MINIREVIEW

Pharmacological Targeting of the Integrated Protein Kinase B, Phosphatase and Tensin Homolog Deleted on Chromosome 10, and Transforming Growth Factor-β Pathways in Prostate Cancer

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Received October 26, 2008; accepted December 2, 2008

ABSTRACT

Prostate cancer is a highly heterogeneous disease in which a patient-tailored care program is much desired. Central to this goal is the development of novel targeted pharmacological interventions. To develop these treatment strategies, an understanding of the integration of cellular pathways involved in both tumorigenesis and tumor suppression is crucial. Of further interest are the events elicited by drug treatments that exploit the underlying molecular pathology in cancer. This review briefly describes the evidence that suggests integration of three established pathways: the tumorigenic phosphoinositide 3-kinase/protein kinase B (AKT) pathway, the tumor suppressive phosphatase and tensin homolog deleted on chromosome 10 pathway, and the tumor suppressive transforming growth factor-β pathway. More importantly, we discuss novel pharmaceutical agents that target key points of integration in these three pathways. These new therapeutic strategies include the use of agents that target iron to inhibit proliferation via multiple mechanisms and suppression of AKT by cytosolic phospholipase A2 inhibitors.

Prostate cancer is the most commonly diagnosed noncutaneous cancer in men and is the second leading cause of death (Assinder and Nicholson, 2007). It is estimated that approximately 186,000 new cases of prostate cancer will be diagnosed in the United States during 2008, accounting for approximately 22,000 deaths (available from the American Cancer Society, http://www.cancer.org).

Radical prostatectomy (surgical removal) is the most common treatment strategy for organ-confined tumors (Steineck et al., 2002). Although this procedure has a 10-year survival of 60%, surgery has a 2% mortality rate, 70% of patients develop erectile dysfunction, and 50% have urine leakage with 2 to 5% of patients being left incontinent (Steineck et al., 2002). The most common treatment option for advanced metastatic prostate cancer is androgen deprivation either by surgical or chemical castration. However, in the majority of patients, the cancer becomes insensitive to androgen deprivation, leading to relapse, and they inevitably die from androgen-independent metastatic prostate cancer (Hussain and Dawson, 2000). Thus, improved treatment options are desperately needed.

The work was supported by the National Health and Medical Research Council [Grant 400939] of Australia and the Australian Research Council [Grant DP0773027].

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.

doi:10.1124/mol.108.053066.

ABBREVIATIONS: PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; AA, arachidonic acid; COX, cyclooxygenase; cPLA2α, cytosolic phospholipase A2α; DFO, desferrioxamine; Dp44mT, di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone; GSK3β, glycogen synthase kinase 3β; HIF-1α, hypoxia inducible factor-1α; LOX, lipooxygenase; mTOR, mammalian target of rapamycin; NDRG1, N-myc downstream regulated gene-1; PIP2, phosphatidylinositol-4,5-bisphosphate; PGE2, prostaglandin E2; EGR-1, early growth response gene; siRNA, small interfering RNA; MK886, 3-(1-(4-chlorobenzyl)-3-t-butylthio-5-isopropylindol-2-yl)-2,2-dimethylpropanoic acid; 311, 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone.
Key to developing improved pharmacological interventions for prostate cancer is an understanding of the integration of pathways known to be involved in the pathophysiology of prostate cancer. This review 1) briefly describes the tumorogenic phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, the phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and transforming growth factor β (TGF-β) tumor suppressive pathways; 2) examines current evidence for the integration at key points of these pathways; and 3) discusses novel pharmaceutical agents that target these key points.

Biochemical Pathways Known to Play a Role in Prostate Cancer

The PI3K/AKT Pathway. A high level of activated (phosphorylated) AKT (pAKT) is correlated with poor prognosis of prostate cancer, whereas in normal prostate tissue, pAKT is undetectable (Wegiel et al., 2008). It is estimated that 30 to 40% of solid tumors have constitutively activated pAKT (pAKT) is correlated with poor prognosis of these key points.

