Review

The power and promise of ''rewiring'' the mitogen-activated protein kinase network in prostate cancer therapeutics

Athanasios G. Papatsoris,[1,2] Michalis V. Karamouzis,[1] and Athanasios G. Papavassiliou[1]

[1]Department of Biological Chemistry, Medical School, University of Athens and [2]2nd Department of Urology, Medical School, University of Athens, Sismanoglio General Hospital, Athens, Greece

Abstract

Prostate cancer is the most frequently diagnosed cancer among men and the second leading cause of male cancer deaths. Initially, tumor growth is androgen dependent and thus responsive to pharmacologic androgen deprivation, but there is a high rate of treatment failure because the disease evolves in an androgen-independent state. Growing evidence suggests that the Ras/mitogen-activated protein kinase (MAPK) signaling cascade represents a pivotal molecular circuitry participating directly or indirectly in prostate cancer evolution. The crucial role of the protein elements comprising this complex signal transduction network makes them potential targets for pharmacologic interference. Here, we will delineate the current knowledge regarding the involvement of the Ras/MAPK pathway in prostate carcinogenesis, spotlight ongoing research concerning the development of novel targeted agents such as the Ras/MAPK inhibitors in prostate cancer, and discuss the future perspectives of their therapeutic efficacy. [Mol Cancer Ther 2007;6(3):811–9]

Introduction

Prostate cancer remains the most common noncutaneous malignancy in the Western world and is the second leading cause of cancer death in males, after lung cancer (1). In spite of these dismal statistics, little progress has been achieved in extending the survival of patients with disseminated disease with current treatment modalities (2). Androgen ablation remains the mainstay of advanced prostate cancer treatment, but progression to hormone-refractory prostate cancer (HRPC) is inevitable and usually occurs within 2 to 5 years. The current treatment options for HRPC are supportive care, salvage endocrine manipulations, radiotherapy, radioactive isotopes, biphosphonates, and chemotherapy (1, 2). Despite the recent advances of new chemotherapeutic regimens in HRPC patients, a 20- to 24-month median survival can only be anticipated (3).

The prostate gland requires both androgens and polypeptide growth factors for proliferation, differentiation, and maintenance of function. In normal prostate epithelial cells, androgens suppress the growth factor stimulatory effects. However, in prostate cancer cells, this is deregulated, allowing external stimuli to interact through membrane receptors with androgens and enhance their growth and proliferation. The transduction of the membrane-bound receptor activation signal to the nucleus is achieved through numerous intracellular biochemical cascades. All these signal transduction pathways are often distorted during prostate carcinogenesis. The Ras/mitogen-activated protein kinase (MAPK) signaling pathway has long been identified as a convergence point for numerous (normal and pathologic) signaling inputs, rendering it an appealing target for therapeutic intervention (4).

MAPK Signaling Network and Carcinogenesis

A diverse array of growth factors, cytokines, and proto-oncogene products deliver their growth-eliciting stimuli through the activation of the small G protein Ras and subsequently of the MAPK network (Fig. 1). The Ras subfamily of small GTP-binding proteins direct signal transduction between the membrane and the nucleus. They are activated when bound to GTP and are inactive when bound to GDP. These states are regulated by the balance between the intrinsic GTPase activity of the proteins: their interactions with inhibitory proteins and with activating proteins that regulate the exchange of GDP for GTP. The Ras subfamily consists of three functional members: H-Ras, N-Ras, and K-Ras. K-Ras is the isoform that is most frequently mutated in human cancers (5). Nonetheless, *ras* gene mutations alone are not sufficient for malignant transformation (6). Carcinogenesis is a multistep process characterized by the gradual accumulation of genetic and epigenetic aberrations resulting in deregulation of cellular homeostasis, allowing premalignant lesions to develop into invasive tumors (7).

Ras proteins function as molecular switches that facilitate transduction of extracellular signals. Ras mediates the activation of a plethora of effector pathways that modulate

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Requests for reprints: Athanasios G. Papavassiliou, Department of Biological Chemistry, Medical School, University of Athens, 75 M. Asias Street, 11527 Athens, Greece. Phone: 30-210-7791207; Fax: 30-210-7791207. E-mail: papavas@med.uoa.gr

