New Advances on Prostate Carcinogenesis and Therapies: Involvement of EGF-EGFR Transduction System

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INTRODUCTION

The prostate cancers (PCs) are among the major causes of death because therapeutic treatments are not effective against advanced and metastatic forms of this cellular hyperproliferative disorder. In fact, although androgen-deprivation therapies permit to cure localized PC forms, the metastatic PC cells have acquired multiple functional features that confer to them resistance to ionizing radiations and anticarcinogenic drugs currently used in therapy. The present review describes last advances on molecular mechanisms that might be responsible for sustained growth and survival of PC cells. In particular, emphasis is on intracellular signaling cascades which are involved in the mitogenic and antiapoptotic effects of epidermal growth factor EGF-EGFR system. Of therapeutic interest, recent advances and prospects for development of new treatments against incurable forms of metastatic PC forms are also discussed.

Keywords: Prostate carcinogenesis; EGF-EGFR system; Tumor growth and metastasis; Apoptotic death; Anticarcinogenic therapies
Androgens and Their Receptors

The androgenic hormones are potent mitogenic and survival factors for normal and cancer epithelial cells of the prostate gland (Lalani et al., 1997; Culig et al., 2000; Feldman and Feldman, 2001; Huang and Tindall, 2002). The androgens mediate their biological functions through their cognate nuclear receptors ARs. In fact, the binding of androgens to ARs transforms them to active transcription factors that regulate the mitogenic gene expression by interacting with specific gene promoter elements as well as through interactions with other transcription factors such as ETS and AP-1. Moreover, the activation of ARs by their ligands as well as androgen interaction with other mitogenic signals might lead to up-regulation of EGF, TGF-α and EGFR expression levels (Liu et al., 1993; Myers et al., 1999; Li et al., 2002). In this context, 5α-dihydrotestosterone (α-DHT), an androgenic product derived from hydrolysis of testosterone by 5α-reductase which is one of the principal hormones implicated in the maintenance of prostate functions, appears to induce the PC cell proliferation in association with EGF by down-regulating p27Kip1 (Fig. 1) (Ripple et al., 1997; Ye et al., 1999).

In addition, the androgens including α-DHT also assume important functions for the survival of PC cells by inducing transcriptional up-regulation of antiapoptotic Bcl-2 protein levels (Fig. 1) (Wang et al., 2000; Banerjee et al., 2002). In this context, it is interesting to notice that α-DHT has been proposed to confer resistance to radiation, TNF-α, Fas ligand and etoposide-induced apoptosis in hormone-dependent PC cells by down-regulating expression levels of proapoptotic Bcl-2 family members including Bax protein and caspases (Kimura et al., 2001; Coffey et al., 2002).

Several studies indicated that the activation of ARs might also be mediated in an androgen-independent manner via distinct compensatory mechanisms involving up-regulation of signal transduction pathways of diverse growth factors (Klocker et al., 1999; Sadar, 1999; Cox et al., 2000; Feldman and Feldman, 2001; Dotzlaw et al., 2002; Huang and Tindall, 2002). Indeed, the stimulation of EGFR, insulin-like growth factor-I (IGF-I) and Protein Kinase A (PKA) cascades appears to lead to recruitment of proteins that interact and activate ARs in the absence of androgenic hormones. For instance, it has been reported that the inhibition of PKA might attenuate the activation of ARs induced by forskolin (Nazareth and Weigel, 1996). Moreover, it has been observed that cAMP analog or coexpression of N-terminus of hARs

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FIGURE 1 Possible intracellular signaling pathways mediated through EGF-EGFR transduction system which might be involved in the sustained growth and survival of PC cells. The physiological consequences of the activation of each cascade on the cellular behaviors are also indicated. Notice the possible implication of signaling cascades mediated through EGFR-erbB2 and EGFRvIII mutant during the PC development.
(Dotzlaw et al., 2002). Strikingly, these androgen-independent pathways seem to be functional in androgen-responsive PC cells such as LNCaP and therefore, these cells are not strictly dependent of androgens for their growth and survival. In fact, certain ligand-independent activators of ARs might also act synergistically with low concentrations of androgens to induce a higher rate of PC cell growth. Notably, long-term androgen deprivation has been observed to results in LNCaP subtype showing enhanced transcriptional activity of ARs in the presence of low amounts of androgenic hormones (Klocker et al., 1999). Moreover, in advanced PC forms, the transcription of prostate specific antigen (PSA) gene also escapes the regulation by androgens and might be stimulated by PKA activation (Sadar et al., 1999). In addition, the androgen-insensitive and metastatic PC cells also possess multiple growth and survival pathways distinct from well-documented androgen-mediated survival pathways that might be activated of autocrine and paracrine fashion by growth factors including EGF. Altogether, these observations suggest that the compensatory mechanisms might be initiated in order to counterbalance the loss of AR activity induced by temporal removing androgenic hormones.

