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REVIEW

Prostate Cancer

Personalized prostate cancer therapy based on systems analysis of the apoptosis regulatory network

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Targeting the androgen receptor axis provides only temporary relief for advanced prostate cancer, which often evolves into androgen-independent disease. The wide variety of signaling mechanisms connected with the pathophysiology of androgen-independent prostate cancer poses both conceptual and practical challenges for the design of efficient therapies. Analysis of apoptosis regulation in prostate cancer suggests the potential value of a systems approach that integrates information on the topology of the antiapoptotic signaling network, the signal transduction pathways that inhibit apoptosis, and the expression of proteins of the Bcl2 family. This approach could be used to identify patients most likely to respond to treatments with drugs that inhibit the signaling pathways controlling apoptosis.

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CASTRATION IS A MAINSTREAM THERAPY THAT EVENTUALLY FAILS

Androgen ablation therapy, introduced by Charles Huggins in 1941, remains the most effective systemic treatment for prostate cancer. In 90% of cases, the disease initially responds to androgen ablation therapy. However, after 2–3 years it eventually recurs as androgen-independent prostate cancer. Metastatic castration-resistant prostate cancer (CRPC) is characterized by poor prognosis and a median survival of 30 months.[1–3] Existing therapies with Radium 223, abirateron, taxanes, and immunotherapy with sipuleucelT only modestly extend overall survival.[4-6]

Since prostate epithelial cells depend on androgen for proliferation and survival, resistance to androgen ablation could be explained by aberrant activation of the androgen receptor (AR) axis. Several therapeutic strategies for complete androgen ablation have been developed, including combinations of gonadotropin-releasing hormone analogues (e.g. leuprolide) that cause continuous stimulation of the pituitary gland; abiraterone acetate (an inhibitor of androgen biosynthesis); and Enzalutamide (MDV3100), an AR antagonist that prevents binding of androgens to AR, nuclear translocation, and chromatin binding of AR. However, even with complete blocking of AR signaling, disease will eventually progress.[7,8] This progression could be explained by (1) persistent activation of AR-induced genes and derepression of AR-inhibited genes or (2) activation of alternative signaling pathways. These two possibilities may not be mutually exclusive and could be complementary.[3]

Several signaling mechanisms connected with AR-independent prostate cancer have been recently reviewed.[3] These include the constitutively active PI3-kinase (PI3K)/Akt and RAS/MAPK modules; overexpression of c-myc; deregulated transforming growth factor-β signaling that switches from inhibiting to supporting growth and survival of prostate cells; activation of WNT/β-catenin signaling that drives epithelial-mesenchymal transition; the insulin-like growth factor-1 pathway, known to be involved in proliferation, survival, and migration; activation of the fibroblast growth factor-1 pathway involved in prostate organogenesis and prostate cancer via paracrine mechanisms; hepatocyte growth factor/c-MET signaling, which is involved in cell growth, motility, and angiogenesis; and elevated caveolin CAV1, which is involved in multiple signaling pathways.

In the light of this evidence, it is becoming increasingly apparent that in addition to inhibiting the AR pathway, targeting AR-independent signaling mechanisms is necessary to extend survival of patients with CRPC.

APOPTOSIS IS THE BEST THERAPEUTIC TARGET

The wide palette of androgen-independent targets poses both conceptual and practical challenges. Cancer phenotypes have been classified into 6 and later 10 individual "hallmarks of cancer;" each is a manifestation of the tangled web of signals deregulated in cancer cells.[9,10] If every "hallmark" must be targeted pharmacologically, the possibility of successful therapy becomes rather remote. An opposing view presents all cancer "hallmarks" as a consequence of continuous proliferation and restricted apoptosis,[11,12] which suggests that targeting a few key "hallmarks" could bring about a cure. Following this logic, a focus on apoptosis presents several advantages:

- Inducing apoptosis eliminates the need to consider other signaling pathways: dead cells do not proliferate, migrate, or metabolize;
- The basic principles of apoptosis regulation are relatively well understood;

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- Short-term inhibition (6–24 h) of antiapoptotic mechanisms is sufficient to induce apoptosis, whereas continuous inhibition of proliferation, migration, or metabolism is needed to suppress corresponding hallmark phenotypes; and
- Induction of apoptosis is an evolutionarily refined (and thus, most effective) mechanism of cancer cell elimination.