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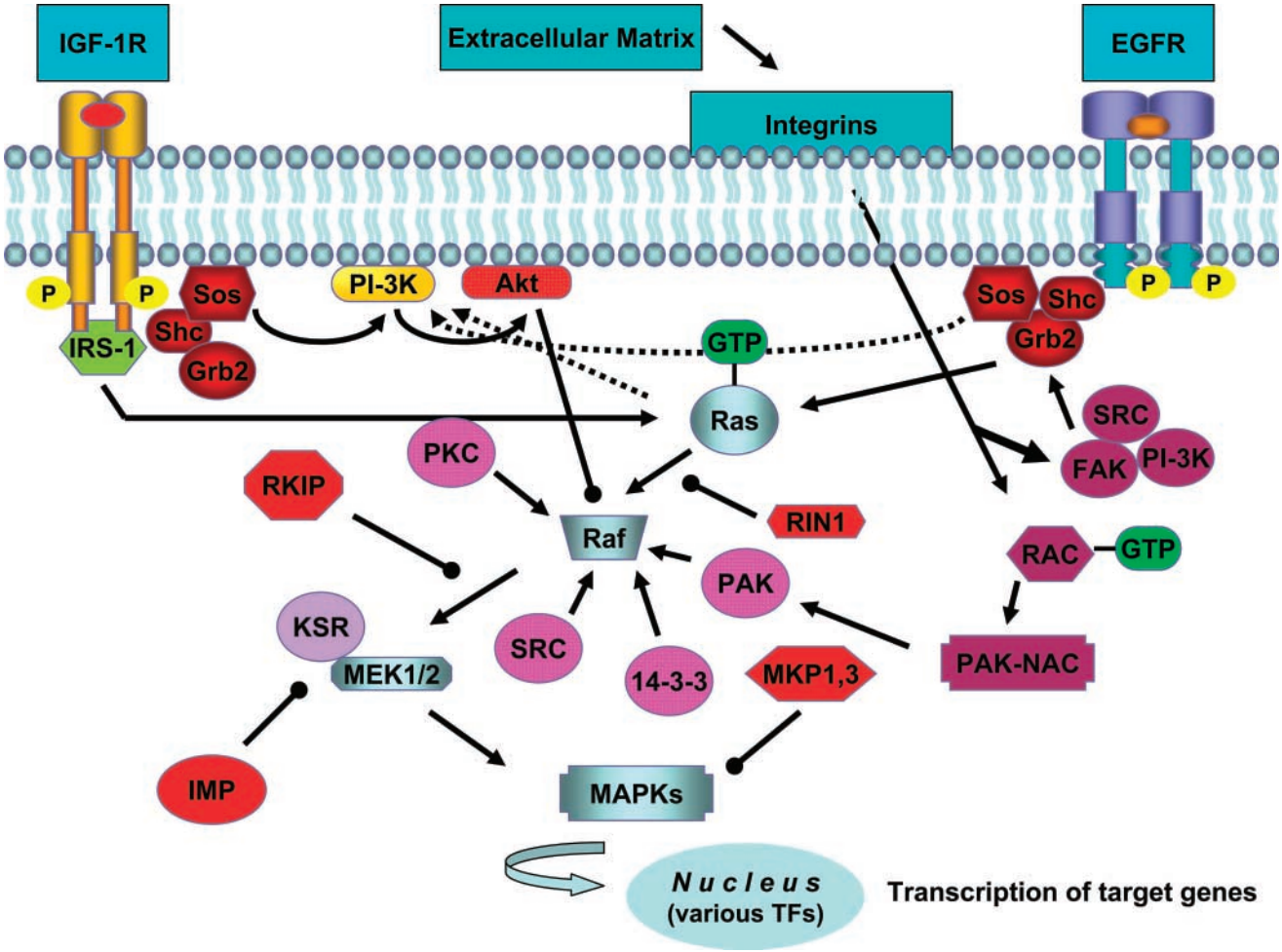

Figure 1. Overview of the Ras/MAPK signaling network. Ligand binding to the extracellular domain of receptors like IGF-1R and EGFR signals conformational changes that induce receptor autophosphorylation and stimulation of its TK activity. Activation of a number of downstream signaling pathways is subsequently mediated by recruitment and tyrosine phosphorylation of adaptor molecules (such as Grb2). In cooperation with Grb2, the guanine-nucleotide exchange factor Sos potentiates Ras, by catalyzing the replacement of GDP for GTP. GTP-bound Ras triggers the kinase activity of Raf and its downstream effector molecules. Raf phosphorylates MEK, which in turn phosphorylates and activates MAPKs (ERKs). Notably, a plethora of negative regulators exist in this pathway, including RKIP (interferes with MEK phosphorylation), RIN1 (competes with Raf for binding to activated Ras), IMP (interacts with KSR and prevents recruitment of MEK to activated Raf), and the MKP family of phosphatases (reduce activation status of the Raf signaling cascade). PI-3K recruits and activates (via a series of intermediary steps) PDK1 which, in turn, phosphorylates and potentiates Akt (the latter can also act as negative regulator of Raf). However, there also positive modulators of Raf activity, such as PKC, SRC, PAK, and 14-3-3, that are capable of inducing Raf in a Ras-independent manner. Importantly, the Ras/MAPK pathway can become activated by binding of integrins to specific extracellular matrix molecules, with subsequent activation of PI-3K and FAK. Stimulation of this pathway results in activation of the GTP-bound protein RAC, which then interacts with the NAC-PAK complex, thereby leading to PAK and Raf activation. Potentiated MAPKs translocate into the nucleus where they modulate the activation status of a wide gamut of transcription factors, thus affecting gene expression programs. FAK, focal adhesion kinase; GRB2, growth factor receptor-binding protein 2; IMP, impedes mitogenic signal propagation; IRS-1, inslulin receptor substrate-1; KSR, kinase suppressor of Ras; MKP, MAPK phosphatase; P, phosphate groups; PAK, p21-activated kinase; PDK1, phosphatidylinositol-dependent kinase 1; PI-3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; RIN1, Ras and Rab interactor 1; RKIP, Raf kinase inhibitor protein; SOS, son of sevenless; TF, transcription factors; TK, tyrosine kinase.