**Autocrine and Paracrine Regulation of PC Growth**

The overexpression of EGFR and the autocrine secretion of EGF and TGF-α constitute one of principal autoregulatory loops that permits a sustained growth of several PC cell types (De Bellis et al., 1996; Seth et al., 1999; De Miguel et al., 1999; Kim et al., 1999, 2001; Torring et al., 2002). In particular, the expression levels of EGFR and its ligands EGF and TGF-α in PC cells appear generally to enhance during the disease progression to more malignant hormone-independent and metastatic PC forms (Maddy et al., 1989; Glynne-Jones et al., 1996; Myers and Grizzle, 1997; Gil-Diez de Medina et al., 1998; Di Lorenzo et al., 2002). For instance, this autocrine loop contributes to the growth of metastatic PC cells such as androgen-independent DU145 and PC3 cells observed in the absence of exogenous growth factors while it is not detected in normal and PC cells such as androgen-sensitive LNCaP cells. This has been associated with the higher expression levels of EGFR, EGF and TGF-α in androgen-independent and metastatic DU145 and PC3 cells than those detected in androgen-sensitive LNCaP cells (MacDonald and Habid, 1992; Sherwood et al., 1998). Importantly, a study carried out by immunohistochemistry has also permitted to perceive that a higher rate of disease relapse is prevalent in EGFR-positive patients as in EGFR-negative patients after radical prostatectomy (Di Lorenzo et al., 2002). Moreover, the prostate tumor cells have been reported to express the proto-oncogene c-erbB2 (HER2) as well as type III mutant EGFR designated EGFRe which shows a tyrosine kinase activity in absence of ligand and mediates the growth of several human cancer cells (Moscatello et al., 1995; Myers and Grizzle, 1997; Schwartz et al., 1999; Olapade-Olaopa et al., 2000; Di Lorenzo et al., 2002).

In addition, the autocrine growth stimulation of PC cells by EGF and TFG-α might also be induced via up-regulation of cleavage by metalloproteases of transmembrane pro-EGF-like ligands into their active forms (Marinissen and Gutkind, 2001; Kranenburg and Moolenaar, 2001; Pierce et al., 2001; Gschwind et al., 2001). For instance, the metalloprotease BB94 inhibitor has been shown to counteract the high constitutive levels of EGF/F tyrosine phosphorylation in unstarved PC3 cells which was mediated through an autocrine loop as well as the bombesin-induced transactivation of EGFR (Prenzel et al., 1999). Moreover, an autocrine secretion for IGF-I, interleukin-6 (IL-6), prostaglandin E2 (PGE2) and lysophosphatidic acid (LPA) has been shown to be functional in PC cells (Chen et al., 1999; Chen and Hughes-Fulford, 2000; Xie et al., 2002). Notably, the connections between the intracellular signaling pathways activated through the receptors for EGF and IGF-I seem to lead to paracrine and autocrine stimulation of PC cell proliferation by activating MAPK cascade and releasing pro-EGF-like ligands on cell surface (Putz et al., 1999; Lin et al., 1999). On the other hand, several neuropeptides and paracrine factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), TGF-β, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), IL-1, IL-8 and endothelin-1 might also contribute to PC growth (Pirtskhalavishvili and Nelson, 2000). Thus, multiple complex cross-talks between the autocrine and paracrine growth factor loops might be involved in sustained growth observed for metastatic PC cells.

**Cellular Functions of EGF-EGFR Transduction System**

The EGFR signaling transduction pathways activated by EGF and TGF-α are recognized as convergent points between diverse intracellular signaling cascades and they play a pivotal role for sustained growth and survival of PC (Prenzel et al., 2000; Kue and Daaka, 2000; Harper et al., 2002). Notably, the autocrine and paracrine secreted EGF and TGF-α can interact specifically with transmembrane EGFR on the PC cells and thereby, induce their proliferation. In fact, the specific binding of EGF and TGF-α to EGFR might lead to the EGFR dimerization or heterodimerization with erbB2 followed by their activation through a transphosphorylation mechanism. This results in the stimulation through activated EGFR of distinct intracellular signaling pathways including the phosphatidylinositol 3'-kinase (PI3K), mitogen-activated protein kinase (MAPK) and phospholipase C-γ (PLC-γ) cascades that ultimately lead to the changes in cellular behaviors associated with oncogenic phenotypes of PC cells (Fig. 1) (Kim et al., 1999; Lu et al., 2000; Bello-DeOcampo et al., 2001; Barton et al., 2001; Graff, 2002; Harper et al., 2002). In particular, the activation of PI3K/Akt serine/threonine
kinase and classical Ras/MEK/MAPK cascades through EGF-EGFR system appear to lead to the changes in expression levels of numerous mitogenic genes involved PC cell growth by down-regulating p27<sup>Kip1</sup> levels concomitant with a stimulation of cyclin-dependent protein kinases (CDKs). In support with this, several studies revealed that the inhibition of EGFR signaling cascades which results in an inhibition of the growth of androgen-responsive MDA Pca 2a, MDA Pca 2b and LNCaP cells as well as androgen-independent DU145 and PC3 cells is accompanied by a blockade of the progression of cells from G1 into S phase by up-regulating p27<sup>Kip1</sup> protein which in turn inhibits the CDKs (Peng et al., 1996; Wu et al., 1996; Zi et al., 1998; Ye et al., 1999; Mimeault et al., 2002; Karashima et al., 2002). Moreover, the activation of PI<sub>3</sub>K has been reported to lead to the stimulation of ribosomal pp70<sup>S6K</sup> serine/threonine kinase pathway which might enhance the translation of important growth and angiogenic factors including IGF-II and VEGF (Graff, 2002; Ghosh et al., 2002). In addition, the activation of MAPK and PLC-γ cascades appears also to result in changes in cell motility during the PC development (Chen et al., 1999; Harper et al., 2002). Hence, the activation of these different cascades through EGF-EGFR system might contribute to uncontrolled growth and invasion of PC cells during the diverse stages of tumor development.

