0026-895X/09/7503-429–436$20.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 75:429–436, 2009

Vol. 75, No. 3
53066/3437809
Printed in U.S.A.

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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 heterogenous 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 A_2-α 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; $cPLA_2$-α, cytosolic phospholipase A2-α; 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; PIP_3, phosphatidylinositol-3,4,5-triphosphate; PLA_2-α, phospholipase A2-α; PTEN, phosphatase and tensin homolog deleted on chromosome 10; RR, ribonucleotide reductase; Tf, transferrin; TfR1, transferrin receptor 1; TGF-β, transforming growth factor-β; TGF-βRI, transforming growth factor-β receptor I; BpT, 2-benzoylpyridine thiosemicarbazone; PIP_2, 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.

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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 tumorigenic phosphoinositide 3-kinase/protein kinase B (P13K/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 (Samuels and Ericson, 2006).

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 (PIP_2) to form phosphatidylinositol-3,4,5-triphosphate (PIP_3; Manning and Cantley, 2007). PIP_3 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 G_1/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 G_1 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 (Grünwald 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, *Pten*-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 PIP_3, converting it back to PIP_2 (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 Kyprianou, 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 Kyprianou, 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).

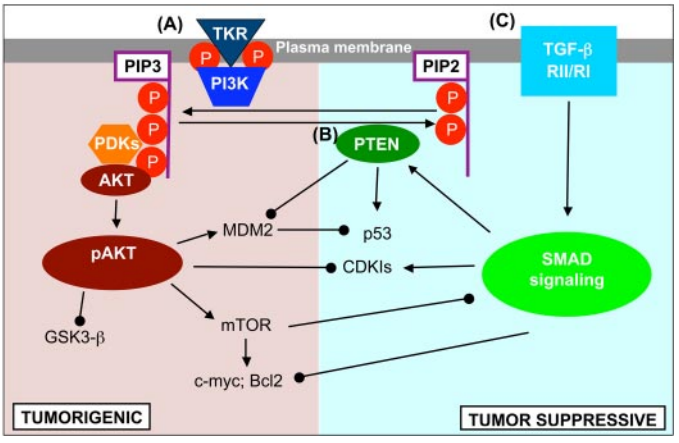

Fig. 1. Overview of the AKT, PTEN, and TGF-β pathways illustrating key points of cross-talk that influence tumorigenic and tumor-suppressive cell responses. The integration of these pathways is important for understanding the development of novel therapeutics. A, the PI3K/AKT pathway is stimulated by activation of PI3K upon ligand binding to a tyrosine kinase receptor. Phosphorylation of PIP_2 to PIP_3 allows for 3-phosphoinositide-dependent kinase 1 phosphorylation of AKT to active pAKT. Activated AKT elicits tumorigenic effects through stimulation of its downstream effectors, mTOR and MDM2, as well as suppression of GSK3β and the cyclin-dependent kinase inhibitors p15, p21, and p27. B, PTEN promotes tumor suppression through antagonism of AKT by dephosphorylation of PIP_3, stimulation of p53 activity, and suppression of MDM2. C, TGF-β tumor suppression is mediated by TGF-β-induced formation of its receptor complex (receptor 2/receptor 1) and subsequent activation of the SMAD signaling cascade. SMAD signaling induces expression of tumor-suppressive PTEN and the cyclin-dependent kinase inhibitors p15, p21, and p27 but suppresses tumorigenic c-Myc and antiapoptotic *Bcl2* expression. Activation of mTOR by pAKT suppresses TGF-β-activated SMAD signaling.

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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 Kyprianou, 1999) but increases expression of the cyclin-dependent kinase inhibitors p15, p21, and p27 (Fig. 1) (Guo and Kyprianou, 1998; Li et al., 2006). However, AKT activation has the opposite effect (Vignot 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 A_2-α ($cPLA_2$-α), 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; Porkka 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

