein, which are the polyphenolic non-steroidal plant compounds acting as SERMs, have also been observed to prevent and/or counteract PC carcinogenesis in animal models in vivo (20,189,192). However, most of the SERMs may also induce their anticarcinogenic effects through negative modulation of other cascades, such as AR and ERRs, or growth factor signaling including EGFR and IGF-1R (20,192–194). Therefore, additional trials on the specific mechanism(s) of action of SERMs appear to be necessary to clearly establish the clinical benefit and optimal regimen for using these agents alone or in combination as chemopreventive and endocrine therapeutic treatments.

Chemotherapy

Chemotherapy represents another option for patients with HRPC which has spread outside of prostate gland. The highly metastatic small-cell carcinoma or NE cell tumors are rare types of PC that respond better to chemotherapy than hormone therapy. These tumors are usually treated with etoposide and cisplatin (195). For locally advanced stages of PC, preclinical trials have revealed the potential benefits of the use of cytotoxic drugs prior to surgery or as adjuvant therapy with the antiandrogen treatment after surgery (39,196,197). The standard chemotherapeutic agents for patients with HRPC include either a combination of mitoxantrone and prednisone or taxols such as docetaxel and prednisone or estramustine. These combinations have been reported to improve the quality of life for patients with pain, offering pain relief instead of just treatment alone. These drugs, however, showed low survival benefits (Table II) (31–34,197). Similarly, other chemotherapeutic drugs such as etoposide, vinblastin and platinum compounds have also been observed to induce a very weak antitumoral activity against the androgen-independent PC forms. The response rates are <20%, which is principally due to dose-limiting toxicities (DLTs). Orally bioavailable platinum (IV) complex, satraplatin (as known as JM-216 or BMS-182751), which shows an antitumoral activity superior to cisplatin and carboplatin, is being used for preclinical trials to estimate its efficacy as a second-line treatment for HRPC (32,198). Moreover, it has recently been reported that the combination of estramustine, docetaxel and suramin could represent a highly effective regimen for HRPC with modest toxicities, mainly due to hematological and gastroenteral toxicities (199). Of clinical interest, the data from a phase II study carried out with a long-term 5 year follow-up have also revealed that 34.8% of patients with HRPC survived >2 years with a treatment consisting of oral estramustine plus oral etoposide (200).

Altogether, these observations indicated that the current chemotherapeutic regimens used remain ineffective against HRPC forms due, in part, to the DLT of drugs. Therefore, this underlines the importance of undertaking additional preclinical trials for optimizing the administration modes and regimen options of conventional chemotherapeutic drugs. The establishment of new combinations of cytotoxic drugs also seems essential for the development of a more effective treatment against metastatic and androgen-independent PC forms.

New molecular targeting therapies

Since the progression from the androgen-dependent PC into more aggressive and metastatic forms often leads to disease relapse, several novel therapeutic strategies have been investigated for improving treatments against metastatic HRPCs. The recent identification of distinct deregulated cellular targets in PC cells directly involved in prostatic carcinogenesis will allow us to target several signaling elements in tumor cells to counteract PC progression. For instance, the molecular targeting of distinct oncogenic signaling pathways, including EGF–EGFR, IGF-1R, hedgehog, Wnt/β-catenin and apoptotic cascades such as caspase and ceramide cascades, which have been reported to be deregulated during the progression of PCs, represents a new therapeutic approach which is highly promising (Table II) (3,14,18,21,23,25,201–203). In addition, the use of new strategies involving immunotherapy or chemoinducible gene therapy, which may enhance antitumor activity of chemotherapeutic drugs, has also provided interesting results (204–206). More specifically, the combination of
doxorubicine with cDNA encoding human tumor necrosis factor-α (TNF-α), Ad.Egr-TNF.11D, allowed a reversal of the resistance of PC3 cells to doxorubicine. This resulted in a significant decrease of tumor growth in vivo as compared with single agents (206). Moreover, it has also been noted that diverse chemotherapeutic drugs may induce the expression of Ad.Egr-TNF.11D by increasing reactive oxygen intermediary levels. Long-term treatment with telomerase inhibitors and telomere shortening has also been observed to inhibit the growth of DU145 and LNCaP cells in vivo and in vitro. It also sensitizes the DU145 cells to cisplatin and carboplatin (207).