Activation of a tyrosine kinase receptor, such as the insulin-like growth factor receptor, activates phosphatidylinositol-3 kinase (PI3K) (Fig. 1A). Thereafter, PI3K catalyzes phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) to form phosphatidylinositol-3,4,5-triphosphate (PIP3; Manning and Cantley, 2007). PIP3 acts as a platform and brings AKT in close proximity to 3-phosphoinositide-dependent kinases that subsequently phosphorylate and activate AKT. Activated AKT results in signaling cascades involving various downstream signaling proteins (Gao et al., 2003; Fig. 1A).

Activated AKT affects many targets and can inhibit glycogen synthase kinase 3β (GSK3β; Fig. 1A), which normally prevents up-regulation of cellular proliferation due to increased cyclin D1 degradation and suppression of expression, possibly via cytosolic (free) β-catenin (de la Taille et al., 2003; Lee et al., 2007). An excessive rate of cyclin D1 production promotes cell cycle progression (Chen et al., 1998) as a result of increased levels of cyclin D1/cyclin-dependent kinase 4 complexes that promote G1/S progression. On reaching appropriate levels, these complexes phosphorylate retinoblastoma protein and cause the dissociation of the E2F transcription factor, which heralds the progression of the cell cycle from G1 to S phase (Yu et al., 2007).

Another key effector downstream of pAKT is the serine/threonine kinase known as the mammalian target of rapamycin (mTOR; Fig. 1A) (Vignot et al., 2005). It plays a central role in protein translation, cell proliferation, and evasion of apoptosis (Vignot et al., 2005). Activation of AKT also helps to evade apoptosis directly by phosphorylation and inactivation of the proapoptotic protein, Bad (Grünewald et al., 2002).

The PTEN Pathway. Fifty percent of prostate cancers display a loss of PTEN (Facher and Law, 1998). Re-expression of normal PTEN in prostate cancer cell lines causes apoptosis (Davies et al., 1999; Sharrard and Maitland, 2000). Furthermore, Ptten-null mice have increased numbers of tentative prostate stem cells and develop prostate cancer with pathological changes similar to human disease (Wang et al., 2006). The major tumor suppressive activity of PTEN is via antagonism of the AKT pathway by dephosphorylation of PIP3, converting it back to PIP2 (Cantley and Neel, 1999). PTEN thus modulates AKT signaling, with opposite effects from PI3K on cell proliferation and survival (Fig. 1B).

Interaction of PTEN with p53 also plays a significant role in tumor suppression (Fig. 1B) (Stambolic et al., 2001). PTEN suppresses the expression of the p53 repressor MDM2 (Mayo et al., 2002). Furthermore, nuclear PTEN interacts directly with p53 to enhance p53-mediated cell cycle arrest and apoptosis in prostate cancer cells (Mayo et al., 2002; Chang et al., 2008) (Fig. 1B).

The Tumor-Suppressive TGF-β Pathway. TGF-β acts on normal prostate epithelial cells and some prostate cancer cell lines to inhibit proliferation and induce apoptosis (Wilding, 1991; Sutkowski et al., 1992). In prostate cancer specimens, it is common to find down-regulation of TGF-β receptors (Shariat et al., 2004). In particular, down-regulation of the type II receptor is associated with aggressive tumors (Shariat et al., 2004).