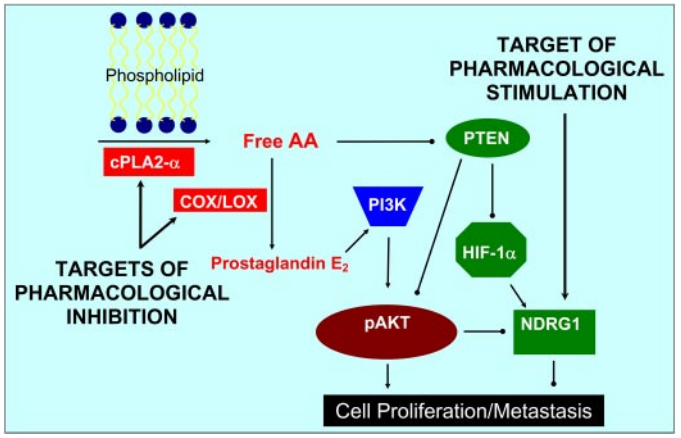

Fig. 2. The metastasis suppressor NDRG1 and the potentially tumorigenic fatty acid AA provide targets for pharmacological modulation of cancer cell proliferation. $cPLA_2$-α releases AA into the cytosol from membrane phospholipids. AA is converted to eicosanoids (e.g., PGE2) by lipooxygenases and cyclooxygenases (COX and LOX, respectively) that indirectly stimulate phosphorylation of AKT (pAKT). On the other hand, AA can directly stimulate formation of pAKT, leading to increased cellular proliferation. Pharmacological inhibitors of cPLA2-α, COX, or LOX would prevent this activity. Furthermore, AA can reduce the activity of PTEN, blocking its antagonism of the AKT pathway, and may also reduce expression of NDRG1. Because PTEN is down-regulated in many cancers, stimulation by pharmacological intervention (e.g., iron chelators) can lead to increased expression of the metastasis suppressor NDRG1 and subsequently the inhibition of proliferation and metastasis.

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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 $\omega 6$ 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 A_2, 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; Wildroudt 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 AKT phosphorylation (Hsu et al., 2000) and by down-regulating cyclin D1 (Patel et al., 2005). The potential biological significance of the regulation between $cPLA_2$-α and PI3K has been highlighted in a recent review (Wymann and Schneiter, 2008).

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 A_2-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 G_1 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 42/6 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

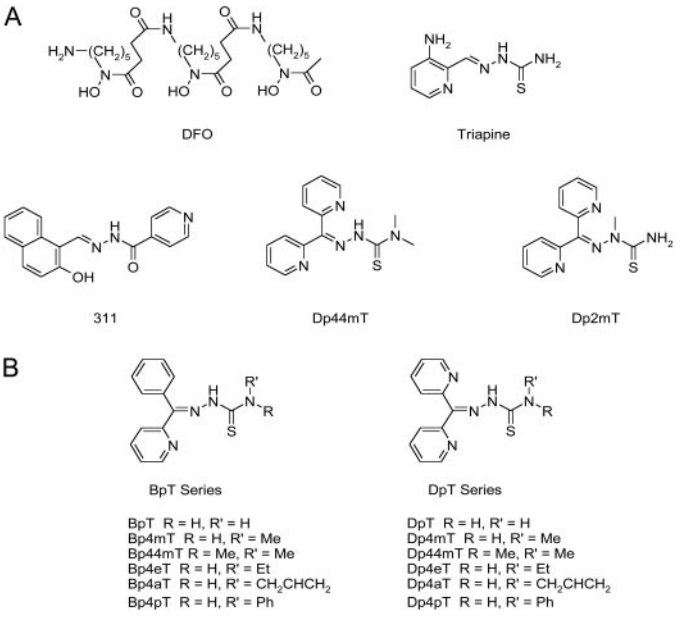

Fig. 3. Structures of iron chelators discussed in this review. DFO and Triapine are both chelators examined in clinical trials for their antitumor activity (Kalinowski and Richardson, 2005; Kalinowski et al., 2007). Development of novel chelators that improve upon the efficacy of DFO led to the aroylhydrazone 311. Thereafter, chelators of the DpT series were generated, including Dp44mT, which markedly inhibits tumor growth and the control chelator dipyridylketone 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).

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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 aroylhydrazone 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 (IC_{50} 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 (Faurschou 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-

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sue-, species- and/or genotype-specific manner (Patel et al., 2008a). Of these proteins, cPLA$_2$-α is the most important, because it is the only family member that cleaves AA selectively from membrane phospholipids (Ghosh et al., 2006). It is noteworthy that in prostate cancer, there is a clear loss of the cellular cPLA$_2$ inhibitors annexin A1 and A2 (Paweletz 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 cPLA$_2$-α could be the rate-limiting step in eicosanoid synthesis, cPLA$_2$-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 cPLA$_2$-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 cPLA$_2$-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 cPLA$_2$-α as a therapeutic target in prostate cancer, the expression levels of cPLA$_2$-α have been examined in prostate cancer cell lines (Patel et al., 2008b). In these studies, androgen-sensitive LNCaP cells expressed less cPLA$_2$-α mRNA and protein than the androgen-independent PC-3 cell line. Comparison was made by examining the immunostaining of phospho-cPLA$_2$-α (Ser^{505}) 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-cPLA$_2$-α staining intensity in cells that displayed androgen-independence. When cPLA$_2$-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 cPLA$_2$-α inhibitor efipladib (also referred to as Wyeth-1; Ni et al., 2006) (Fig. 4), decreased p-cPLA$_2$-α and mitochondria-active cell numbers in a dose-dependent manner. An accumulation of cells in G_0/G_1 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).

cPLA$_2$-α and its Effect on AKT and p53. To determine whether the AKT pathway could be affected by inhibition of cPLA$_2$-α, a recent study examined LNCaP and PC-3 cells treated with cPLA$_2$-α siRNA (Patel et al., 2008b). Compared with control cells transfected with scrambled siRNA, cPLA$_2$-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 cPLA$_2$-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 bromoenol lactone effectively blocked epidermal growth factor-induced cellular proliferation. This was associated with G_o/G_1 stage cell cycle arrest proceeded by increased p53 and p21 and decreased expression of the p53 antagonist MDM2 (Sun et al., 2008). Bromoenol 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.