**EGFR signaling inhibitors**

Many new therapeutic strategies targeting EGFR and its ligands have been investigated. This will serve to counteract the progression and relapse of PC as well as improve the cytotoxic effects induced by antiandrogens, conventional chemotherapeutic agents and irradiation therapy. Among the EGFR signaling inhibitors, there are the monoclonal antibodies including cetuximab (as known as IMC-C225 or Erbitux), antisense oligonucleotides or immunotoxins directed against EGFR, gefitinib or TGF-α and selective EGFR tyrosine kinase inhibitors such as erlotinib (OSI-774), PD182905, CI-1033 and gefitinib (ZD1839 or Iressa) (Table II) (3). Numerous in vitro and in vivo studies have indicated that these pharmacological agents may inhibit the growth of androgen-dependent MDA PCa 2a and 2b, LNCaP-C33, CWR22 and relapsed CWR22R cells as well as androgen-independent and highly metastatic LNCaP-C81, DU145, PC3 and PC-3M-LN4 cells (3,18,111, 112,208–210). Most of these drugs also induced apoptotic cell death and inhibited the invasion and metastasis of PC cells and the angiogenesis process. More particularly, the use of antisense oligonucleotides specific for TGF-α or EGFR has notably revealed that this treatment inhibited the growth of highly metastatic PC3 cells in vitro and in vivo. The specific sequential treatment with these antisense oligonucleotides plus paclitaxel, cyclophosphamide or mitoxantrone also resulted in synergistic growth inhibitory effects as compared with individual agents (209). Similarly, the EGFR-related peptide, which may act as a negative regulator of EGFR signaling by increasing the sequestration of EGFR ligands, has also been reported to induce an inhibition of the growth and apoptosis in PC3 cells (211). Since gefitinib or ErbB2 inhibitor TAK165 have been reported to inhibit the transactivation activity of AR induced through ErbB2-mediated MAPK activation in PC3 cells, it appears that this type of treatment could be particularly effective for counteracting the development of androgen-independent PC states (126). It has been observed that the combination of gefitinib plus the non-steroidal AR antagonist bicalutamide resulted in an additive inhibitory effect on the growth of LNCaP, CWR22R and DU145-AR cells (185). Additionally, a novel dual EGFR/ErbB2 kinase inhibitor, GW572016 (as known as lapatinib), has also been reported to be more effective than gefitinib at inhibiting the androgen-independent PC3MM2 cells in bone. This therapy also induced a massive rate of apoptotic cell death in PC cells and tumor endothelial cells, concomitant with a reduction of lymph node metastases as compared with mono- and bi-therapies (218).

**Hedgehog and Wnt/β-catenin signaling inhibitors**

The selective blockade of hedgehog signaling by SMO inhibitor cyclopamine or antiSHH antibody has notably revealed that this treatment induced the arrest of the growth, apoptotic death and decreased the invasiveness of metastatic androgen-sensitive and androgen-independent PC cells in vitro and in vivo (11,21,53). Our recent results have also revealed that a combination of cyclopamine and gefitinib resulted in an arrest of the growth and a greater rate of apoptotic death in LNCaP, DU145 and PC3 cells as compared with individual drugs (18). Similarly, several types of strategies have also been proposed to counteract Wnt/β-catenin signaling including the use of a selective Wnt antibody, Wnt protein inhibitors or repressors disrupting nuclear TCR/β-catenin complexes (14,131,219). A recent study has effectively revealed that the overexpression of the Wnt-inhibitory factor WIF-1, an inhibitor of Wnt proteins in PTEN-deleted PC3 cells, resulted in a down-regulation of the Akt pathway and sensitized these cells to the apoptotic effect induced by paclitaxel (131). Gefitinib has also been reported to enhance the cytotoxic effects induced by diverse chemotherapeutic agents such as platinum compounds, cisplatin and carboplatinum, and paclitaxel on PC3 tumors in vivo (214). The continued treatment of DU145 cells with gefitinib has also been reported to result in a decrease in their sensitivity to this agent due to an up-regulation of the IGF-1R signaling cascade (215). Therefore, the simultaneous use of the aforementioned selective inhibitors of EGFR with the agents targeting IGF-1R signaling could be more appropriate in long-term therapies as individual agents. Among available IGF-1R inhibitors, the human monoclonal antibody A12 directed against IGF-R1 has been reported to induce cell-cycle arrest in the G1 phase and tumor apoptosis in androgen-dependent LNCaP 35 xenografts and growth arrest in the G2 phase in androgen-independent LNCaP 35 V cells (Table II) (216). It has also been noted that A12 down-regulated the AR-regulated gene expression in LNCaP 35 V, suggesting that this agent could effectively prevent relapse after androgen deprivation therapy. The inhibition of IGF-1R by using the siRNA technique has also been observed to inhibit the Akt and MAPK cascades and enhance the sensitivity of LNCaP, DU145 and PC3 cells to mitoxantrone, etoposide, nitrogen mustard and ionizing radiation (217). Altogether, these observations suggest that the combined use of selective inhibitors of EGFR and IGF-1R could represent a more effective approach against metastatic HRPC forms.