In spite, knowledge acquired about the EGFR signaling cascades involved in prostatic carcinogenesis, the specific function(s) assumed by activated EGFR-erbB2 heterodimer and EGFRvIII mutant in the progression of PCs have not been established precisely. Therefore, it will be important to estimate whether the mitogenic signaling cascades activated in the PC cell types overexpressing EGFR, erbB2 and EGFRvIII are amplified and associated with more aggressive and invasive PC forms. In this context, it has been observed that EGFR-erbB2 heterodimer expressed in several cancer cell types possesses a higher EGF binding affinity and a weaker rate of degradation after ligand binding than those observed for EGFR homodimer and this seems to be related with the tumor progression (Karunagaran et al., 1996; Worthylake et al., 1999; Xia et al., 1999).

**Modulation of EGF-EGFR Signal Transduction by GPCRs**

Many neuropeptides such as vasoactive intestinal peptide (VIP), calcitonin, bombesin, serotonin, and bradykinin as well as lipid growth factor lysophosphatidic acid (LPA) have been reported to participate in conjunction with EGF-EGFR transduction system to PC cell growth by interacting with their cognate GPCRs on PC cell surface appears to result in the stimulation of adenylate cyclase (AC) activity followed by releasing cAMP which acts as an intracellular second messenger by activating cAMP-dependent PKA (Fig. 2). In fact, PKA is an intracellular enzyme with serine/threonine-specific kinase activity that assumes different functions during the cell growth and differentiation. Two isoforms of PKA have been identified and designated to as PKA-I (type I) and PKA-II (type II) which contain identical catalytic subunits but distinct cAMP-binding regulatory subunits termed to as RIα and RIIβ, respectively (Nesterova et al., 2000). In particular, the expression levels of PKA-I have been observed to be enhanced in several human cancer cell lines including PC cells (Cho et al., 2000; Nesterova et al., 2000). Interestingly, an extracellular form of PKA (ECPKA) has also been observed in many cancer cells including PC cells (Cho et al., 2000; Cvijic et al., 2000). The secreted ECPKA is an active form of enzyme constituted only by free catalytic subunit which also participates to cancer cell growth.

The mitogenic effect induced via PKA signaling cascade also depends on other cascades activated by growth factors such as EGF that control the cellular expression levels of PKA-I/PKA-II and/or available effectors involved in PKA pathway (Fishman et al., 1997; Ciardiello and Tortera, 1998; Miller, 1998; Chen et al., 1999; Putz et al., 1999; Tortora and Ciardiello, 2002). For instance, EGF activation in PC cells which leads to activation of Ras might result in stimulation of Raf-1/MEK/MAPK pathway as well as B-Raf/MEK/MAPK mitogenic cascade which is also activated by PKA via Rap-1 response element (Fig. 2) (Chen et al., 1999). Moreover, it has been proposed that activated PKA might act as a selective activator of B-Raf but an inhibitor of Ras and Rap-1 in several cell types including PC cells (Hafner et al., 1994; Vosslet et al., 1997). Importantly, it has also been observed that the cellular expression levels of Raf-1, Rap-1 and B-Raf effectors involved in these two mitogenic cascades might vary with experimental conditions and cell types studied. Notably, the expression level of B-Raf which is more elevated in EGF-stimulated LNCaP than in PC3 cells has been associated with a higher degree of activation of MAPK.

**Role of PKA**

The interaction of certain neuropeptides such as calcitonin and bombesin with their cognate GPCRs on PC cell surface has been identified and designated to as PKA-I (type I) and PKA-II (type II) which contain identical catalytic subunits but distinct cAMP-binding regulatory subunits termed to as RIα and RIIβ, respectively (Nesterova et al., 2000). In particular, the expression levels of PKA-I have been observed to be enhanced in several human cancer cell lines including PC cells (Cho et al., 2000; Nesterova et al., 2000). Interestingly, an extracellular form of PKA (ECPKA) has also been observed in many cancer cells including PC cells (Cho et al., 2000; Cvijic et al., 2000). The secreted ECPKA is an active form of enzyme constituted only by free catalytic subunit which also participates to cancer cell growth.

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cascade in LNCaP (Chen et al., 1999). In addition, it has been proposed that PKA-I might interact with the activated EGFR through its binding to SH3 domain of adaptor protein Grb2 (Tortora et al., 1997). Hence, these complex bi-directional interactions between the different PKA forms and EGFR signaling pathway might lead to distinct effects on MAPK activity in PC cells.

Interestingly, the blockade of PKA cascade by a specific inhibitor, Rp-cAMPs or 8-Cl-cAMP, PKA-I subunit-directed antisense oligonucleotide or endocannabinoid anandamide has also been observed to be accompanied by down-regulating expression levels of many growth factor receptors including EGFR, c-erb B-2, c-erb B-3, long form prolactin receptor (PRLr) and nerve growth receptor (trk) in several human cancer cells from prostate, ovary, colon and breast (Alper et al., 1999; Melck et al., 2000; Mimeault et al., 2002). However, the molecular mechanism(s) involved in this inhibitory effect of PKA have not been established precisely. In this context, it has been proposed that the activation of MAPK activity which might occur in time after treatment with a PKA inhibitor such as anandamide might contribute to down-regulation of PRLr and trk in certain cell types (Melck et al., 1999). Thus, further works on PKA functions could aid to make the light on complex regulatory mechanisms of MAPK cascade in PC cells.