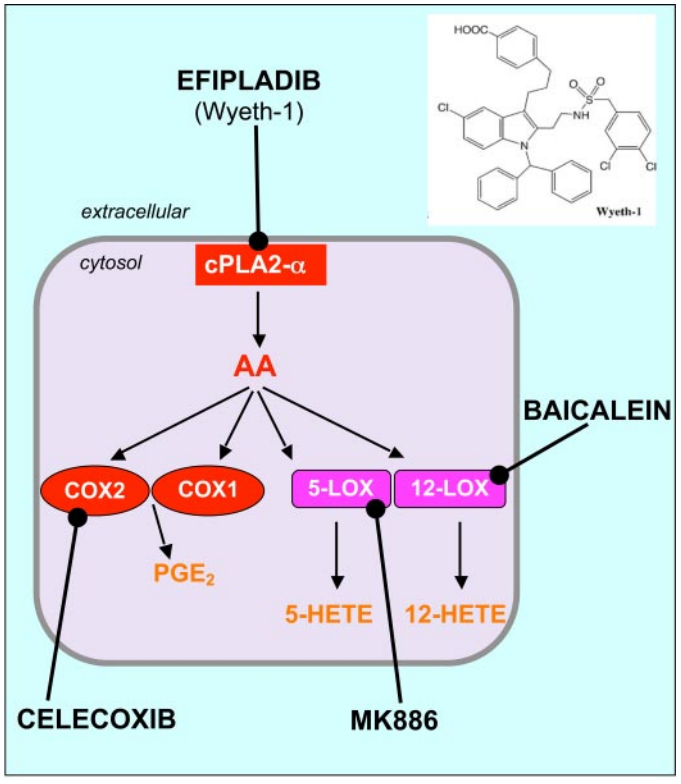

Fig. 4. Inhibitors of key enzymes of the AA pathway. The enzyme cPLA$_2$-α liberates AA from the plasma membrane. The release of AA can be tumorigenic leading to activation of AKT. However, this enzyme can be inhibited by Efipladib (see insert for structure) (Ni et al., 2006). The AA can also be metabolized further by COX-1 and -2, leading to prostaglandins (e.g., PGE2). COX-1 and -2 inhibitors are well known and include celecoxib. Liberated AA can also be acted upon by 5-lipooxygenase (5-LOX) or 12-lipooxygenase (12-LOX), leading in turn to the hydroxyeicosatetraenoic acids 5-HETE and 12-HETE. The inhibitors MK886 and baicalein prevent the activity of 5-LOX and 12-LOX, respectively.

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 cPLA$_2$-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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References

Assinder SJ and Nicholson HD (2007) Prostate disease: prostate hyperplasia, prostate cancer, and prostatitis, in *Male Reproductive Dysfunction: Pathophysiology and Treatment* (Kandeel FR, Swerdloff RS, Pryor JL eds) pp 499–518, Informa Healthcare, New York.

Bandyopadhyay S, Pai SK, Gross SC, Hirota S, Hosobe S, Miura K, Saito K, Commes T, Hayashi S, Watabe M, et al. (2003) The Drg-1 gene suppresses tumor metastasis in prostate cancer. *Cancer Res* **63:**1731–1736.

Bandyopadhyay S, Pai SK, Hirota S, Hosobe S, Takano Y, Saito K, Piquemal D, Commes T, Watabe M, Gross SC, et al. (2004a) Role of the putative tumor metastasis suppressor gene Drg-1 in breast cancer progression. *Oncogene* **23:**5675–5681.

Bandyopadhyay S, Pai SK, Hirota S, Hosobe S, Tsukada T, Miura K, Takano Y, Saito K, Commes T, Piquemal D, et al. (2004b) PTEN up-regulates the tumor metastasis suppressor gene *Drg-1* in prostate and breast cancer. *Cancer Res* **64:**7655–7660.

Becker EM, Lovejoy DB, Greer JM, Watts R, and Richardson DR (2003) Novel aroylhydrazone iron chelators differ in their iron chelation efficacy and anti-proliferative activity: Identification of a new class of potential anti-proliferative agents. *Br J Pharmacol* **138:**819–830.

Berger AP, Kofler K, Bektic J, Rogatsch H, Steiner H, Bartsch G, and Klocker H (2003) Increased growth factor production in a human prostatic stromal cell culture model caused by hypoxia. *Prostate* **57:**57–65.

Bohnsack BL and Hirschi KK (2004) Nutrient regulation of cell cycle progression. *Annu Rev Nutr* **24:**433–453.