The dietary agents such as silymarin, genistein and epigallocatechin 3-gallate (EGCG) present in milk thistle, soy beans and green tea, respectively, have also been reported to reduce the growth of LNCaP, DU145 and PC3 cells by inhibiting EGFR signaling and inducing p27Kip1 and p21waf1. Furthermore, genistein and ECGC also induced a significant rate of apoptotic death of PC cells (187,188). Additionally, the combination therapy using oral EGFRI, PDGFRI, PKI166 and ST1571, with an intraperitoneal injection of paclitaxel, has been observed to inhibit tumor growth of highly metastatic PC3MM2 cells in bone. This therapy also induced a massive rate of apoptotic cell death in PC cells and tumor endothelial cells, concomitant with a reduction of lymph node metastases as compared with mono- and bi-therapies (218).
Apoptotic cascade activators

The progression of PC generally involves the development of hormone-refractory states of PC cell populations which are characterized by a deregulated expression and/or activity of apoptotic signaling pathway elements (3,23,25,201,202). This is principally due to an enhanced stimulation of diverse survival signaling including Akt and NF-κB pathways induced by combined actions of distinct hormones and growth factors (Figures 2 and 3). The overexpression of Bcl-2, Myc-1 and clusterin oncogene products or the down-regulation both of tumor suppressor genes, such as p53 and PTEN, and pro-apoptotic proteins including Bax, an apoptosis inhibitor of protein (IAP), ceramide and caspase may also protect certain PC cell populations against triggering of the intracellular cascades leading to apoptotic/necrotic cell death (3,5,23,25,27,28,201,220). Since these deregulated signaling cascades also appear to be involved in the resistance of certain metastatic PC cells to antiandrogen therapy, chemotherapy and irradiation, targeting these signaling elements represents a promising approach for improving the cytotoxic effects induced by current treatments (Table II). The inhibition of the checkpoint regulators has notably been reported to sensitize p53-defective PC3 cells to cytotoxic effects induced by the DNA-damaging agent, doxorubicin (220). Targeting bcl-2 or clusterin cell survival genes, which are overexpressed after androgen deprivation, by using antisense oligonucleotides also synergistically enhanced the antitumoral effects induced by paclitaxel on androgen-independent Shionogi tumors and human PC xenografts in vivo (27,28). The inhibition of survivin, which is an apoptosis inhibitor of the IAP family, has also been observed to enhance the sensitivity of LNCaP, DU145 and PC3 cells to flutamide, cisplatin and paclitaxel-induced apoptosis in vitro and in vivo (221–223). This indicates then that targeting survivin could be an alternative to enhance the therapeutic effects that are induced by anti-androgens and chemotherapy.