TGF-β signaling is activated by binding to the TGF-β receptor II and subsequent recruitment and phosphorylation of TGF-βRI (Shi and Massagué, 2003) (Fig. 1C). This activates a SMAD signaling cascade (Ten Dijke et al., 2002), resulting in the up-regulation of cyclin-dependent kinase inhibitors (Guo and Kyriianou, 1998; Li et al., 2006) and down-regulation of c-Myc (Massagué et al., 2000), leading to suppressed cellular proliferation (Fig. 1C). Apoptosis is promoted by TGF-β-induced expression of proapoptotic Bax, down-regulation of antiapoptotic Bcl-2, and increased expression and activation of the effector caspases (Guo and Kyriianou, 1999). Additional apoptotic cross-talk with the androgen receptor also occurs depending on the mutational status of this protein (Shariat et al., 2004; ZHU et al., 2008).
Interactions of the PI3K/AKT, PTEN, and TGF-β Pathways: Implications for Pharmacological Targeting

TGF-β and PTEN Integration Act to Oppose AKT. pAKT suppresses TGF-β-activated SMAD-signaling (Fig. 1), whereas PTEN inhibits AKT suppression (Song et al., 2003). In turn, TGF-β induces PTEN expression through its classic SMAD-dependent pathway (Fig. 1C) and stimulates a tumor-suppressive response (Chow et al., 2007). Considering this, it is known that iron chelation up-regulates TGF-β (Yoon et al., 2002), and this may potentially increase PTEN levels, although further studies are needed to investigate this. As mentioned above, SMAD activation suppresses oncogenic c-Myc (Massagué et al., 2000) and Bcl-2 expression (Guo and Kyriianou, 1999) but increases expression of the cyclin-dependent kinase inhibitors p15, p21, and p27 (Fig. 1) (Guo and Kyriianou, 1998; Li et al., 2006). However, AKT activation has the opposite effect (Vignon et al., 2005) (Fig. 1).

The Metastasis Suppressor N-myc Downstream Regulated Gene-1: A Possible Common Point of AKT, PTEN, and TGF-β Integration. It has been shown that expression of both the N-myc downstream regulated gene-1 (NDRG1) and PTEN are repressed in prostate tumors (Bandyopadhyay et al., 2004b). Forced expression of PTEN up-regulates NDRG1 expression in the prostate cancer cell lines PC-3 and DU145 via suppression of the AKT pathway (Bandyopadhyay et al., 2004a) (Fig. 2). It is therefore hypothesized that AKT activation acts to inhibit NDRG1 expression, whereas PTEN antagonizes this effect (Fig. 2).

Up-regulation of NDRG1 expression after hypoxia or cellular Fe-depletion is driven, at least in part, by hypoxia inducible factor-1α (HIF-1α) (Le and Richardson, 2004), which stimulates NDRG1 expression (Kovacevic and Richardson, 2006). It is noteworthy that increased HIF-1α levels during hypoxia correlate with increased TGF-β expression (Berger et al., 2003), mediating further potential integration between the AKT, PTEN, and TGF-β pathways. In fact, as already discussed, there is up-regulation of TGF-β after iron chelation (Yoon et al., 2002) that may act to increase PTEN, which subsequently could up-regulate NDRG1 expression. PI3K/AKT and PTEN have completely opposite effects on the activity of HIF-1α (Emerling et al., 2008). PTEN inhibits the transcriptional activity of HIF-1α, whereas AKT induces HIF-1α transcriptional activity via suppression of the fork-head transcription factor FOXO3a (Emerling et al., 2008). This effect of tumor-suppressive PTEN inhibiting HIF-1α activity can be seen as both logical and counterintuitive. For instance, the PTEN-mediated decrease in HIF-1α would lead to the appropriate decrease in expression of one of its classic targets, vascular endothelial growth factor 1, that promotes angiogenesis and tumor progression. However, paradoxically, PTEN would also potentially lead to decreased expression of the metastasis suppressor, NDRG1, which can be up-regulated by a HIF-1α-dependent mechanism (Le and Richardson, 2004). However, NDRG1 can also be up-regulated by an HIF-1α independent process (Le and Richardson, 2004), and it is probable that the regulation of this metastasis suppressor is complex. Moreover, it may be that HIF-1α serves more than one function, and this needs to be examined in detail. Indeed, overexpression of HIF-1α has been found in various cancers, and down-regulation of this molecule is currently evaluated as a therapeutic target for cancer treatment (Greenberger et al., 2008; Groot et al., 2008; Yasui et al., 2008).