Brenner RR (1974) The oxidative desaturation of unsaturated fatty acids in animals. *Mol Cell Biochem* **3:**41–52.

Burr GO and Burr MM (1929) A new deficiency disease produced by rigid exclusion of fat from the diet. *J Biol Chem* **82:**345–367.

Buss JL, Torti FM, and Torti SV (2003) The role of iron chelation in cancer therapy. *Curr Med Chem* **10:**1021–1034.

Cangul H (2004) Hypoxia upregulates the expression of the NDRG1 gene leading to its overexpression in various human cancers. *BMC Genet* **5:**27.

Cantley LC and Neel BG (1999) New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci U S A* **96:**4240–4245.

Chang CJ, Mulholland DJ, Valamehr B, Mosessian S, Sellers WR, and Wu H (2008) PTEN nuclear localization is regulated by oxidative stress and mediates p53-dependent tumor suppression. *Mol Cell Biol* **28:**3281–3289.

Chaudry AA, Wahle KW, McClinton S, and Moffat LE (1994) Arachidonic acid metabolism in benign and malignant prostatic tissue in vitro: effects of fatty acids and cyclooxygenase inhibitors. *Int J Cancer* **57:**176–180.

Chen Y, Martinez LA, LaCava M, Coghlan L, and Conti CJ (1998) Increased cell growth and tumorigenicity in human prostate LNCaP cells by overexpression to cyclin D1. *Oncogene* **16:**1913–1920.

Chetcuti A, Margan SH, Russell P, Mann S, Millar DS, Clark SJ, Rogers J, Handelsman DJ, and Dong Q (2001) Loss of annexin II heavy and light chains in prostate cancer and its precursors. *Cancer Res* **61:**6331–6334.

Chow JY, Quach KT, Cabrera BL, Cabral JA, Beck SE, and Carethers JM (2007) RAS/ERK modulates TGFbeta-regulated PTEN expression in human pancreatic adenocarcinoma cells. *Carcinogenesis* **28:**2321–2327.

Clark JD, Schievella AR, Nalefski EA, and Lin LL (1995) Cytosolic phospholipase A2. *J Lipid Mediat Cell Signal* **12:**83–117.

Cooper CE, Lynagh GR, Hoyes KP, Hider RC, Cammack R, and Porter JB (1996) The relationship of intracellular iron chelation to the inhibition and regeneration of human ribonucleotide reductase. *J Biol Chem* **271:**20291–20299.

Covey TM, Edes K, and Fitzpatrick FA (2007) Akt activation by arachidonic acid metabolism occurs via oxidation and inactivation of PTEN tumor suppressor. *Oncogene* **26:**5784–5792.

Cummings BS (2007) Phospholipase A_2 as targets for anti-cancer drugs. *Biochem Pharmacol* **74:**949–959.

Darnell G and Richardson DR (1999) The potential of analogues of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents III: the effect of the ligands on molecular targets involved in proliferation. *Blood* **94:**781–792.

Davies MA, Koul D, Dhesi H, Berman R, McDonnell TJ, McConkey D, Yung WK, and Steck PA (1999). Regulation of Akt/PKB activity, cellular growth, and apoptosis in prostate carcinoma cells by *MMAC/PTEN*. *Cancer Res* **59:**2551–2556.

de la Taille A, Rubin MA, Chen MW, Vacherot F, de Medina SG, Burchardt M, Buttyan R, and Chopin D (2003) Beta-catenin-related anomalies in apoptosis-resistant and hormone-refractory prostate cancer cells. *Clin Cancer Res* **9:**1801–1807.

Dong Z, Arnold RJ, Yang Y, Park MH, Hrncirova P, Mechref Y, Novotny MV, and Zhang JT (2005) Modulation of differentiation-related gene 1 expression by cell cycle blocker mimosine, revealed by proteomic analysis. *Mol Cell Proteomics* **4:**993–1001.

Dongiovanni P, Valenti L, Ludovica Fracanzani A, Gatti S, Cairo G, and Fargion S (2008) Iron depletion by deferoxamine up-regulates glucose uptake and insulin signaling in hepatoma cells and in rat liver. *Am J Pathol* **172:**738–747.

Dunn LL, Rahmanto YS, and Richardson DR (2007) Iron uptake and metabolism in the new millennium. *Trends Cell Biol* **17:**93–100.

Elford HL, Freese M, Passamani E, and Morris HP (1970) Ribonucleotide reductase and cell proliferation. I. Variations of ribonucleotide reductase activity with tumor growth rate in a series of rat hepatomas. *J Biol Chem* **245:**5228–5233.

Emerling BM, Weinberg F, Liu JL, Mak TW, and Chandel NS (2008) PTEN regulates p300-dependent hypoxia-inducible factor 1 transcriptional activity through FOXO3a. *Proc Natl Acad Sci U S A* **105:**2622–2627.