In addition, the stimulation of GPCRs by bradykinin and LPA might lead to the activation of matrix metalloproteases involved in processing of transmembrane EGF-like ligand precursors and thereby allows an autocrine activation of EGFR by releasing growth factors on the PC cell surface and subsequent MAPK activation (Fig. 2) (Dong et al., 1999; Prenzel et al., 1999; Kranenburg and Mooleenaar, 2001; Pierce et al., 2001; Gschwind et al., 2001; Pierce et al., 2001; Kranenburg and Moolenaar, 2001; Pierce et al., 2001; Gschwind et al., 2001; Daaka, 2002; Kue et al., 2002). However, the identification of metalloproteases involved in this mechanism and the role played by intracellular signaling response elements such as PKC, Ca^{2+} and Src are not yet well documented. In this context, it has been proposed that the members of the family of

**Role of PKC and Src**

The specific binding of certain ligands of GPCRs might also result in PC cell growth via the activation of PKC and Src tyrosine kinase (Kranenburg and Mooleenaar, 2001; Marinissen and Gutkind, 2001; Gschwind et al., 2001; Pierce et al., 2001). For instance, the interaction of bradykinin with G_{\alpha_q}-coupled B_{2} receptor subtype expressed on the PC3 cells has been reported to lead to MAPK activation through the stimulation of phospholipase C (PLC) and generation of inositol 1,4,5-triphosphate (IP_{3}) and diacylglycerol (DAG) (Barki-Harrington and Daaka, 2001). In fact, IP_{3} can induce the release of Ca^{2+} ions from endoplasmic reticulum (ER) into cytosol whose Ca^{2+} ions act in conjunction with DAG as second messengers by activating PKC-\beta via its translocation to plasma membrane (Fig. 2). In contrast, the activation of G_{\beta\gamma}-coupled LPA receptors seems rather to result in stimulation of MAPK cascade through the phosphorylation of EGFR by activated Src (Daaka, 2002; Kue et al., 2002). In this matter, it has also been reported that the mitogenic effects induced by bradykinin and LPA via the activation of MAPK cascade in PC3 cells might be inhibited by blocking EGFR.

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**FIGURE 2** Proposed regulatory effects induced through G-protein coupled receptors on EGF-EGFR mitogenic signaling pathways in PC cells.
zinc-dependent proteases designated to as a disintegrin and metalloprotease (ADAM) might be implicated in the processing of EGF-like growth factor precursors in the PC cells (Werb and Yang, 1998).

ANTIAPOPTOTIC EFFECTS OF EGF-EFGR SYSTEM

EGF has been reported to induce its antia apoptotic effects in many normal and cancer cells including the PC cells by interacting with EGFR localized within the plasma membrane microdomains designated to as caveolae and raft structures which also contain several important cellular mediators such Ras, PI3K and sphingolipids as well as cholesterol (Coroneos et al., 1995; Anderson, 1998; Shaul and Anderson, 1998; Brown and London, 2000; Mimeault, 2002; Mimeault and Bonenfant, 2002; Van Meer and Lisman, 2002; Zhuang et al., 2002). In particular, the EGF-EGFR system appears to trigger an antia apoptotic signaling cascade, a cell type-dependent manner, by activating PI3K which is localized at proximity to EGFR within the membrane microdomains (Zhuang et al., 2002). In particular, it has been proposed that the cell survival function assumed by activated PI3K might be mediated through the activation of Akt followed by phosphorylation of mitochondrial proapoptotic Bad protein by activated Akt (Fig. 1) (Datta et al., 1997; Graff, 2002). In fact, the phosphorylated Bad form seems unable to homodimerize with Bcl-2 and Bcl-XL and therefore, these antia apoptotic proteins stay active (Datta et al., 1997). In this context, the constitutive signaling response mediated through PI3K/Akt pathway appears to be important for survival of certain PC cells such as LNCaP but not for DU145 and PC3 while MAPK and androgen response pathways did not seem to be required for survival of all these PC cell types (Lin et al., 1999; Edamatsu et al., 2000). Indeed, it has been observed that LNCaP cells rapidly undergo apoptotic death when treated with a specific PI3K inhibitor such as wortmannin or LY294002 which acts by decreasing Akt activity while the androgenreceptor Casodex or specific MEK inhibitor, PD98059, were unable to induce the apoptotic death. Importantly, it has also been noticed that the apoptosis induced by inhibiting PI3K in LNCaP might be attenuated by activating EGFR by EGF suggesting that other PI3K-independent cell survival signals are also activated by EGF (Lin et al., 1999). On basis of these observations, it appears that the antia apoptotic signaling cascades which are mediated through EGF-EGFR system might be responsible at least in part for resistance of certain PC cells to apoptotic effects of specific PI3K inhibitors.

In addition, EGF-EGFR system appears also to mediate its antia apoptotic effect in certain cell types including PC cells by lowering cellular ceramide levels via the inhibition of aSMase activity and/or activation of acidic ceramidase which catalyzes the hydrolysis of ceramide into sphingosine and free fatty acids (Coroneos et al., 1995; Del Peso et al., 1997; Payne et al., 1999; Mimeault et al., 2002). As a matter of fact, it has been observed that the inhibition of acidic ceramidase activity by specific inhibitors such as N-oleylethanolamine potentiates the apoptotic response induced in the PC cells by diverse cytotoxic agents in the presence of EGF (Mimeault et al., 2002). Thus, since it has been noticed that EGFR and acidic ceramidase are overexpressed in metastatic PCs (Seelan et al., 2000), it is likely that the stimulatory effect of EGF-EGFR system on intrinsic activity of acidic ceramidase might also contribute greatly to assume the cancer cell survival. In this context, it is interesting to notice that the application of exogenous C2 or C6 ceramide to diverse metastatic cancer cells including LNCaP, DU145 and PC3 cells has been observed to result in an increase of apoptotic/necrotic cell death (Engedal and Saatcioglu, 2000; Gallardo et al., 2000; Gewies et al., 2000).