The Arachidonic Acid and Eicosanoid Signaling Pathway: A Possible Regulator of AKT and PTEN. Arachidonic acid (AA) is an ω6 polyunsaturated essential fatty acid (Burr and Burr, 1929). As such, it cannot be synthesized by cells de novo. Rather, it is obtained from the diet or synthesized from its precursor linoleic acid (Brenner, 1974). In resting cells, AA is stored within the phospholipid bilayer of the cell membrane (Hughes-Fulford et al., 2001). Stimulation of cells by a number of diverse agonists leads to the activation of intracellular phospholipases, namely cytosolic phospholipase A2α (cPLA2α), that then releases AA from the cell membrane (Clark et al., 1995; Niknami M et al., 2008). When AA is released, it can either become reincorporated into the membrane phospholipid bilayer, diffuse out of the cell, or be metabolized via the lipooxygenase (LOX) or cyclooxygenase (COX) enzymes to eicosanoids such as prostaglandin E2 (PGE2) (Fig. 2; Niknami et al., 2008).

Previous studies have demonstrated that the eicosanoid synthetic rates from labeled AA are significantly higher in malignant than in benign prostate tissue, suggesting an increased AA flux through the COX and LOX pathways (Chaudry et al., 1994; Faas et al., 1996). Eicosanoids can contribute to cancer progression by promoting cell proliferation, motility, invasion, and angiogenesis (Nie et al., 1998, 2001; Pidgeon et al., 2002; Pörkka and Visakorpi, 2004). Other evidence also supports a role for eicosanoids in prostate carcinogenesis. For example, eicosanoid inhibitors effectively reduce the size of prostate cancer xenografts (Hsu et al., 2000). Treatment of the androgen-independent human prostate carcinoma cell line, PC-3, with linoleic acid stimulates growth (Tjandrawinata et al., 1997). Furthermore, a study by Hughes-Fulford and associates (2001) demonstrated that linoleic acid, arachidonic
acid, and PGE2 all stimulate prostate tumor growth and alter gene expression in PC-3 cells. In mice, diets high in corn oil (hence rich in the AA precursor linoleic acid) markedly stimulate the growth of human prostate cancer xenografts (Wang et al., 1995). Together, these studies suggest a stimulatory effect of dietary ω6 fatty acid on prostate tumor cell growth, which may be critical for the development and progression of cancer.

In humans, it was shown that an increase in serum prostate-specific antigen levels after prostatectomy or radiotherapy was slowed in patients treated with COX-2 inhibitors (Pruthi et al., 2006; Smith et al., 2006). However, an increased risk of thrombosis was found in patients treated with COX-2 inhibitors as a result of the sparing effect on COX-1 (Marcus et al., 2002). In addition, COX-2 inhibitors inhibit the production of the vasodilator prostacyclin with no effect on the synthesis of the vasoconstrictive thromboxane A2, thus potentially tipping the balance toward vasoconstriction and thrombosis (Funk and FitzGerald, 2007).

In the past few years, it has become clear that AA and/or eicosanoids can also lead to increased AKT activation. In pancreatic cancer cells, AA and/or eicosanoids can augment AKT activation indirectly by increasing the inactivation of PTEN by oxidation (Covey et al., 2007; Fig. 2). Alteration of PLA2 can directly regulate PI3K/AKT in the prostate (Hsu et al., 2000; Patel et al., 2005; Hughes-Fulford et al., 2006) and vascular smooth muscle cells (Li and Malik, 2005a,b; Wilkens and Freeman, 2006). Inhibition of COX-2 by celecoxib has been shown to induce apoptosis in both androgen-responsive LNCaP and androgen-unresponsive PC-3 cells by blocking the production of COX-2 and increasing the synthesis of the vasoconstrictive thromboxane A2, thus potentially tipping the balance toward vasoconstriction and thrombosis (Funk and FitzGerald, 2007).