Faas FH, Dang AQ, Pollard M, Hong XM, Fan K, Luckert PH, and Schutz M (1996) Increased phospholipid fatty acid remodeling in human and rat prostatic adenocarcinoma tissues. *J Urol* **156:**243–248.

Facher EA and Law JC (1998) PTEN and prostate cancer. *J Med Genet* **35:**790.

Faurschou A and Gniadecki R (2008) TNF-alpha stimulates Akt by a distinct aPKC-dependent pathway in premalignant keratinocytes. *Exp Dermatol* **17:**992–997.

Fu D and Richardson DR (2007) Iron chelation and regulation of the cell cycle: Two mechanisms of post-transcriptional regulation of the universal cyclin-dependent kinase inhibitor $p21^{CIP1/WAF1}$ by iron-depletion. *Blood* **110:**752–761.

Funk CD and FitzGerald GA (2007) COX-2 inhibitors and cardiovascular risk. *J Cardiovasc Pharmacol* **50:**470–479.

Gao N, Zhang Z, Jiang BH, and Shi X (2003) Role of PI3K/AKT/mTOR signaling in the cell cycle progression of human prostate cancer. *Biochem Biophys Res Commun* **310:**1124–1132.

Ghosh M, Tucker DE, Burchett SA, and Leslie CC (2006) Properties of the group IV phospholipase A2 family. *Prog Lipid Res* **45:**487–510.

Green DA, Antholine WE, Wong SJ, Richardson DR, and Chitambar CR (2001) Inhibition of malignant cell growth by 311, a novel iron chelator of the pyridoxal isonicotinoyl hydrazone class: effect on the R2 subunit of ribonucleotide reductase. *Clin Cancer Res* **7:**3574–3579.

Greenberger LM, Horak ID, Filpula D, Sapra P, Westergaard M, Frydenlund HF, Albaek C, Schrøder H, and Ørum H (2008) A RNA antagonist of hypoxia-inducible factor-1{alpha}, EZN-2968, inhibits tumor cell growth. *Mol Cancer Ther* **7:**3598–3608.

Groot AJ, Gort EH, van der Wall E, van Diest PJ, and Vooijs M (2008) Conditional inactivation of HIF-1 using intrabodies. *Cell Oncol* **30:**397–409.

Grünwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB, and Hidalgo M (2002) Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. *Cancer Res* **62:**6141–6145.

Guo Y and Kyprianou N (1998) Overexpression of transforming growth factor (TGF) β1 type II receptor restores TGF-β1 sensitivity and signalling in human prostate cancer cells. *Cell Growth Diff* **9:**185–193.

Guo Y and Kyprianou N (1999) Restoration of transforming growth factor β signalling pathway in human prostate cancer cells suppresses tumorigenicity via induction of caspase-1-mediated apoptosis. *Cancer Res* **59:**1366–1371.

Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, and Chen CS (2000) The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. *J Biol Chem* **275:**11397–11403.

Hughes-Fulford M, Chen Y, and Tjandrawinata RR (2001) Fatty acid regulates gene expression and growth of human prostate cancer PC-3 cells. *Carcinogenesis* **22:**701–707.

Hughes-Fulford M, Li CF, Boonyaratanakornkit J, and Sayyah S (2006) Arachidonic acid activates phosphatidylinositol 3-kinase signaling and induces gene expression in prostate cancer. *Cancer Res* **66:**1427–1433.

Hussain A and Dawson N (2000) Management of advanced/metastatic prostate cancer: 2000 update. *Oncology* **14:**1677–1688; discussion 1688, 1691–1694.

Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, and Kaelin WG Jr (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* **292:**464–468.

Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim Av, Hebestreit HF, Mukherji M, Schofield CJ, et al. (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* **292:**468–472.

Jordan A and Reichard P (1998) Ribonucleotide reductases. *Ann Rev Biochem* **67:**71–98.

Kalinowski DS and Richardson DR (2005) The evolution of iron chelators for the treatment of iron overload disease and cancer. *Pharmacol Rev* **57:**547–583.

Kalinowski DS, Yu Y, Sharpe PC, Islam M, Liao YT, Lovejoy DB, Kumar N, Bernhardt PV, and Richardson DR (2007) Design, synthesis and characterization of novel iron chelators: Structure-activity relationships of the 2-benzoylpyridine thiosemicarbazone series and their 3-nitrobenzoyl analogs as potent anti-tumor agents. *J Med Chem* **50:**3716–3729.

Kovacevic Z, Fu D, and Richardson DR (2008) The iron-regulated metastasis suppressor, Ndrg-1: identification of novel tumor cell-specific targets. *Biochim Biophys Acta* **1783:**1981–1992.

Kovacevic Z and Richardson DR (2006) The metastasis suppressor, Ndrg-1: a new ally in the fight against cancer. *Carcinogenesis* **27:**2355–2366.