REGULATION OF APOPTOSIS BY A NETWORK OF SIGNALLING PATHWAYS

Normal prostate epithelial cells undergo apoptosis after androgen levels are decreased.[13] Thus, apoptosis is a default pathway for normal prostate cells after androgen withdrawal. In contrast, androgen-independent prostate cancer cells remain viable in the absence of androgen. Androgen independence could be explained by defects in execution of apoptosis or by acquired antiapoptotic mechanisms that control the decision to undergo apoptosis. So far, the second proposition has been confirmed experimentally since all prostate cancer cell lines retain the ability to die by apoptosis.[27]

What is known about androgen-independent antiapoptotic signals, and how could they be targeted? Activation of the PI3K/Akt signaling pathway was one of the first signal transduction mechanisms shown to inhibit apoptosis in numerous cell types, including prostate cancer cells.[14,15] Activation of PI3K signaling occurs when external growth factors trigger the recruitment of PI3K to the plasma membrane, where it phosphorylates phosphatidylinositol at the 3-d position. Phosphatidylinositol 3 phosphate in turn engages serine/threonine protein kinases like PDK1 and Akt through binding to their PH domains, as well as the mammalian target of rapamycin complex2 (mTORC2). The phosphorylation of Akt at T308 by PDK1 and at S473 by mTORC2 is required for complete activation of Akt.[16] Akt in turn phosphorylates several substrates, including the BAD and transcription factors of FOXO family connected with apoptosis inhibition. PI3K signaling is negatively regulated by the lipid phosphatase PTEN, which dephosphorylates phosphatidylinositol 3 phosphate. Loss of PTEN phosphatase is one of the most common intrinsic mechanisms of constitutive activation of PI3K/Akt signaling that makes cells independent from extracellular stimuli.[17]

Studies of clinical samples and established prostate cancer cell lines have shown that a substantial proportion of prostate cancers have relied on PI3K signaling for their growth and survival. The loss of PTEN that leads to constitutive activation of the PI3K pathway has been documented in 30% of primary and 60% of androgen-independent metastatic cancers.[18] A prostate-restricted knockout of PTEN in mice triggers development of metastatic prostate cancer.[19] A recent comprehensive sequence analysis showed that almost all advanced prostate cancers possess mutations leading to increased activity of the PI3K pathway.[20]

Beside PI3K/Akt other signaling pathways were implicated in antiapoptotic signalling. The epidermal growth factor receptor (EGFR)/GRB2 signaling pathway was among the first linked to apoptosis inhibition.[21] In prostate cancer cells, activation of EGFR inhibited apoptosis induced by PI3K inhibitors.[14,22] Subsequently, at least two signal transduction pathways downstream of EGFR RAS/ERK and Rac/PAK were shown to inhibit apoptosis.[23] Other RTK and nonreceptor tyrosine kinases of the Src family also have been implicated in androgen-independent prostate cancer.[24] Yet another signaling pathway activated by the G-protein coupled receptor (GPCR) agonists vasoactive intestinal peptide and epinephrine that leads to activation of protein kinase A (PKA) was connected to inhibition of apoptosis (**Figure 1**).[25,26] This brief overview of androgen-independent signaling pathways that inhibit apoptosis illustrates the challenges that may complicate both analysis and selection of effective treatment.

TOPOLOGY OF THE ANTIAPOPTOTIC NETWORK

Analyses of apoptosis regulation in prostate cancer cell lines indicate that the decision for apoptosis to occur is made at the level of Bcl-family proteins. Thus, increased expression of antiapoptotic proteins (e.g. Bcl_{XL} in PC3 cells), loss of pro-apoptotic proteins (BAX in Du145 cells), or activation of signaling pathways that phosphorylate BAD and upregulate Mcl-1 have been reported.[27] These tissue culture experiments suggest that combined loss of Mcl-1 expression and BAD dephosphorylation triggers apoptosis in most prostate cancer cells.

Mcl-1 in prostate cells has a short half-life (~3 h). Comparable time is needed for complete dephosphorylation of BAD in PTEN-deficient cells treated with PI3K inhibitors. Thus, both BAD phosphorylation and Mcl-1 expression are subjects of dynamic regulation by antiapoptotic signals.[27]

Analysis of signaling pathways that inhibit apoptosis in prostate cancer cells reveals a complex network that converges on phosphorylation of BAD and regulation of Mcl-1 expression. Constitutive PI3K signaling leads to phosphorylation of BAD at S112 and S136 (amino acid numbers are based on the mouse sequence). Phosphorylation of S136 is mediated by Akt, whereas S112 does not match consensus sequence RxRxxS/T and is only partially Akt-dependent. EGF activates two parallel signaling pathways that converge on S112 and S136. S112 phosphorylation is mediated via the RAS/ERK module, although proximal S112 kinase that phosphorylates BAD remains at large. Phosphorylation of S136 is mediated by the Rac/PAK module. Yet another signaling pathway