cell proliferation, apoptosis, and other cellular processes (Fig. 1). Cell proliferation can be enhanced by activated membrane growth factor receptors (GFR) and/or through binding of integrins to specific extracellular matrix molecules (8). Activation of the MAPK pathway has been found to be associated with activator protein 1–regulated induction of cyclin D1 and down-regulation of endogenous cyclin-dependent kinase inhibitors (9). It has been postulated that the intensity and duration of the signaling through this pathway ultimately determines whether a cell undergoes differentiation, proliferation, cell cycle arrest, or apoptosis (4). A second pathway in which Ras is involved is the phosphatidylinositol-3 kinase/Akt pathway that mainly modulates apoptosis (10). Furthermore, Ras may signal through Tiam1, a protein that regulates Rec, which in turn activates various proteins such as RhoB and nuclear factor-κB (11). RhoB is implicated in several cellular functions, including cell adhesion and apoptosis. Nuclear

factor-κB is a transcriptional regulator of genes that are decisive for cell survival and immune responses. To become functionally active, Ras protein has to undergo several posttranslational modifications, with farnesylation of the COOH-terminal cysteine by the enzyme farnesyl transferase (FTPase) being the most important. Yet, both K-Ras and N-Ras can also be geranylgeranylated as an alternative way of prenylation (12).

The Raf serine/threonine kinases (A-Raf, B-Raf, and C-Raf) are the principal effectors of Ras in the MAPK pathway (12). Although all Raf proteins can activate the MAPK cascade, they seem to have distinct downstream phosphorylation targets. GTP-bound Ras interacts with Raf and mobilizes the inactive protein from the cytoplasm. Once the Ras-Raf complex is translocated to the cell membrane, Ras activates Raf. The regulatory mechanisms of various Ras isoforms differ in that A-Raf and C-Raf require additional phosphorylation reactions for activation, whereas B-Raf has a much higher level of basal kinase activity. Although primarily triggered by Ras, Raf may also be stimulated by Ras-independent mechanisms and thus propagate signals through diverse cascades that mediate proliferation, angiogenesis, metastasis, and survival (ref. 13; Fig. 1). Physiologically, Raf activity is modulated by binding of growth factors, hormones, and cytokines to receptors on the cell surface. Oncogenic Raf, however, is able to ignite the MAPK pathway, leading to constitutive proliferation independently of mitogenic stimulation (14). Activating Raf, especially B-Raf, mutations have been identified in various solid tumors such as prostate cancer (15).

Potentiated Raf principally propagates signaling by phosphorylating the two dual-specificity MAPK kinases: MEK1 and MEK2 (Fig. 1). The Raf isoforms are the best-characterized MEK1 and MEK2 activators, and all Raf species activate MEK1, whereas only B-Raf and C-Raf activate MEK2. Although both A-Raf and C-Raf are capable of triggering other downstream molecules independently of MAPK pathway activation, MEK1 and MEK2 are the only known substrates of B-Raf (16, 17).

It is generally accepted that the respective downstream substrates of MEK1 and MEK2 are extracellular signal-regulated kinases (ERK1/2), Jun NH_2-terminal kinases (JNK1–JNK3), and p38 kinases (α, β, γ, and δ isoforms), which upon activation translocate to the nucleus whereby they induce an array of nuclear regulatory proteins (Fig. 1). Effectors include the transcription factors Elk-1, activator protein 1, Myc, and others that ultimately regulate genes encoding proteins with crucial roles in proliferation, angiogenesis, metastasis, and resistance to anticancer treatments (17).

Role of MAPK Signaling Network in Prostate Cancer Evolution

Increases in autocrine and paracrine growth factor loops are among the most commonly reported changes, correlated with prostate cancer progression from a localized and androgen-dependent to a disseminated and androgen-independent state. In advanced prostate cancer, epidermal growth factor receptor (EGFR), insulin-like growth factor-1 receptor (IGF-1R), and other GFRs have been frequently shown to be overexpressed, and an intensive cross-talk of these receptors has been increasingly pointed out (17–19).

Various studies have shown that polypeptide growth factor signal transduction pathways can evoke androgen receptor (AR) activation, suggesting that the increase in growth factor and GFR expression could be directly implicated in prostate cancer progression to androgen independence (20). Growth factor stimulation has been reported to render androgen response element–driven promoters hypersensitive to, or independent of, androgens (21). These effects seem to be mediated by tyrosine kinase receptors and their downstream signaling effectors through regulated changes in phosphorylation of the AR (20). Current data reveal that Ras/MAPK influences on AR function are possible through phosphorylation of AR-associated proteins such as the transcription cofactors (ref. 21; Fig. 2).