Larrick JW and Cresswell P (1979) Modulation of cell surface iron transferrin receptors by cellular density and state of activation. *J Supramol Struct* **11:**579–586.

Le NT and Richardson DR (2002) The role of iron in cell cycle progression and the proliferation of neoplastic cells. *Biochim Biophys Acta* **1603:**31–46.

Le NT and Richardson DR (2004) Iron chelators with high anti-proliferative activity up-regulate the expression of a growth inhibitory and metastasis suppressor gene: A novel link between iron metabolism and proliferation. *Blood* **104:**2967–2975.

Lee HK, Kwak HY, Hur J, Kim IA, Yang JS, Park MW, Yu J, and Jeong S (2007) Beta-catenin regulates multiple steps of RNA metabolism as revealed by the RNA aptamer in colon cancer cells. *Cancer Res* **67:**9315–9321.

Li F and Malik KU (2005a) Angiotensin II-induced Akt activation is mediated by metabolites of arachidonic acid generated by CaMKII-stimulated $Ca2^{+}$-dependent phospholipase A2. *Am J Physiol Heart Circ Physiol* **288:**H2306–H2316.

Li F and Malik KU (2005b) Angiotensin II-induced Akt activation through the epidermal growth factor receptor in vascular smooth muscle cells is mediated by phospholipid metabolites derived by activation of phospholipase D. *J Pharmacol Exp Ther* **312:**1043–1054.

Li Z, Chen Y, Cao D, Wang Y, Chen G, Zhang S, and Lu J (2006) Glucocorticoid upregulates transforming growth-β type II receptor enhances TGF-β signalling in human prostate cancer PC-3 cells. *Endocrinology* **147:**5259–5267.

Liang SX and Richardson DR (2003) The effect of potent iron chelators on the regulation of p53: examination of the expression, localization and DNA-binding activity of p53 and the transactivation of WAF1. *Carcinogenesis* **24:**1601–1614.

Lovejoy DB and Richardson DR (2002) Novel "hybrid" iron chelators derived from aroylhydrazones and thiosemicarbazones demonstrate high anti-proliferative activity that is selective for tumor cells. *Blood* **100:**666–676.

Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

Manning BD and Cantley LC (2007) AKT/PKB signaling: navigating downstream. *Cell* **129:**1261–1274.
Marcus AJ, Broekman MJ, and Pinsky DJ (2002) COX inhibitors and thromboregulation. *N Engl J Med* **347:**1025–1026.
Massagué J, Blain SW, and Lo RS (2000) TGFβ signalling in growth control, cancer, and heritable disorders. *Cell* **103:**295–309.
Mayo LD, Dixon JE, Durden DL, Tonks NK, and Donner DB (2002) PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. *J Biol Chem* **277:** 5484–5489.
McMahon S, Charbonneau M, Grandmont S, Richard DE, and Dubois CM (2006) Transforming growth factor β1 induces hypoxia-inducible factor-1 stabilization through selective inhibition of PHD2 expression. *J Biol Chem* **281:**24171–24181.
Ni Z, Okeley NM, Smart BP, and Gelb MH (2006) Intracellular actions of group IIA secreted phospholipase A2 and group IVA cytosolic phospholipase A2 contribute to arachidonic acid release and prostaglandin production in rat gastric mucosal cells and transfected human embryonic kidney cells. *J Biol Chem* **281:**16245–16255.
Nie D, Che M, Grignon D, Tang K, and Honn KV (2001) Role of eicosanoids in prostate cancer progression. *Cancer Metastasis Rev* **20:**195–206.
Nie D, Hillman GG, Geddes T, Tang K, Pierson C, Grignon DJ, and Honn KV (1998) Platelet type 12-lipoxygenase in a human prostate carcinoma stimulates angiogenesis and tumor growth. *Cancer Res* **58:**4047–4051.
Niknami M, Patel M, Witting PK, and Dong Q (2008) Cytosolic phospholipase A_2-α. *Int J Biochem Cell Biol* doi: 10.1016/j.biocel.2008.07.017.
Nurtjahja-Tjendraputra E, Fu D, Phang JM, and Richardson DR (2007) Iron chelation regulates cyclin D1 expression via the proteasome: a link to iron-deficiency mediated growth suppression. *Blood* **109:**4045–4054.
Nyholm S, Mann GJ, Johansson AG, Bergeron RJ, Gräslund A, and Thelander L (1993) Role of ribonucleotide reductase in inhibition of mammalian cell growth by potent iron chelators. *J Biol Chem* **268:**26200–26205.
Patel MI, Kurek C, and Dong Q (2008a) The arachidonic acid pathway and its role in prostate cancer development and progression. *J Urol* **179:**1668–1675.
Patel MI, Singh J, Niknami M, Kurek C, Yao M, Lu S, Maclean F, King NJ, Gelb MH, Scott KF, et al. (2008b) Cytosolic phospholipase A2-α: A potential therapeutic target for prostate cancer. *Clin Cancer Res* **14:**8070–8079.
Patel MI, Subbaramaiah K, Du B, Chang M, Yang P, Newman RA, Cordon-Cardo C, Thaler HT, and Dannenberg AJ (2005) Celecoxib inhibits prostate cancer growth: evidence of a cyclooxygenase-2-independent mechanism. *Clin Cancer Res* **11:** 1999–2007.
Paweletz CP, Ornstein DK, Roth MJ, Bichsel VE, Gillespie JW, Calvert VS, Vocke CD, Hewitt SM, Duray PH, Herring J, et al. (2000) Loss of annexin 1 correlates with early onset of tumorigenesis in esophageal and prostate carcinoma. *Cancer Res* **60:**6293–6297.
Pidgeon GP, Kandouz M, Meram A, and Honn KV (2002) Mechanisms controlling cell cycle arrest and induction of apoptosis after 12-lipoxegenase inhibition in prostate cancer cells. *Cancer Res* **62:**2721–2727.
Porkka KP and Visakorpi T (2004) Molecular mechanisms of prostate cancer. *Eur Urol* **45:**683–691.
Pruthi RS, Derksen JE, Moore D, Carson CC, Grigson G, Watkins C, and Wallen E (2006) Phase II trial of celecoxib in prostate-specific antigen recurrent prostate cancer after definitive radiation therapy or radical prostatectomy. *Clin Cancer Res* **12:**2172–2177.
Richardson D, Ponka P, and Baker E (1994) The effect of the iron(III) chelator, desferrioxamine, on iron and transferrin uptake by the human malignant melanoma cell. *Cancer Res* **54:**685–689.
Richardson DR (2002) Iron chelators as therapeutic agents for the treatment of cancer. *Crit Rev Oncol Hematol* **42:**267–281.
Richardson DR (2005) Molecular mechanisms of iron uptake by cells and the use of iron chelators for the treatment of cancer and other diseases. *Curr Med Chem* **12:**2711–2729.
Richardson DR and Baker E (1990) The uptake of iron and transferrin by the human malignant melanoma cell. *Biochim Biophys Acta* **1053:**1–12.
Richardson DR, Kalinowski DS, Lau S, Jansson PJ, and Lovejoy DB (2008) Cancer cell iron metabolism and the development of potent iron chelators as anti-tumor agents. *Biochim Biophys Acta* doi: 10.1016/j.bbagen.2008.04.003.
Richardson DR and Milnes K (1997) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents II. The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone. *Blood* **89:**3025–3038.
Richardson DR and Ponka P (1997) The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. *Biochim Biophys Acta* **1331:**1–40.
Richardson DR, Sharpe PC, Lovejoy DB, Senaratne D, Kalinowski DS, Islam M, and Bernhardt PV (2006) Dipyridyl thiosemicarbazone chelators with potent and selective anti-tumor activity form iron complexes with marked redox activity. *J Med Chem* **49:**6510–6521.
Richardson DR, Tran EH, and Ponka P (1995) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents. *Blood* **86:**4295–4306.
Samuels Y and Ericson K (2006) Oncogenic PI3K and its role in cancer. *Curr Opin Oncol* **18:**77–82.
Shariat SF, Kattan MW, Traxel E, Andrews B, Zhu K, Wheeler TM, and Slawin KM (2004) Association of pre- and post-operative plasma levels of transforming growth factor-$beta_1$ and interleukin 6 and is soluble receptor with prostate cancer progression. *Clin Cancer Res* **10:**1992–1999.