PROSTATIC CANCER THERAPIES

Among the more current treatments used for PCs are the androgen-deprivation therapies, radiotherapy and chemotherapy (Aquilina et al., 1997; Bruchovsky et al., 2000; Demers et al., 2001; Kelloff et al., 2001; Hellerstedt and Pienta, 2002). In particular, the most of antiandrogens used in therapy for benign and localized forms of prostatic tumors antagonize the production and/or biological actions of the testosterone and its most potent metabolite, α-DHT. For instance, the specific inhibitors of steroid degradation enzyme, 5α-reductase such as the finasteride are used for the treatment of benign PCs (Sadar et al., 1999; Bruchovsky et al., 2000; Van Coppenolle et al., 1999; Liu et al., 2002a,b; Torring et al., 2002). Moreover, several androgen antagonists of ARs including bicalutamide and flutamide are used as therapeutic adjuvant after surgery or irradiation in particular cases where a high risk in the recurrence of PCs is prevalent (Sadar et al., 1999; Dotlaw et al., 2002). However, although these therapies are effective for patients with localized and androgen-responsive PCs, they are rarely curative against advanced states of PCs and therefore, the development of other therapeutic strategies for prevention and treatment of extraprostatic cancers that are recurrent and incurable is essential. Thus, certain monotherapies and combination therapies which represent the promising strategies for treatment of advanced and metastatic PC forms will be reported below (Table I).

Therapies by Blocking EGFR

The high biological importance of EGFR in the development of numerous cancers including PCs has led to the design of a multitude of drugs and specific antibodies that have been used as tools in biochemical studies to block EGFR signal transduction cascades and as
new pharmaceutical substances for treatment of cancers (Barton et al., 2001; Bergan et al., 2001; Kim et al., 2001; Chan et al., 2002; Herbst, 2002; Naruse et al., 2002). As a consequence, the chemistry of inhibitors of EGFR signaling pathways is highly developed and has been extensively reported in several previous reviews. Then, only the more potent and selective inhibitors of EGFR-EGFR system used of a current manner as pharmacological tool or which are undergoing phase I, II and III preclinical studies are presented here.

EGFR Tyrosine Kinase Activity Inhibitors

Certain natural products such as silymarin, genistein, epigallocatechin 3-gallate and curcumin have been shown to induce an arrest of PC cell growth by inhibiting EGF-EGFR transduction system (Zi et al., 1998; Ye et al., 1999; Dorai et al., 2000; 2001). In particular, it has been reported that curcumin induces apoptotic death of LNCaP cells and inhibits in vivo the angiogenic process which is associated with the progression of localized PCs into metastatic forms (Dorai et al., 2000; 2001). Of particular interest, it has also been shown that diverse compounds which interact with the adenosine-triphosphate binding site of EGFR (erbB1) and thereby, inhibit EGFR tyrosine kinase activity such as quinazoline-derived drugs PD153035 and PD182905 (Parke-Davis) and ZD1839 “Iressa” (AstraZeneca) as well as the dual erbB1/erbB2 tyrosine kinase inhibitor, PKI-166 and pan-erbB (erbB1 or EGFR, erbB2, erbB3 and erbB4) tyrosine kinase inhibitor, CI-1033 are able to induce an arrest of the growth and apoptotic death of diverse cancer cells including PC cells (Tortora et al., 1999; 2000; Ciardiello, 2000; Ciardiello et al., 2000; Meric et al., 2000; Barton et al., 2001; Slichenmyer et al., 2001; Albanell et al., 2002; Chan et al., 2002; Herbst, 2002; Harper et al., 2002; Naruse et al., 2002; Mellinghoff et al., 2002; Mimeault et al., 2002). Among them, ZD1839 presents very interesting therapeutic properties because it shows a high anticarcinogenic potency and has a good bioavailability and acceptable tolerability in vivo in human (Baselga et al., 2002; Ranson, 2002). Moreover, ZD1839 induces an arrest of the growth and regression of a variety established tumors in nude mice including DU145 and PC3 xenografts (Barton et al., 2001). In addition, ZD1839 has also been reported to inhibit the tyrosine kinase activity of erbB2 expressed in many metastatic cancers (Moasser et al., 2001; Moulder et al., 2001). In fact, it has been proposed that the inhibition of EGFR tyrosine kinase activity by ZD1839 could result to an inactive form of EGFR-erbB2 heterodimer and inhibition of the growth of human tumor cells expressing erbB2 and EGFR. Thus, on the basis of these observations, it will be important to estimate whether the therapies with selective EGFR inhibitor such ZD1839 are also efficacy to inhibit the growth of metastatic PC cells which are characterized by high levels of EGFR, erbB2 and EGFRvIII.