Virtually all of the GFRs that are up-regulated in prostate cancer potentiate Ras and its cognate network for a portion of their activity (19). However, Ras and Raf mutations are infrequent in prostate cancer (15, 22). Therefore, it could be hypothesized that wild-type Ras and/or Raf are activated as a consequence of autocrine and paracrine growth factor stimulation during prostate carcinogenesis. Nevertheless, it should be noted that it is as yet undefined whether the different Ras isoforms have diverse roles during prostate carcinogenesis. It has been suggested that activation of Ras through the MAPK pathways can modulate androgen-dependent gene expression; on the other hand, it has also been documented that Ras activation might be sufficient for the progression of prostate cancer cells towards androgen independence (6, 23). The cellular response to androgen is mediated by ARs, which are members of the nuclear receptor superfamily. ARs have been shown to exert their transcriptional effects either through the androgen response elements or via activator protein 1–binding elements (12-*O*-tetradecanoylphorbol-13-acetate response elements; ref. 24; Fig. 2). In addition, further to the classic nuclear ''androgenic'' effects, it is increasingly clear that AR signaling effects may take place in a ligand-independent manner, via cross-talk with membrane GFRs such as EGFR and IGF-1R (ref. 25; Fig. 2). Thus, various molecular mechanisms converge in the Ras/MAPK cascade and contribute to both androgen-dependent and androgen-independent evolution of prostate cancer.

Several studies have shown that MAPK activity correlates with the progression to an increasingly advanced and hormone-independent disease (26, 27). The effects of activated (phosphorylated) ERK on prostate cancer cells can either be reducing apoptosis or, more commonly, enhancing cellular proliferation. Conceivably, the relative activation of the ERK species (ERK1 and ERK2) can have variable cellular effects in prostate carcinogenesis. Elevation of prostate-specific antigen is also an ERK-sensitive phenomenon in androgen-independent conditions, and this might explain the continuing increase of prostate-specific