Pharmacological Targeting of the Integrated AKT, PTEN, and TGF-β Pathways

Prostate cancer accounts for significant morbidity and mortality with current therapies being far from adequate. Therefore, new therapeutic approaches are essential. Because the tumorigenic AKT and tumor-suppressive PTEN and TGF-β pathways have potential and established points of integration, this indicates the possibility of specifically targeting these pathways (Fig. 2). In this section, we describe novel pharmacological agents that have been shown to do this. First, we describe the development of iron chelators that act on a number of molecular targets, including NDRG1, cyclin D1, and TGF-β. Second, we examine the suppression of AKT by cytosolic phospholipase A2-α inhibitors.

Targeting Iron for Inhibition of Tumor Growth. Iron is vital for life, because it is an essential component of many proteins and enzymes that are involved in cell growth and replication (Bohnsack and Hirschi, 2004; Dunn et al., 2007). For example, iron is involved in the rate-limiting step of DNA synthesis catalyzed by ribonucleotide reductase (RR) (Jordan and Reichard, 1998). In addition, without iron, cells cannot proceed from the G1 phase to the S phase of the cell cycle (Yu et al., 2007). Depleting cells of iron by chelators such as desferrioxamine (DFO) results in cell cycle arrest (Buss et al., 2003; Kalinowski and Richardson, 2005).

Iron is transported by transferrin (Tf), which binds to the transferrin receptor 1 (TfR1) and donates its iron to cells (Richardson and Ponka, 1997). TfR1 is expressed at high levels in tumor cells, reflecting their greater need for iron than normal cells (Larrick and Cresswell, 1979; Sutherland et al., 1981; Richardson and Baker, 1990). The importance of TfR1 and iron uptake in growth is shown by the ability of the anti-TfR1 monoclonal antibody 426 to inhibit cancer cell growth by blocking Tf-binding to TfR1 and preventing iron uptake (Trowbridge and Lopez, 1982). Furthermore, tumor cells express higher levels of the iron-containing enzyme, RR, which is a crucial target for chelators (Nyholm et al., 1993; Cooper et al., 1996). The greater level of RR in cancer cells explains, at least in part, their greater sensitivity to iron chelators than normal cells (Elford et al., 1970; Richardson, 2002). Indeed, the cytotoxic agent hydroxyurea acts on RR via a mechanism independent of iron chelation, showing its utility as a useful target. However, hydroxyurea has limited potency because of its short half-life, low affinity for RR, and the fact that tumor cells develop resistance to it (Green et al., 2001; Richardson, 2002).

Iron Chelators: A Novel Class of Antitumor Agents. Many in vitro and in vivo investigations and clinical trials have shown clearly that iron chelators are effective antitumor agents (Buss et al., 2003; Kalinowski and Richardson, 2005). The classic iron chelator, DFO (Fig. 3A), that is used for treating iron overload is the best studied in terms of its antitumor efficacy (Kalinowski and Richardson, 2005; Richardson et al., 2008). However, because of its limited membrane permeability and short half-life, DFO has shown modest antitumor activity (Richardson et al., 1994; Kalinowski and Richardson, 2005). There has been continuing efforts to improve the efficacy of iron chelators as cancer treatments. Indeed, the potential of iron chelators to act as antitumor

Fig. 3. Structures of iron chelators discussed in this review. DFO and Triapine are both chelators examined in clinical trials for their antitumor activity (Kalinowski and Richardson, 2005; Kalinowski et al., 2007). Development of novel chelators that improve upon the efficacy of DFO led to the arylhydrazone 311. Thereafter, chelators of the DpT series were generated, including Dp44mT, which markedly inhibits tumor growth and the control chelator dipyridyldiketone 2-methylthiosemicarbazone (Dp2mT), which does not bind iron. Further development of these chelators resulted in synthesis of the effective BpT series of ligands (Kalinowski et al., 2007).
agents was confirmed by the entrance of the chelator Triapine into clinical trials (Yu et al., 2006; Fig. 3A).