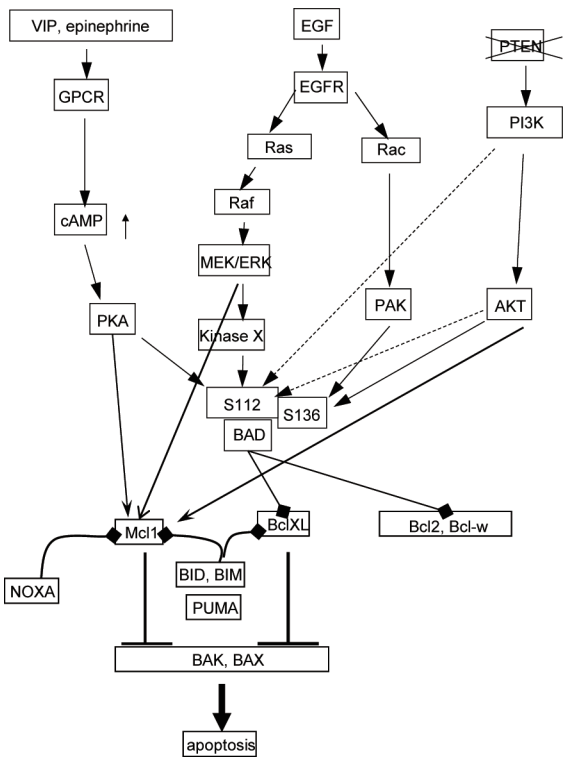

Figure 1: Topology of apoptosis regulatory network. Network of antiapoptotic signalling pathways where active PI3-kinase, receptor tyrosine kinases, and G-protein coupled receptors converge on BAD and Mcl-1. Expression pattern of the Bcl2 family of proteins determines whether BAD dephosphorylation and loss of Mcl-1 will induce rapid apoptosis.

activated by GPCR agonists leads to PKA-dependent phosphorylation of BAD at S112 (**Figure 1**).[23,25,26]

Experiments with inhibitors of protein synthesis have shown that the half-life of Mcl-1 in prostate cells could be extended by signals from PI3K and GPCR pathways. Information about the topology of the apoptosis regulatory network identifies BAD phosphorylation and Mcl-1 expression as readouts of activation of upstream signaling pathways, which could be used as predictors of the efficacy of therapies that target these pathways.

A SYSTEMS APPROACH IS NEEDED TO IDENTIFY PATIENTS WHO WILL RESPOND TO INHIBITORS OF APOPTOSIS REGULATORY NETWORK

Substantial advances have been made in developing inhibitors of signaling pathways that control protein synthesis and phosphorylation. Several inhibitors of PI3K, EGFR, and GPCR antagonists, and the general protein synthesis inhibitor omacetaxine have been approved for clinical use.[28,45] Therefore, targeting signaling pathways that control BAD phosphorylation and Mcl-1 expression is a realistic strategy for inducing apoptosis in androgen-independent prostate tumors.

However, clinical trials of kinase inhibitors in prostate cancer brought disappointing results: no reports on extended patient survival were published after several clinical trials of inhibitors of PI3K and receptor tyrosine kinases.[29–36] Two possible explanations for these results are the ineffectiveness of inhibitors against their intended targets or existence of compensatory mechanisms that alleviate the intended noxious effects on cancer cells.

Doses of kinase inhibitors are limited by toxicity because signal transduction pathways are not unique for cancer cells and play important roles in normal physiology. To achieve more efficient inhibition of target enzymes in the prostate gland, and reduce toxicity for other tissues, two strategies were designed. The first was based on generation of latent prodrugs that could be activated in prostate tumors by prostate-specific antigen (PSA) protease.[37–41] This approach could be used to increase prostate tumor selectivity of kinase inhibitors, as was recently tested with the PI3K inhibitor LY294002.[41] The second approach is based on the generation of chimera toxins with antibodies that recognize prostate-specific membrane antigen (PSMA).[42,43,46] Combinations of PSMA-targeted bacterial toxins that inhibit protein synthesis, and latent PSA-activated kinase inhibitors could accomplish simultaneous inhibition of Mcl-1 expression and BAD phosphorylation in a prostate-selective fashion (**Figure 2**).