Anti-EGFR Antibodies and Antisense Oligonucleotides

Several monoclonal antibodies (MAbs) directed against the extracellular domain of EGFR which act by blocking the binding of EGF and TGF-α to EGFR as well as antisense oligonucleotide (AS) blocking the EGFR expression have been shown to reduce the rate of autophosphorylation of EGFR and thereby, inhibit the basal and EGF-stimulated proliferation of PC cells such as DU145 and PC3 through a G1 arrest in cellular cycle (Peng et al., 1996; Wu et al., 1996; Kim et al., 2001). Interestingly, inoculation of antibody ImClone(IMC)-C225 or antisense oligonucleotide directed against EGFR in human tumor established from androgen-independent PC cells in nude mice has been observed to lead to inhibition of tumor growth and angiogenesis as well as tumor and endothelial cell apoptosis and hemorrhagic necrosis (Rubenstein et al., 1998; Karashima et al., 2002). Of therapeutic interest, the use of human anti-EGFR monoclonal antibody, ABX-EGF has also permitted to perceive that this agent exhibits only its antitumor activity on the human tumor xenographs established from neoplastic cells including PC3 which express EGFR levels superior to 17000 EGFR molecules per cells (Yang et al., 2001). This suggests then that inhibition of EGFR signaling pathways might constitute a promising strategy for the development of new preventive and effective therapies to counteract the progression of numerous human cancers including PCs which are dependent of EGFR transduction pathways for the sustained growth and survival of transformed cells.

Other Therapies

Therapies by Inhibiting PKA

Since PKA signaling cascade influences a multitude of cell reactions which are involved in conjunction with EGF-EGFR system in the growth and survival of cancer cells, a lot of potent and selective inhibitors of this enzyme have also been developed. Of particular therapeutic interest, it has been observed that the inhibition of PKA activity by specific inhibitors such as H-89, Rp-cAMPs or 8-C1-cAMP induces in vitro and in vivo a growth inhibition of various human metastatic cancer cells including PC cells (Fig. 2) (Melck et al., 2000; Mimeault et al., 2002; Tortora and Ciardiello, 2002). Moreover, PKA inhibitors, Rp-cAMPs and H-89 have been observed to down-regulate P-glycoprotein (Pgp)-mediated multidrug resistance in prostate tumors (Wartenberg et al., 2000). In addition, use of antisense oligonucleotides targeting R1a subunit of PKA-I has been observed to results in a reduction of PKA-I activity that was accompanied by an arrest of the cancer cell growth concomitant with a compensatory increase of PKA-Ⅱб level (Nesterova et al., 2000). Importantly, it has also been observed that PKA-I antisense oligonucleotide inhibition results in vitro and in vivo in an arrest of the growth and apoptotic death of PC3 cells by up-regulating apoptotic
proteins Bax, Bak and Bad and inhibiting antiapoptotic Bcl-2 by hyperphosphorylation (Cho et al., 2002). Thus, since it has been reported that PKA could be involved in the activation of ARs in the absence of androgens (Sadar et al., 1999; Cox et al., 2000; Dotzlaw et al., 2002), it appears that PKA inhibition might represent a therapeutic strategy to counteract androgen-independent and metastatic PC forms.

**Therapies by Inhibiting Arachidonic Acid Cascades**

The blockade of the eicosanoid production from arachidonic acid cascade (AA) metabolism by using specific inhibitors of lipooxygenases (5-LOX and 12-LOX) and cyclooxygenases (COX-1 and COX-2) pathways has also been observed to result in an arrest of the growth and massive apoptotic death of PC cells in vitro and in vivo as well as in a reduction of tumor angiogenesis (Myers and Ghosh, 1999; Cuendet and Pezzuto, 2000; Attiga et al., 2000; Nie et al., 2000; Bakhle, 2001; Shureiqi and Lippman, 2001; Kellof et al., 2001; Johnson et al., 2002). In fact, the cytotoxic effects of these inhibitory agents seem to be mediated at least in part of the cellular AA accumulation that leads to ceramide generation through the hydrolysis of sphingomyelin as well as by down-regulating expression of antiapoptotic Bcl-2 protein (Tanji et al., 2000; Kirschenbaum et al., 2001; Pidgeon et al., 2002).

In particular, several new drugs which specifically inhibit only COX-1 or COX-2 activity have been developed based on structural differences between COX-1 and COX-2 and although these isoenzymes differ only within their active sites by valine/isoleucine substitutions at two positions (Cuendet and Pezzuto, 2000; Attiga et al., 2000; Liu et al., 2000; Shureiqi and Lippman, 2001). The selective COX-2 inhibitors offer a greater promise as effective chemopreventive and therapeutic agents because they decrease the risks of secondary effects associated with the inhibition of COX-1 and they act preferably on metastatic PC cells that highly express this enzyme compared to normal and low-grade PC cells (Kirschbaum et al., 2001). For instance, COX-2 inhibitors including NS398 and Etodolac suppress the proliferation and induce apoptosis of LNCaP and PC3 cells while they have no cytotoxic effect on PrSc normal prostate cells (Liu et al., 2000; Kamijo et al., 2001). Importantly, COX-2 cyclooxygenase is also considered as a key enzyme for development of PC cancers because it is involved in control of tumor neovascularization. Indeed, the sustained induction of COX-2 in PC cells seems to result in an increase of the synthesis of AA product PGE_2 which is involved in hypoxia-induced up-regulation of expression of potent angiogenic factors such as vascular endothelial growth factor (VEGF) (Liu et al., 2000; Kirschbaum et al., 2001; Fujita et al., 2002). In fact, it has been proposed that PGE_2 might induce its angiogenic effect in promoting the translocation of hypoxia-inducible factor from cytosol to nucleus (Liu et al., 2002a,b). In this manner, the broad COX inhibitor ibuprofen and selective COX-2 inhibitor NS398 have been reported to counteract the angiogenic process associated with the development of PCs (Attiga et al., 2000; Kirschbaum et al., 2001). Among the selective COX-2 inhibitors, celecoxib and rifocoxib are also undergoing preclinical evaluation in human for the treatment of PCs. Interestingly, since hormonal deprivation therapies seem to be accompanied by selective expression of COX-2 (Tanji et al., 2000; 2001), the inhibitors of this enzyme represent then potent adjuvant agents for a more effective treatment of recurrent PCs.