**DpT and 2-Benzoylpyridine Thiosemicarbazone Chelators Possess Marked and Selective Antitumor Activity.** The development of novel aryloxazoline chelators such as compound 311 (Fig. 3A) have resulted in compounds that show far greater antitumor and iron chelation efficacy than DFO (Richardson et al., 1995; Richardson and Milnes, 1997; Darnell and Richardson, 1999). More recently, novel chelators of the DpT class have been generated (Fig. 3A) (Lovejoy and Richardson, 2002; Becker et al., 2003; Yuan et al., 2004; Richardson et al., 2006; Whitnall et al., 2006). Some of these chelators are far more effective than DFO at entering cells, inducing cellular iron efflux, and preventing iron uptake from Tf (Yuan et al., 2004). A control DpT analog known as Dp2mT (Fig. 3A) that does not bind iron has also been synthesized and demonstrates that the mechanism of activity of these compounds is due to their ability to bind metal ions (Yuan et al., 2004).

Studies in vivo showed that dipyridylketone 4,4-dimethylthiosemicarbazone (Dp44mT) significantly decreased tumor weight in mice bearing the chemotherapy-resistant lung carcinoma to 47% of the control after only 5 days (Yuan et al., 2004). It is significant that no changes in animal weight or hematological indices were found, demonstrating that the chelator acts selectively to inhibit tumor growth (Yuan et al., 2004). In addition, Dp44mT has been shown to be highly effective in vivo using a panel of human tumor xenografts and a wide range of cultured tumor cells in vitro (Whitnall et al., 2006). It is important to note that little or no toxicity was seen in normal tissues at optimal doses.

More recent studies have resulted in the development of the 2-benzoylpyridine thiosemicarbazone (BpT) class of chelators (Fig. 3B), which show a general increase in antitumor activity relative to the DpT group of ligands (Kalinowski et al., 2007). In these compounds, the noncoordinating 2-pyridyl group of the DpT ligands is replaced with a phenyl ring (Kalinowski et al., 2007), which increases the lipophilicity of the molecule that probably allows greater access to the tumor microenvironment (Tredan et al., 2007). These chelators show selectivity against tumor cells being far less active in normal fibroblasts (IC50 value, 1.86 to >6.25 μM; Kalinowski et al., 2007). In addition, relative to the DpT ligands, the BpT series shows a marked increase in redox activity that is vital for antitumor efficacy (Kalinowski et al., 2007).

**Iron Chelators Affect Multiple Molecular Targets.** Studies have revealed that the successful antitumor activity of iron chelators is due to their effects on multiple molecular targets (Le and Richardson, 2004; Fu and Richardson, 2007; Nurtjahja-Tjendraputra et al., 2007). These include the classic target RR (Le and Richardson, 2002; Richardson, 2005) and their ability to 1) up-regulate the iron-regulated metastasis suppressor NDRG1 (Le and Richardson, 2004; Kovacevic et al., 2008); 2) prevent iron uptake from transferrin in vitro (Yuan et al., 2004); 3) increase iron efflux from cells (Yuan et al., 2004); and 4) affect the expression of molecules involved in cell cycle progression that can inhibit proliferation and lead to apoptosis (e.g., p53, cyclin D1, p21\(^{WAF1/CIP1}\)) (Liang and Richardson, 2003; Yuan et al., 2004; Fu and Richardson, 2007). It is noteworthy that the novel mechanism of action of these drugs means that they overcome resistance to established chemotherapeutics (Whitnall et al., 2006).