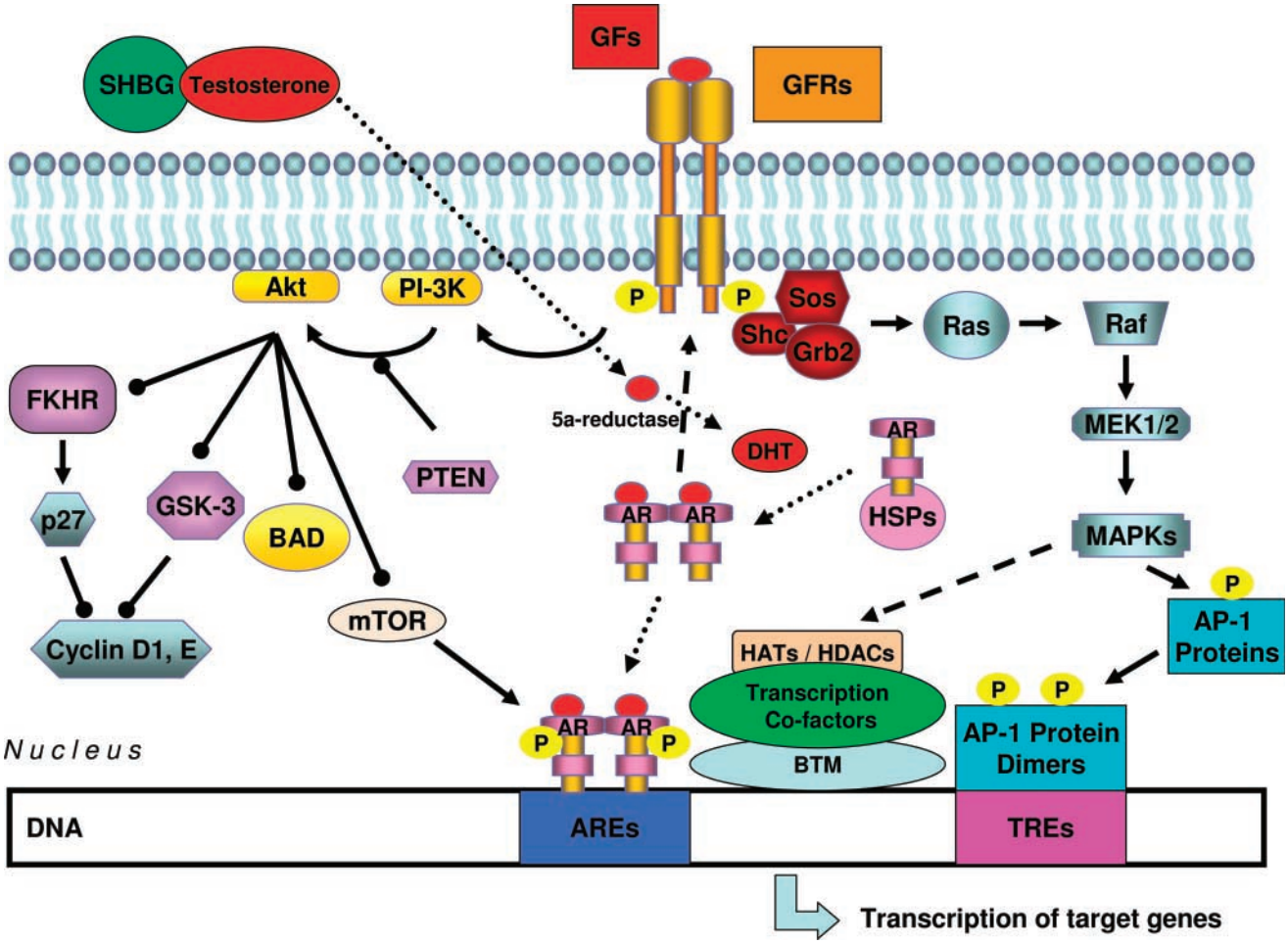

Figure 2. Interactions of ARs with the Ras/MAPK signaling network. Testosterone circulates in the blood bound to albumin and SHBG and exchanges with free testosterone. Free testosterone enters prostate cells and is converted to DHT by the enzyme 5α-reductase. Binding of DHT to the AR induces dissociation from HSPs and receptor phosphorylation. DHT-bound ARs enter the nucleus as homodimers that bind to AREs on target gene promoters, in their classic ''genomic'' mode of action. Promoter-associated ARs enhance gene transcription through interaction with transcription cofactors and the BTM [transcription cofactors operate as multi-protein complexes and are usually divided into two types: chromatin-modifying cofactors (HATs and HDACs) and general cofactors that associate with and/or influence the activities of components of the BTM]. Ligand stimulation of the ARs can also function through interaction with other transcription factors such as AP-1 (Jun-Jun and Jun-Fos protein complexes). Following homodimerization/heterodimerization, AP-1 proteins bind to the so-called TREs in the promoter/enhancer regions of target genes that include important modulators of many cellular processes. AP-1 activity is mainly controlled via phosphorylation by different kinases (primarily MAPKs), which influence DNA-binding capacity, trans-activation potential, and stability of AP-1 proteins. In general, AP-1 proteins have both overlapping as well as unique roles and function in a tissue- and/or cell-specific manner. ''Nongenomic'' effects of androgens might also occur through the ARs located at or adjacent to the plasma membrane, which may require the presence of adaptor proteins that target the ARs to the membrane. Activation of the membrane ARs might lead to a rapid change in cellular signaling molecules and stimulation of membrane GFR TK activity, which in turn triggers GFR signaling cascades. AP-1, activator protein-1; ARE, androgen response elements; BAD, Bcl associated dimmer; BTM, basal transcriptional machinery; DHT, dihydrotestosterone; FKHR, forkhead transcription factor; GF, growth factors; GSK-3, glycogen synthase kinase-3; HAT, histone acetyltransferases; HDAC, histone deacetylases; HSP, heat shock proteins; mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SHBG, sex hormone-binding globulin; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TRE, TPA response elements.

antigen in HRPC (28). Although the presence of active JNK in normal prostate tissues is somewhat controversial, most studies have shown that JNK expression or activation is increased in prostate cancer cells (29). JNK is generally activated by cellular stress, and numerous molecules are able to phosphorylate, hence potentiate, the enzyme (30). JNK activation is not a prerequisite for apoptosis, as shown in multiple studies employing cognate inhibitors or dominant-negative JNK-harboring cell lines. Despite overwhelming data indicating a correlation between JNK activation and apoptosis, several studies imply that JNK inactivation might also have a role in growth and proliferation control (31). Similar to JNK, p38 MAPK is a kinase primarily induced by external stresses and shares stronger activity in the prostatic stroma with ERK. Several authors have postulated that the determination of the phosphorylated ERK to phosphorylated p38 MAPK ratio might be valuable in predicting the *in vivo* behavior of solid

tumors, including prostate cancer (32). However, epithelial p38 MAPK can be found active in both benign hyperplasia and prostate cancer (29, 33).

It should be emphasized that the Ras/MAPK cascade is not exclusively responsible for growth, survival, and androgen responsiveness of prostate cancer cells (34). As depicted in Fig. 1, Ras and its transduction network is a multi-effector signaling system. Hence, it seems rationale that the progression of prostate cancer in the androgen independence state is mediated by a plethora of molecular pathways (35). For example, the significance of increased focal adhesion kinase expression and augmented SRC activity in prostate cancer progression, partly due to deregulation of MAPK activation, has been underscored by recent preclinical data combined with the fact that Ras and/or Raf mutations are rare in prostate cancer.

MAPK Signaling Network as a Potential Target in Prostate Cancer

The diversity of signaling during prostate carcinogenesis predicts that attempts to use a single ligand/receptor pair as a therapeutic target will not be generally effective due to their well-characterized extensive cross-coupling (36). For example, EGFR has been a major research focus of anticancer strategies in the last years. However, the inhibition of the EGFR tyrosine kinase has been, thus far, disappointing in prostate cancer therapeutics (37). The heterogeneity of prostate cancer seems to require a treatment ''algorithm'' designed to reduce this diversity. The sequential use of agents targeting different molecular targets is one option. However, such an approach would probably require the ability to effectively characterize tumors and select the subgroup of patients most likely to benefit. An alternative approach to identify optimal targets for prostate cancer treatment would be to pinpoint the downstream signaling intermediates that are shared by these diverse molecular cascades. The Ras/MAPK pathway represents one of those sites of regulatory convergence; therefore, its constituents seem suitable candidate targets for therapeutic intervention. Three components of this pathway have received, thus far, most of the scientific interest as potential targets for pharmacologic intervention: Ras, Raf, and MEK (4, 13). Various novel agents targeting these proteins are in preclinical and early clinical evaluation in various human tumors, including prostate cancer.