Development of prostate-specific therapies is justified because most patients with advanced prostate cancer are older men, for whom preservation of the prostate gland is a low priority. Optimal therapeutic doses should be based on monitoring kinase inhibition within tumors, which may require repeated biopsies or development of probes for noninvasive monitoring of kinase activity. Currently, inhibitor efficacy is measured by surrogate markers. Thus, efficacy of EGFR inhibitors is tested by analyzing phosphorylation of EGFR in hair follicles,[44] and inhibitors of PI3K are tested by analyzing Akt phosphorylation or FDG-glucose uptake.[47] However, new information about the topology of antiapoptotic signaling network in prostate cells suggests that phosphorylation of BAD and Mcl-1 expression also should be used to predict whether inhibiting upstream kinases will lead to induction of apoptosis.

Experiments in a panel of prostate cancer cell lines showed that efficacy of apoptosis induction depends on expression of other proteins of Bcl2 family. For example, overexpression of Bcl_{XL} or loss of BAX decreases sensitivity to apoptosis. Conversely, knocking down Bcl_{XL} or restoring BAX expression sensitizes cells to apoptosis.[27] Thus, even

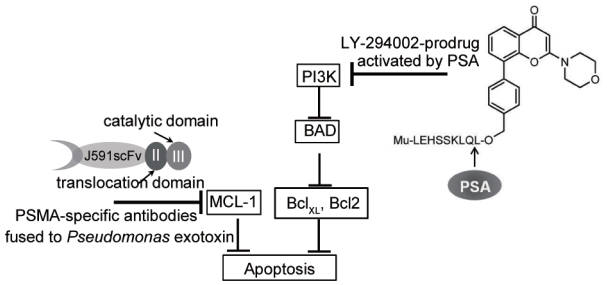

Figure 2: Prostate-selective therapeutics designed to induce rapid apoptosis by dephosphorylating BAD and downregulating Mcl-1. Inhibition of PI3-kinase with latent inhibitor activated by prostate-specific antigen protease leads to dephosphorylation of BAD. This allows BAD to bind and neutralize antiapoptotic proteins Bcl_{XL} and Bcl2, but not Mcl-1. Mcl-1 protein is characterized by rapid turnover. Chimera of antigen binding domain of J591PE antibodies against prostate-specific membrane antigen and translocation and catalytic domains of *Pseudomonas aeruginosa* exotoxin selectively inhibits protein synthesis in prostate cells and downregulate Mcl-1 expression. Combined loss of BAD phosphorylation and Mcl-1 expression induces rapid apoptosis in PTEN-deficient prostate cancer cells.[27,43]

successful inhibition of antiapoptotic signaling pathways that decrease Mcl-1 expression and BAD phosphorylation will induce apoptosis only in a subset of prostate tumors.

Hence, the following strategies could be suggested for selecting patients most likely to respond to inhibitors of antiapoptotic pathways: (1) examine expression of Bcl2 proteins to identify tumors that will respond by apoptosis to BAD dephosphorylation and loss of Mcl-1 expression; (2) determine whether inhibitors block intended signaling pathways; and (3) determine whether BAD is dephosphorylated and Mcl-1 is downregulated upon treatment with inhibitors.

Most information on apoptosis and signal transduction in prostate cancer has been gathered from experiments in cell lines. In well-defined tissue culture conditions, defined signaling pathways are activated by purified ligands. Yet *in vivo*, cancer cells are exposed to a plethora of endocrine signals and signals from the tumor microenvironment. As a result, redundant mechanisms that control BAD phosphorylation and Mcl-1 expression could contribute to inhibiting apoptosis. Therefore, systems analysis of multiple signaling pathways and Bcl2-family proteins should be combined with modeling of the network of apoptosis regulatory signals. Such modeling will help identify tumors and patients who will respond to inhibitors of antiapoptotic signals.

A wide armamentarium of signal transduction inhibitors has been developed over last 20 years. These inhibitors could be further improved by targeting with PSMA-specific antibodies and by converting inhibitors into latent pro-drugs activated by PSA. Prostate-selective inhibitors could be used to test whether targeting the apoptosis regulatory network will extend lives of patients with advanced prostate cancer. The main challenge is to develop techniques for analyzing the apoptosis regulatory network in prostate tumors that will help to select patients who will respond to treatments that inhibit BAD phosphorylation and Mcl-1 expression.

COMPETING INTERESTS

The author declares no competing interests.

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