**Iron Chelators Up-Regulate the Growth and Metastasis Suppressor NDRG1.** As described above, NDRG1 expression is up-regulated by hypoxia (Le and Richardson, 2004). Several studies have also shown that NDRG1 is up-regulated by iron depletion (Le and Richardson, 2004; Dong et al., 2005). The transcription factor HIF-1α is expressed in response to hypoxia and iron depletion and has been found to up-regulate NDRG1 (Cangul, 2004; Le and Richardson, 2004). However, the up-regulation of NDRG1 by iron-depletion is mediated by both HIF-1α-independent and -dependent mechanisms (Le and Richardson, 2004).

It has been shown that iron depletion using chelators stabilizes HIF-1α by inhibiting the activity of prolyl hydroxylase that acts to degrade this transcription factor (Ivan et al., 2001; Jaakkola et al., 2001). More recently, in cancer cell types, it has been suggested that DFO up-regulates COX-2 through an extracellular signal-regulated kinase signaling mechanism (Tanji et al., 2001; Woo et al., 2006). The elevated activity of COX-2 is believed to increase HIF-1α (Woo et al., 2006). It is of interest that TGF-β1 increases HIF-1α protein stability by decreasing HIF-1α-associated prolyl hydroxylase through the SMAD signaling pathway (McMahon et al., 2006). This observation follows the intriguing finding by others that TGF-β1 is up-regulated by DFO (Yoon et al., 2002), and this may lead to increased PTEN that inhibits the AKT pathway. As described above, PTEN and PI3K/AKT have completely opposite effects on the activity of HIF-1α and hence probably also NDRG1 expression (Emerling et al., 2008).

It is of interest that DFO has been shown to inhibit pAKT in the premalignant keratinocyte cell line HaCaT (Faurouch and Gniadecki, 2008). In contrast, DFO was shown recently to increase the phosphorylation status of AKT and its targets FoxO1 and GSK3β in HepG2 hepatoma cells (Dongiovanni et al., 2008). It remains to be determined whether the up-regulation of NDRG1 by iron chelation in cancer cells is influenced by the inhibition of pAKT. It has also been shown that the early growth response gene (EGR-1) transcription factor mediates the expression of NDRG1 in response to hypoxia and iron depletion in some cells through direct binding to the NDRG1 promoter (Zhang et al., 2007). In addition, it was found recently that EGR-1 regulates the HIF-1α gene during hypoxia (Sperandio et al., 2008), suggesting another mechanism that may modulate NDRG1 expression. There seems to be many points of integration between pathways that act to regulate NDRG1 and its metastasis suppressor function. However, increased expression of EGR-1 is found in prostate cancer cells (Thigpen et al., 1996), suggesting an alteration of the coupling mechanism between EGR-1 and NDRG1. Thus, the role of EGR-1 in the up-regulation of NDRG1 and HIF-1α and the link between the three proteins clearly need to be investigated further, particularly in the context of prostate cancer. Because many studies have shown that the up-regulation of NDRG1 plays a vital role in preventing metastatic spread in cancer (Bandyopadhyay et al., 2003, 2004a,b), the up-regulation of NDRG1 by iron chelators is of significance. In summary, novel iron chelators show great promise as future anticancer agents through their effect on multiple targets such as the metastasis suppressor NDRG1.

**Targeting Arachidonic Acid and Eicosanoid Formation for Suppression of Tumor Growth.** The phospholipases can be classified into four classes on the basis of their nucleotide and amino acid sequence homology (Patel et al., 2008a). These enzymes are differentially expressed in a tis-
of prostate cancer, there is a clear loss of the cellular cPLA2-α inhibitors annexin A1 and A2 (Pawel et al., 2000; Chetcuti et al., 2001), suggesting that increased activity of these enzymes may be important in neoplasia.