FTPase Inhibitors

Ras family proteins regulate important GFR-induced signaling pathways contributing to cellular differentiation and proliferation. Key to the functioning of Ras proteins is the posttranslational farnesylation of the NH_2 terminus of Ras by FTPase (38). Prostate cancer has been reported to exhibit Ras deregulation, whereas it has been shown that Ras mutations is not a prerequisite for FTPase inhibitors (FTPI) activity (39). This has been confirmed in a recently published phase I clinical trial, whereby the administration of a FTPI in advanced cancer patients with documented K-*ras* mutations did not result into clinical responses (40). Although agents that either down-regulate *ras* expression or reverse Ras activation have not been developed yet, several FTPIs are currently under clinical evaluation (41).

Preclinical evaluation of numerous small-molecule FTPIs with diverse chemical structure has shown promising antiproliferative, proapoptotic, and antiangiogenic activities (42). For instance, combined efficacy was shown with FTPIs SCH66336 and SCH58500 in androgen-insensitive DU145 prostate cancer cells (43). Another novel Ras antagonist (farnesylthiosalicylate) can bind to the Ras membrane-binding site and release Ras into the cytoplasm where it is degraded, resulting in reduction of MAPK activity by up to 80% (44). Moreover, FTPIs could antagonize the growth-enhancing properties of Ras-related small GTP-binding proteins such as Rho and Rac. The Rho GTPase family affects various cellular processes like proliferation and apoptosis. Notably, the Rho kinase inhibitor Y-27632 has been shown to hamper tumor growth and angiogenesis in androgen-insensitive prostate cancer cells (45). Nevertheless, serious concerns about these agents arouse when investigations revealed that higher concentrations of FTPIs were required to inhibit oncogenic K-Ras compared with the wild-type Ras, and that both K-Ras and N-Ras proteins can alternatively be geranylgeranylated, thus escaping the FTPase inhibition (12, 46).

The preclinical promising results stimulated many phase I to III clinical trials of FTPIs in HRPC, either as single-agent therapy or in combination with chemotherapeutic agents (42). Tipifarnib (R115777) has been the most broadly studied FTPI in a wide variety of tumor types with, unfortunately, poor clinical outcome and myelosuppression and neurotoxicity being the major toxicities (42). Phase I/II trials in advanced cancer patients have also been done with lonafarnib (SCH66336), BMS-2146642, and L778123 with similar efficacy results (47, 48).

Despite the high expectations based on preclinical observations, FTPIs did not display the anticipated anticancer activity in prostate cancer. There is also a little doubt that these drugs actually inhibit FTPase activity in patients. Investigation is now focused on the search for farnesylated proteins that are required for disease pathogenesis and are strongly inhibited by the aforementioned drugs. Alternatively, the real targets of FTPIs might not be farnesylated but also geranylgeranylated species. Thus, geranylgeranyl transferase (GGTase) inhibitors and dual prenyl transferase inhibitors are under development. For example, recently a novel oral dual prenyl transferase inhibitor (AZD3409) showed significant *in vitro* anticancer potential in prostate cancer cells (49).

Raf Inhibitors

Given the high percentage of cancers with constitutively active Raf, various treatment strategies are being developed, such as antisense oligonucleotides and small-molecule kinase inhibitors. In addition, other therapeutics that indirectly target Raf are being evaluated, such as inhibitors of chaperone proteins stabilizing Raf and histone deacetylase inhibitors reducing *raf* expression (50, 51).

Antisense technology has been used for the development of Raf kinase selective inhibitors. The Raf kinase inhibitor ISI 5132 has been evaluated in phase I/II trials in HRPC patients and was found to lack significant effects on the prostate-specific antigen response and to bear a non-tolerable toxicity (52).

Several classes of low-molecular-mass compounds have been designed and evaluated for Raf inhibition. Besides Raf kinase blockade, these molecules can also inhibit a wide range of other kinases because of the structure homology between the kinase families. Although this multiple inhibitory effect of small-molecule drugs might be advantageous, it would also enhance their toxicity profile. The first orally active inhibitor of Raf kinase that has been most evaluated is BAY 43-9006 (sorafenib), which was found to be a potent competitive inhibitor of ATP binding in the catalytic domains of Raf-1 and wild-type and mutated B-Raf. Preclinical studies have suggested that BAY 43-9006 inhibits tumor progression by blocking cellular proliferation that is dependent on activation of the MAPK pathway and/or impairing tumor angiogenesis through interaction with vascular endothelial growth factor receptors 2 and 3, platelet-derived growth factor receptor, c-kit, Flt-3, and p38 (53). Many of these molecules have been implicated in the development and progression of HRPC. BAY 43-9006 has been assessed in phase I studies in patients with prostate cancer (54). The principal dose-related toxicities were diarrhea, vomiting, skin rash, fatigue, hypertension, and palmar-plantar erythrodysesthesia. Subsequent phase II/III studies had intriguing results, whereas the combination of sorafenib and chemotherapeutic agents has shown promising results in several solid tumors (13, 55). Clinical trials are now evaluating the effect of BAY 43-9006 either as single agent or in combination with chemotherapy in HRPC patients by using clinical and radiographic evidence for disease progression (55, 56). Apart from sorafenib, other small-molecule competitive inhibitors of the ATP-binding site of Raf proteins, such as L-779450 and SB2033580, are being developed and evaluated in preclinical and early clinical studies (2).