Akt and NF-κB survival pathway inhibitors

Several works have indicated the substantial benefit of inhibiting PI3K/Akt and NF-κB survival signaling pathways to restore the sensitivity of metastatic PC cells to currentlyused chemotherapeutic drugs. The inhibition of Akt downstream signaling element, mTOR-regulated 70 kDa S6 [p70(s6k)] kinase, by rapamycin or CCI-779 has been reported to inhibit the growth and clonogenic survival of wild-type PTEN DU145 and PTEN-mutant PC3 cells in vitro as well as the growth of xenografts derived from these cells in vivo (224). The inhibition of the PI3K/Akt signaling pathway also sensitized PC cells to diverse cytotoxic drugs such as staurosporine, doxorubicin, and vincristine (75,225–227). It has also been reported that the inhibition of the PI3K/AKT pathway by using a specific PI3K activity inhibitor LY294002 may enhance the sensitivity of the long-term androgen-ablated LNCaP- abl cell subline to the cytotoxic effects induced by chemotherapeutic drugs such as etoposide. Then this agent type could be particularly beneficial for increasing the sensitivity of androgen-independent PC cells to current chemotherapeutic treatments (128). A cholesterol synthesis inhibitor, simvastatin, which induced a decrease of the cholesterol content in lipid rafts, has also been reported to inhibit the Akt pathway. This caused apoptotic death in caveolin-1 and PTEN-mutant LNCaP cells (228).

On the other hand, it has also been reported that the inhibition of the NF-κB cascade by acetyl-boswellic acids, whose compounds inhibit IκB kinase activity, was accompanied by down-regulating antiapoptotic proteins Bcl-2 and Bcl-x(L) and resulted in an inhibition of the growth and apoptotic death of PC3 cells in vitro and in vivo (229). The inhibition of NF-κB signaling by using super repressor IκB also sensitized the PC3 cells to the cytotoxic effect induced by TNF (153). Interestingly, it has also been reported that the overexpression of prostate apoptosis responsive-4 (Par-4) element induced the apoptotic death of androgen-independent DU145 and PC3 cells while the androgen-sensitive LNCaP cells were resistant to this treatment (230). Moreover, Par-4 in vivo also caused the regression of tumor established from PC3 cells in nude mice by inhibiting NF-κB activity and stimulating Fas and FasL-induced caspase-8 activation. Therefore, this suggests that the induction of Par-4 pro-apoptotic signaling cascade could represent an effective strategy against the metastatic and androgen-independent PC forms.

Additionally, the simultaneous inhibition of multiple tumorigenic signals in PC cells by certain dietary agents also represents an interesting strategy for the prevention and treatment of metastatic PC forms. Dietary agents, genistein and BAY 11–7085 have been observed to induce a decrease in NF-κB, PIM-2 and the defender against cell death 1 expression. These agents counteract the survival effect induced via NF-κB signaling in prostate PNI cells (87). Clinical trials with non-steroidal antiinflammatory drugs which may inhibit the growth of PC cells in vitro and in vivo by down-regulating the expression and/or activity of distinct survival factors as well as certain angiogenic factors are also undergoing in order to estimate their benefit for chemopreventive strategies for PC (231).

Ceramide cascade activators

The modulation of the cellular ceramide levels by activating the enzymes involved in its synthesis and/or inhibiting its metabolic transformation represents another promising approach for promoting the cytotoxic effects induced by androgen deprivation and available chemotherapeutic agents (Table II) (3,23,24). Although etoposide and paclitaxel have been reported to induce apoptosis in PKCβ-positive LNCaP and DU145 cells through cellular ceramide accumulation by activating de novo synthesis and a neutral sphingomyelinase pathway, the PKCβ-negative PC3 cells were significantly less sensitive to the cytotoxic effects of these drugs (232). The use of neutral endopeptidases, which inhibit the PKCβ degradation, has notably been observed to restore the sensitivity of PC3 cells to these chemotherapeutic agents by increasing cellular ceramide levels (233). Additionally, the results from our recent work revealed that the inhibition of the acidic ceramidase, whose enzyme is overexpressed in PC cells, by using N-oleoylthanolamine (OE), also promoted the apoptotic/necrotic effects induced by diverse cytotoxic drugs such as anandamide and EGFR inhibitors, PD153035 and genifinib, in LNCaP, DU145 and PC3 cells through an enhanced elevation of cellular ceramide level (211,234,235). The inhibition of the acidic ceramidase by using the ceramide analog B13 also sensitized the androgen-insensitive PC xenografts established in animal model in vivo to the radiation treatment (26). The inhibition of the expression of the antiapoptotic CLN3 protein, which is overexpressed in LNCaP, PC3 and DU145 cells by using adeno virus-expressing antisense
CLN3 construct (Ad-As-CLN3), also caused an increase in endogenous ceramide production via the de novo ceramide synthesis that resulted in enhanced apoptosis (236). Several works have also revealed that the sensitivity of PC cells which are highly resistant to γ-irradiation-induced cell death, may be enhanced by agents that are able to induce ceramide production, such as and TNF-α and agonistic Fas antibody, CH-11 (237). On the other hand, it is interesting to note that a novel ceramide analogue N-oleoyl serinol (S18) has been shown to inhibit the formation of stem cell-derived tumors or teratomas induced by engraftment of embryoid body-derived cells (EBCs) into mouse brain (238). Indeed, S18 was able to specifically eliminate the pluripotent EBCs expressing Par-4 by triggering apoptotic cell death.