Rationale for Targeting cPLA2-α. Inhibition of AA metabolism has become an attractive new target for treating cancer (Cummings, 2007). Because the cleavage of AA by cPLA2-α could be the rate-limiting step in eicosanoid synthesis, cPLA2-α is a strong candidate to target in the treatment of prostate cancer. Considering the role of AA signaling in promoting mutagenesis, mitosis, angiogenesis, and metastasis, it can be reasoned that a better outcome may be achieved with cPLA2-α inhibitor(s) than COX or LOX inhibitors alone (Fig. 4). This is because the latter approaches only suppress the production of COX and LOX metabolites (Figs. 2 and 4). In contrast, blockade of the cPLA2-α enzyme will decrease the supply of substrate to all eicosanoid-producing enzymes (Fig. 4). For this same reason, inhibition of cPLA2-α should not induce the side effects of COX-2 selective inhibitors such as thrombosis.

The Efficacy of Targeting cPLA2-α in Prostate Cancer Cells In Vitro and In Vivo. In an effort to assess the potential of cPLA2-α as a therapeutic target in prostate cancer, the expression levels of cPLA2-α have been examined in prostate cancer cell lines (Patel et al., 2008b). In these studies, androgen-sensitive LNCaP cells expressed less cPLA2-α mRNA and protein than the androgen-independent PC-3 cell line. Comparison was made by examining the immunostaining of phospho-cPLA2-α (Ser505) in androgen-sensitive prostate cancer with samples from the same patient once they had reached androgen-independent status. This latter investigation demonstrated a clear further increase in phospho-cPLA2-α staining intensity in cells that displayed androgen-independence. When cPLA2-α mRNA was silenced with siRNA, there was a decrease in cell proliferation and increase in apoptosis in LNCaP and PC-3 cells. Similar to the results obtained using siRNA, the cPLA2-α inhibitor efipladib (also referred to as Wyeth-1; Ni et al., 2006) (Fig. 4), decreased p-cPLA2-α and mitochondria-active cell numbers in a dose-dependent manner. An accumulation of cells in G0/G1 and a corresponding decrease in S phase were also observed, and this decreased tumor cell proliferation was also found in vivo. In fact, Wyeth-1 reduced PC-3 xenograft growth by approximately 33% within 2 weeks (Patel et al., 2008b).

cPLA2-α and its Effect on AKT and p53. To determine whether the AKT pathway could be affected by inhibition of cPLA2-α, a recent study examined LNCaP and PC-3 cells treated with cPLA2-α siRNA (Patel et al., 2008b). Compared with control cells transfected with scrambled siRNA, cPLA2-α siRNA-treated cells showed a reduction in pAKT and cyclin D1. Increasing concentrations of Wyeth-1 for 72 h resulted in a reduction in pAKT with a simultaneous decrease in cyclin D1 expression (Patel et al., 2008b). This work demonstrated a significant role of cPLA2-α in prostate cancer cell proliferation.

A recent study by Sun and colleagues (2008) has demonstrated that treatment of the LNCaP cell line (wild-type p53) with the cPLA2-α-selective inhibitor bromoeno lactone effectively blocked epidermal growth factor-induced cellular proliferation. This was associated with G0/G1 stage cell cycle arrest proceeded by increased p53 and p21 and decreased expression of the p53 antagonist MDM2 (Sun et al., 2008). Bromoeno lactone also inhibited proliferation of the PC-3 (mutant p53) cell type, indicating that both p53-dependent and -independent pathways can be influenced by cPLA2. These data support our hypothesis that targeting AKT via cPLA2 inhibitors can influence important sites of cross-talk between cellular pathways that regulate proliferation.

Conclusions

We have demonstrated that there are many established and potential key points of integration between the TGF-β, PI3/AKT, and PTEN pathways in tumor cell biology. We highlighted the potential of two new pharmacological approaches of iron chelation and cPLA2-α inhibition. These agents could exploit our suggested points of cross-talk in cell pathways that are commonly disrupted in prostate tumors and many other cancers. Further analysis and verification of these points of integration and their roles in cancer development will undoubtedly result in the development of new pharmaceutical agents for the treatment of prostate cancer and, possibly, other tumors.
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