**Therapies by Activating the Caspase and Ceramide Cascades**

Since the progression of PCs into high malignant grades is often accompanied by the deregulated functions in the apoptotic/necrotic cell death, up-regulating cellular caspase and ceramide levels also represents a potential approach for development of new adjuvant-based therapies (Denmeade and Isaacs, 1996; Herrmann et al., 1997; Chaudhary et al., 1999; Condorelli et al., 1999; Wang et al., 1999; Gallardo et al., 2000; Kelloff et al., 2001; Gleave et al., 2002; Von Haefen et al., 2002). As a matter of fact, the diethyl-maleate (DEM), a thiodenating agent has been shown at a subtoxic dose to sensitize the serum-stimulated LNCaP, DU145 and PC3 cells to cytotoxic effects induced by diverse chemical compounds such as etoposide, cycloheximide and Fas antibody as well as by irradiation (Coffey et al., 2001). In fact, DEM promotes the apoptosis-induced by these antinegenicogenic agents by down-regulating Bcl-2 expression level and this leads to increase of procaspase-3 expression level. Moreover, the evaluation of an antisense oligonucleotide directed against Bcl-2 has indicated that it delays androgen independence and promotes sensibility of PCs to cytotoxic effect of chemotherapeutic agent, mitoxantrone (Chi et al., 2001). In addition, DNA-damaging etoposide (topoisomerase II inhibitor) and microtubule-damaging paclitaxel have been observed to induce an arrest of the growth and apoptotic death of LNCaP and DU145 by inducing cellular ceramide accumulation (Sumitomo et al., 2000). In fact, an early and transient increase of ceramide level via de novo synthesis pathway induced by these cytotoxic agents which is accompanied by stimulating PKC-δ through its mitochondrial translocation, appears to lead subsequently to cytochrome c release, caspase-9 activation, neutral sphingomyelinase (nSMase)-induced ceramide production and apoptotic death of PC cells in a caspase-3-independent manner. In this context, it is noteworthy that calcitonin and bombesin have been reported to counteract the apoptotic effect induced by etoposide in PC cells suggesting that the antagonists of these neuropeptides could also be benefit as adjuvant therapeutic agents (Salido et al., 2002). Importantly, it has also been observed that the ceramide generation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment permits
to sensitize LNCaP cells to apoptotic effect of ionizing radiations (Garzotto et al., 1999).

**Combination Therapies**

Several strategies carried out in order to sensitize human metastatic PC cells that are resistant to cytotoxic effects of antiandrogenic and anticarcinogenic agents have been clinically unsuccessful emphasizing importance to develop new adjuvant-based approaches or combination cytotoxic therapies (Kyprianou, 1994; Karp et al., 1996; Teicher et al., 1997). In this context, several investigations have revealed that a combination of anti-EGFR blocking MAb, selective EGFR tyrosine kinase inhibitor, PKA-I inhibitor and distinct chemotherapeutic agents results in synergistic growth-inhibitory and cytotoxic effects on many cancer cell lines and tumors in vitro and in vivo (Herbst, 2002; Tortora and Ciardiello, 2002). For instance, the mixture of anti-EGFR monoclonal antibody MAb C225, EGFR tyrosine kinase inhibitor ZD1839, antisense oligonucleotide HYB165 directed against Rlα of PKA-I or specific PKA inhibitor 8-Cl-cAMP and diverse cytotoxic drugs including platinum- and taxane-derived compounds as cisplatin, paclitaxel and docetaxel have been demonstrated to induce in vitro a cooperative cytotoxic effect on several types of human cancer cell lines from prostate, breast, ovary, colon, lung and skin. In particular, the synergistic cytotoxic effects observed between antisense oligonucleotide targeting PKA-I, MAb C225 and docetaxel on human breast cancer cells which were accompanied by a greater degree of Bcl-2 phosphorylation lead to a higher rate of apoptotic death than that was observed in the presence of each single agent (Tortora et al., 1997). In this matter, our recent works also indicated that the simultaneous inhibition of EGFR and PKA cascades by using PD153035 and Rp-cAMPs results in vitro in a more massive apoptotic/necrotic cell death of LNCaP, DU145 and PC3 cells as the cytotoxic effects observed for the drugs alone (unpublished observations). In fact, these agents appear to act in synergy by inducing a greater damage to cellular membrane and this is accompanied by mitochondrial cytochrome c release, caspase activation and DNA fragmentation.

Importantly, antibodies directed against EGFR and/or PKA or selective EGFR and PKA inhibitors also sensitize several cancer cell lines to cytotoxic effect of ionizing radiations (Bianco et al., 2000; Tortora and Ciardiello, 2002; Naruse et al., 2002). In particular, it has been observed that the treatment of mice bearing established human cancer xenograft with the radiotherapy (RT), anti-EGFR monoclonal antibody (MAb C225) and antisense oligonucleotide targeting PKA-I at low doses produces a greater antitumor activity and a significant improvement in survival of mice compared to that was observed after treatment with individual agent (Bianco et al., 2000). On the other hand, it has been observed that the Pseudomonas exotoxin-chimeric protein which interacts specifically with EGFR markedly enhances sensibility of human xenografts to radiation killing through the protein synthesis and simultaneous production of ceramide (Seetharam et al., 1998). In addition, preclinical investigations have also revealed that ZD1839 sensitizes a wide variety of tumors including PCs to the cytotoxic effects induced by irradiation (Ranson, 2002).