The benzoquinoid ansamycin antibiotics are derived from *Streptomyces hygroscopicus* and include geldanamycin and herbimycin derivatives. Ansamycins seem to have an antitumor effect based on their action against chaperone proteins, which are responsible for maintaining the active conformation of many proteins, among them Raf kinase (50). Although the effects of these agents on Raf kinase might not be specific, phase I clinical trials in patients with HRPC are under way.

MAPK Inhibitors

The rationale for targeting MEK is similar to that of targeting Raf, as it is a key molecule in the MAPK pathway and in signaling cascades in a plethora of solid tumors, including HRPC. The MEK inhibitors PD098059 and PD184352 were among the first small-molecule compounds that have been developed and tested in preclinical prostate tumor models (57, 58). However, only PD184352 (renamed CI-1040) displayed tumor growth inhibition *in vivo*. Whereas there were promising results of antitumor activity in phase I trials, these were not verified in phase II trials, and further development of this agent was terminated. PD0325901, which is structurally similar to CI-1040, was subsequently developed (4). Phase I trials are evaluating this new more potent MEK inhibitor in patients with prostate cancer. Similarly, the benzimidazole ARRY-142886 has been reported as a potential MEK inhibitor and is currently evaluated in phase I clinical trials (4). Differential regulation of the interaction between MAPKs can be also accomplished with selective inhibition by phosphatases, such as Cdc25A phosphatase (59).

Recently, it was shown that angiotensin II induces not only the phosphorylation of MAPKs but also the signal transducer and activator of transcription pathway in androgen-independent prostate cancer cells. Oral administration of angiotensin II receptor blocker has been found to inhibit the growth of prostate cancer xenografts in a dose-dependent manner (60). The role of these agents in prostate cancer therapeutics is currently under evaluation.

Consumption of dietary genistein (4,5,7-trihydroxyisoflavone) in the form of soy has been associated with lower rates of mortality from prostate cancer. Genistein exhibits activity in several preclinical model systems that relate to cancer prevention and is considered a potential cancer chemoprevention agent. A recent study showed that genistein hinders activation of p38 MAPK, thereby inhibiting processes closely linked to metastasis, and does so at concentrations associated with dietary consumption (61). Vitamin D also seems to exert its preventive action during prostate carcinogenesis through inhibition of p38 MAPK (62). Finally, it has been shown that growth-inhibitory concentrations of the dietary phytochemical resveratrol suppress EGFR-dependent ERK1/2 activation pathways stimulated by EGF and phorbol ester (12-*O*-tetradecanoylphorbol-13-acetate) in human androgen-independent prostate cancer cell lines (63). Thus, MAPK inhibitors might have also a role as chemoprevention agents in prostate carcinogenesis.

Future Perspectives of MAPK Network Targeting in Prostate Cancer

MAPK signaling cascades seem to play divergent roles in the prostate gland. Significant differences have been observed in the activation pattern of all MAPK network components in prostate epithelial and stromal cells, under normal and pathologic conditions. Modulation of MAPK pathways has been shown in several prostate cancer cell lines by growth factors, cytokines, and a variety of agents that control growth and apoptosis of prostate cancer cells. However, structure and function of MAPK signal transduction pathways have not been thoroughly defined in prostate carcinogenesis.

The prostate is a heterogeneous gland comprising several cell types, which regulate each other's function by paracrine mechanisms. Hence, it is important to decipher the role played by MAPK signal transduction pathways in mediating the interaction between various neighboring

prostate cell types. A diverse array of signaling cascades have been identified as activating elements upstream of the MAPK circuitry. In-depth elucidation of the transduction cascades that are specifically activated in normal prostate and in hormone-responsive and HRPC cells is fundamental in unveiling the selective targets for novel rational prostate cancer therapies.

Although Ras proteins have been intensively explored, the complex biology and contribution of other crucial proteins to the cellular pharmacology of FTPIs have probably been underestimated. For example, it has been observed that FTPIs induce apoptosis of cancer cells through inhibition of the phosphatidylinositol-3 kinase/Akt cascade, but Ras does not mediate this inhibition (64). Furthermore, it has been shown that RhoB, an important Ras effector, is both farnesylated and geranylgeranylated; thus, FTPIs would also affect its action (42). The encouraging results of FTPIs in preclinical models were not confirmed in the clinical setting, suggesting that these agents may not represent a promising approach in blocking the signal transduction through the Ras/MAPK pathway in prostate carcinogenesis. The potential of cross-prenylation and the ability of GGTase to restore the function of some Ras proteins whenever FTPase is inhibited seem to be of paramount importance.