Hence, it appears that the molecular targeting of the deregulated genes mediating resistance to drug-induced apoptotic effects in PC cells could promote chemotherapeutic drug-induced apoptosis in androgen-dependent and androgen-independent PC cells. This would delay the progression to HRPC states and reduce the chance of recurrence of malignancy.

Conclusions

Taken together, the recent works have provided new insights into the molecular events involved in prostate carcinogenesis which are controlled by several hormonal and growth factor signaling cascades. In particular, numerous analyses on the deregulated genes which are expressed at higher levels in prostate tumor epithelial and stromal cells relative to normal prostate cells have identified different signaling components that are altered during PC initiation and progression. Certain gene products, including Bcl-2 and MIC-1, constitute potent biomarkers that could be used alone or in combination for an earlier diagnosis and prognosis of PC. The molecular targeting of these deregulated gene products may also help prevent PC initiation and/or counteract the progression of the more aggressive and lethal disease states. The combination of pharmacological agents which are able to selectively inhibit the mitotic and survival signaling pathways and activate the ceramide- and caspase-induced apoptotic cascades represent great promise for the development of a new therapy effective against androgen-independent and metastatic PC forms.

Perspectives and future directions

Several recent studies have indicated that distinct oncogenic products are activated through the stimulation of AR, ERs, EGF–EGFR, IGF-1R, hedgehog and Wnt/β-catenin cascades. They may act in cooperation to confer the more aggressive phenotypes to PC cells; however, the complex molecular interactions between these signaling pathways remain poorly understood and require additional investigations. Further characterization of the roles of these different developmental signaling cascades in normal prostate stem cells, during the tissue regeneration process, and their implications in the oncogenic transformation of stem cells into cancer progenitor cells should shed light on the molecular mechanisms involved in prostate carcinogenesis. This will allow for the development of new combinational therapies involving these signaling elements. The establishment of the specific function(s) assumed by hedgehog and Wnt signaling elements and their interaction with other mitogenic cascades, including AR and EGFR, requires further investigation to understand their real implication in the progression of PC. The use of new identified inhibitors of hedgehog and Wnt/β-catenin signaling, including cyclopalmine or WIF-1, should allow researchers to evaluate whether their specific targeting represents an alternative therapeutic approach. These inhibitors could be incorporated in combination chemotherapy for localized and/or metastatic and recurrent PC forms. Additional studies on the molecular mechanisms associated with the cytotoxic properties of dietary agents which are able to negatively modulate distinct oncogenic signaling cascades, including AR, ER, EGFR and IGF-1R, should also be carried out on different human PC cell models to estimate their potential as chemopreventive and therapeutic agents.

The progression of PC from localized and androgen-dependent states into highly metastatic and androgen-independent forms that are lethal for patients is accompanied by a marked rise of secreted IL-6 and MIC-1 levels by the tumor epithelial cells. Therefore, additional investigations on the signaling cascades activated by these cytokines could also shed light on the molecular mechanisms involved in the development of aggressive and incurable forms of PC. For instance, it will be important to identify the receptor type by which MIC-1 induced these effects on PC cells. Moreover, the in vivo studies on different animal models by using monoclonal antibody directed against MIC-1 or IL-6 should allow researchers to evaluate the importance of these cytokines in the tumor formation and metastasis of PC cells at distant sites of prostate compartment.

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