### TABLE I  Possible strategies for treatment of advanced and metastatic prostate cancer forms by monotherapy and combination therapy

<table>
<thead>
<tr>
<th>Type of strategy</th>
<th>Name of agent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapies by blocking EGFR</strong></td>
<td></td>
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<tr>
<td>Inhibitors of Receptor Tyrosine Kinase Activity (erbB-TKIs):</td>
<td></td>
</tr>
<tr>
<td>EGFR-TKI</td>
<td>ZD1839</td>
</tr>
<tr>
<td>EGFR/erbB2-TKI</td>
<td>PKI-166</td>
</tr>
<tr>
<td>erbB1/erbB2/erbB3/erbB4-TKI</td>
<td>CI-1033</td>
</tr>
<tr>
<td><strong>Monoclonal Antibodies Anti-EGFR (Mabs):</strong></td>
<td></td>
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<tr>
<td>Chimeric MAb directed against mouse and human EGFR</td>
<td>MAb-C225</td>
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<tr>
<td>MAb directed against human EGFR</td>
<td>IMC-C225</td>
</tr>
<tr>
<td><strong>Antisense Oligonucleotide (AS)</strong></td>
<td></td>
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<tr>
<td>AS-EGFR</td>
<td>ABX-EGF</td>
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<tr>
<td><strong>Other Therapies</strong></td>
<td></td>
</tr>
<tr>
<td>Inhibitors of PKA cascade</td>
<td>8-Cl-cAMP</td>
</tr>
<tr>
<td>Selective inhibitor of PKA-I activity</td>
<td>Antagonist of bombesin or calcitonin</td>
</tr>
<tr>
<td>GPCR blocker</td>
<td>AS-PKA-I</td>
</tr>
<tr>
<td>Oligonucleotide antisense</td>
<td>Nordhydroguaiaretic acid</td>
</tr>
<tr>
<td><strong>Inhibitors of arachidonic acid cascade</strong></td>
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</tr>
<tr>
<td>Specific inhibitor of lypoxygenases (LOs)</td>
<td>MK886 or Baicalein</td>
</tr>
<tr>
<td>Selective inhibitor of 5-LO or 12-LO</td>
<td>Ibuprofen</td>
</tr>
<tr>
<td>Specific inhibitor of cyclooxygenases (COXs)</td>
<td>NS398, Eto-dolax, Celecoxib, Rofecoxib</td>
</tr>
<tr>
<td>Selective inhibitor of COX-2</td>
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<tr>
<td><strong>Combination Therapies</strong></td>
<td></td>
</tr>
<tr>
<td>EGFR blocker with</td>
<td>8-Cl-cAMP, AS-PKA-I</td>
</tr>
<tr>
<td>PKA-I blocker</td>
<td>Etoposide, Paclitaxel, TPA AS-Bcl-2</td>
</tr>
<tr>
<td>Activator of ceramide generation</td>
<td>Inhibitors of PDGF, VEGF and FGF receptors (SU-6668)</td>
</tr>
<tr>
<td>Activator of caspase cascades</td>
<td>Selective COX-2 inhibitor</td>
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<tr>
<td>Anti-angiogenic agents</td>
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</tbody>
</table>
In addition, since tumor growth and metastasis of cancer cells are angiogenesis-dependent processes (Mimeault, 2001; Van Moorselaar and Voest, 2002), the development of new combinatorial therapeutic strategies to counteract dissemination of metastatic cancer cells including PC cells could also include the EGFR inhibitor with other antiangiogenic agents. Among them, PDGF receptor antagonist such as SU-101, VEGF receptor inhibitor, SU-5416 as well as the broad agent, SU-6668 which targets PDGF, VEGF and FGF receptors have been shown to block the growth of numerous tumor types including PCs (Bergan et al., 2001). In this context, it is interesting to notice that the combination of specific monoclonal antibodies directed against EGFR (C225) and VEGFR (DC 101) has notably been observed to induce a greater inhibition of growth and angiogenesis as well as a higher rate of apoptosis of colon cancer cells that drugs alone (Shaheen et al., 2001). Moreover, use of different human PC cell lines established from RWPE-1 and characterized by distinct degrees of invasive ability has permitted to perceive that alteration in interactions between cell and extracellular matrix through changes in integrin expression and EGF levels might occur during prostate carcinogenesis (Bello-DeOcampo et al., 2001). Importantly, it has also been observed that an anti-EGF antibody can cause an increase of these interactions which might be associated with a loss of invasive ability of PC cells.

Taken together, these works suggest that the combination of specific inhibitors of EGFR and PKA such as peptide and non-peptide inhibitors, monoclonal antibody, antisense oligonucleotide or immunotoxin with other cytotoxic agents including activators of ceramide accumulation and angiogenic inhibitors could represent the more effective treatments for recurrent PC forms that are resistant to conventional radiotherapy, chemotherapy and androgen-deprivation therapies.

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References


NEW ADVANCES ON PROSTATE CARCINOGENESIS

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