Regarding Raf inhibitors, albeit the early results with BAY 43-9006 have been quite propitious, it does not seem to strengthen Raf as a very promising target, mainly due to its lack of specificity. Instead, the documented clinical activity in patients with renal cancer, its lack of activity as single-agent treatment in melanoma patients, and its ability to enhance the activity of chemotherapeutic drugs may reflect its greater potency at inhibiting vascular endothelial growth factor receptor or other pivotal signal-transducing molecules. Nevertheless, its future role in prostate cancer therapeutics has to be scrupulously investigated. MEK inhibitors seem to represent the highest selective MAPK inhibitors, as they target the more downstream effect of the Ras/MAPK pathway, and their clinical evaluation constitutes one of the major research priorities concerning prostate cancer therapeutics.

As with all molecularly targeted potential anticancer drugs, the question remains whether Ras/MAPK inhibitors could exert their optimal action as single-agent treatment or in combination with other signal transduction inhibitors. For instance, the role of GFRs, such as IGF-1R, in prostate cancer evolution is widely suggested (19). IGF-1R signaling promotes growth in many prostate tumor models. Current studies seek to quantify the presence of the IGF-1R in prostate cancer and to determine a preclinical rationale for therapy with agents such as nordihydroguareacetic acid, which are assessed in early clinical trials (65). Other members of the Ras/MAPK cascade, such as the Sos protein, might also represent attractive antitumor targets (Fig. 1). By disrupting the Ras-Sos interaction, one might expect that signaling through both Ras/MAPK and phosphatidylinositol-3 kinase/Akt pathways would be significantly impaired, thereby inhibiting both proliferation and survival of prostate cancer cells. Moreover, the ability of the Ras/MAPK pathway to regulate proliferation, differentiation, and survival seems to depend upon the amplitude and duration of the MAPK activation (29). Activation of the MAPK cascade has been correlated not only with enhanced progression through the G_1-S but also with the G_2-M transition, particularly following drug- or radiation-induced growth arrest. Recent evidence has also suggested that inhibition of the MAPK pathway sensitizes a broad range of tumor cells to the toxic effects of ionizing radiation and chemotherapeutic drugs (66). Thus, interference with MAPK signaling, and the cell cycle check points in particular, using small-molecule compounds such as the UCN-01, might represent promising strategies for activation of the cell death pathway in prostate cancer (67).

It has been postulated that the caveat of targeting intracellular signaling might be that the same regulatory modules are used in multiple functions; thus, drugs that impede these pathways might display widespread mechanism-induced toxicities. For example, the involvement of Ras/MAPK cascade in immune signaling has raised valid concerns about the potential toxicity of inhibitors directed against this pathway. However, preclinical studies have shown that the complete and unresolved ''switching off'' of the MAPK signaling output is a prerequisite for maximal therapeutic benefit in cancer patients (68).

An additional concern regarding evaluation of Ras/MAPK inhibitors in dose-dependent clinical studies is whether the aim would be a better pharmacodynamic end point than the classic study end point of clinical toxicity. The latter issue takes upfront once again the inherent problems that have to be addressed for the identification of the optimal biological dose of all these novel anticancer agents that hamper aberrant and cancer-specific proliferative and antiapoptotic pathways. These agents may be preferentially cytostatic and produce relatively minimal organ toxicity, compared with conventional chemotherapeutic drugs, making a profound discrepancy between the conventionally used maximum tolerated dose and the ideal optimal biological dose (69). Surrogate biomarkers might offer an alternative way of their activity evaluation.

Unlike the case for cytotoxic agents, patients who benefit from signal transduction therapeutics are likely to share common molecular alterations of the target or the intermediate pathway, such as the Ras/MAPK network (70). This means that a subset of patients with certain tumor molecular features will respond to a certain drug in a more consistent manner, than will an entire patient population of the same prostate cancer grade and stage. Therefore, it might be challenging to test these agents in patients based on specific biological profile of the tumor. Prostate cancer specimen analysis for specific biomarkers is of cardinal importance in the field of signal transduction therapy.

Concluding Remarks: Outlook

AR holds a critical role in prostate cancer evolution. Various cellular adaptation mechanisms that affect AR

signaling have been identified in prostate cancer, although their effect on patients' outcome has not yet been clarified. In HRPC, the signaling pathway of the AR remains functional. Novel treatment options that interfere with androgen signaling have been proposed in experimental systems, albeit their effectiveness in advanced prostate cancer remains to be further investigated.

Activation of Ras/MAPK signaling network is known to affect directly and/or indirectly AR activity. This multipartite network propagates chemical stimuli from the cell surface to the nucleus via sequential kinase signaling and intensive cross-talk. During prostate carcinogenesis, crucial components of this network are deregulated, thus affecting cellular proliferation, apoptosis, and metastasis. Various molecules of the Ras/MAPK network represent appealing selective targets for prostate cancer therapeutics, although several caveats have to be adequately addressed. The great pressure for new effective treatment options in prostate cancer patients should not surpass the necessity for careful, rationally designed randomized studies evaluating the most promising therapeutic pharmaceuticals. Molecularly ''tailored'' treatment of prostate cancer pathogenesis represents the most auspicious, albeit not yet possibly applicable, approach of chemoprevention and/or remedy of prostate neoplasms.

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