Sharrard RM and Maitland NJ (2000) Phenotypic effects of overexpression of the MMAC1 gene in prostate epithelial cells. *Br J Cancer* **83:**1102–1109.
Shi Y and Massagué J (2003) Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* **113:**685–700.
Smith MR, Manola J, Kaufman DS, Oh WK, Bubley GJ, and Kantoff PW (2006) Celecoxib versus placebo for men with prostate cancer and a rising serum prostate-specific antigen after radical prostatectomy and/or radiation therapy. *J Clin Oncol* **24:**2723–2728.
Song K, Cornelius SC, Reiss M, and Danielpour D (2003) Insulin-like growth factor-I inhibits transcriptional responses of transforming growth factor-β by phosphatidylinositol 3-kinase/Akt-dependent suppression of the activation of Smad3 but not Smad2. *J Biol Chem* **278:**38342–38351.
Sperandio S, Fortin J, Sasik R, Robitaille L, Corbeil J, and de Belle I (2008) The transcription factor Egr1 regulates the HIF-1alpha gene during hypoxia. *Mol Carcinog* doi: 10.1002/mc.20454
Stambolic V, MacPherson D, Sas D, Lin Y, Snow B, Jang Y, Benchimol S, and Mak TW (2001) Regulation of PTEN transcription by p53. *Mol Cell* **8:**317–325.
Steineck G, Helgesen F, Adolfsson J, Dickman PW, Johansson JE, Norlén BJ, and Holmberg L (2002) Quality of life after radical prostatectomy or watchful waiting. *N Engl J Med* **347:**790–796.
Sun B, Zhang X, Talathi S, and Cummings BS (2008) Inhibition of Ca^{2+}-independent phospholipase A2 decreases prostate cancer cell growth by p53-dependent and independent mechanisms. *J Pharmacol Exp Ther* **326:**59–68.
Sutherland R, Delia D, Schneider C, Newman R, Kemshead J, and Greaves M (1981) Ubiquitous cell surface glycoprotein on tumor cells is proliferation-associated receptor for transferring. *Proc Natl Acad Sci U S A* **78:**4515–4519.
Sutkowski DM, Fong CJ, Sensibar JA, Rademaker AW, Sherwood ER, Kozlowski JM, and Lee C (1992) Interaction of epidermal growth factor and transforming growth factor beta in human prostatic epithelial cells. *Prostate* **21:**133–143.
Tanji K, Imaizumi T, Matsumiya T, Itaya H, Fujimoto K, Cui X, Toki T, Ito E, Yoshida H, Wakabayashi K, et al. (2001) Desferrioxamine, an iron chelator, upregulates cyclooxygenase-2 expression and prostaglandin production in a human macrophage cell line. *Biochim Biophys Acta* **1530:**227–235.
Ten Dijke P, Goumans MJ, Itoh F, and Itoh S (2002) Regulation of cell proliferation by Smad Proteins. *J Cell Physiol* **191:**1–16.
Thigpen AE, Cala KM, Guileyardo JM, Molberg KH, McConnell JD, and Russell DW (1996) Increased expression of early growth response-1 messenger ribonucleic acid in prostatic adenocarcinoma. *J Urol* **155:**975–981.
Tjandrawinata RR, Dahiya R, and Hughes-Fulford M (1997) Induction of cyclooxygenase-2 mRNA by prostaglandin E2 in human prostatic carcinoma cells. *Br J Cancer* **75:**1111–1118.
Trédan O, Galmarini CM, Patel K, and Tannock IF (2007) Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst* **99:**1441–1454.
Trowbridge IS and Lopez F (1982) Monoclonal antibody to transferrin receptor blocks transferrin binding and inhibits human tumor cell growth in vitro. *Proc Natl Acad Sci U S A* **79:**1175–1179.
Vignot S, Faivre S, Aguirre D, and Raymond E (2005) mTOR-targeted therapy of cancer with rapamycin derivatives. *Ann Oncol* **16:**525–537.
Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, and Wu H (2006) *Pten* deletion leads to expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. *Proc Natl Acad Sci U S A* **103:**1480–1485.
Wang Y, Corr JG, Thaler HT, Tao Y, Fair WR, and Heston WD (1995) Decreased growth of established human prostate LNCaP tumors in nude mice fed a low-fat diet. *J Natl Cancer Inst* **87:**1456–1462.
Wegiel B, Bjartell A, Culig Z, and Persson JL (2008) Interleukin-6 activates PI3K/Akt pathway and regulates cyclin A1 to promote prostate cancer cell survival. *Int J Cancer* **122:**1522–1529.
Whitnall M, Howard J, Ponka P, and Richardson DR (2006) A class of iron chelators with a wide spectrum of potent anti-tumor activity that overcome resistance to chemotherapeutics. *Proc Natl Acad Sci U S A* **103:**14901–14906.
Wilding G (1991) Response of prostate cancer cells to peptide growth factors: transforming growth factor-beta. *Cancer Surv* **11:**147–163.
Wildroudt ML and Freeman EJ (2006) Regulation of Akt by arachidonic acid and phosphoinositide 3-kinase in angiotensin II-stimulated vascular smooth muscle cells. *Biochim Biophys Acta* **1761:**11–16.
Woo KJ, Lee TJ, Park JW, and Kwon TK (2006) Desferrioxamine, an iron chelator, enhances HIF-1alpha accumulation via cyclooxygenase-2 signaling pathway. *Biochem Biophys Res Commun* **343:**8–14.
Wymann MP and Schneiter R (2008) Lipid signalling in disease. *Nat Rev Mol Cell Biol* **9:**162–176.
Yasui H, Ogura A, Asanuma T, Matsuda A, Kashiwakura I, Kuwabara M, and Inanami O (2008) Inhibition of HIF-1alpha by the anticancer drug TAS106 enhances X-ray-induced apoptosis in vitro and in vivo. *Br J Cancer* **99:**1442–1452.
Yoon G, Kim HJ, Yoon YS, Cho H, Lim IK, and Lee JH (2002) Iron chelation-induced senescence-like growth arrest in hepatocyte cell lines: association of transforming growth factor beta1 (TGF-beta1)-mediated p27Kip1 expression. *Biochem J* **366:** 613–621.
Yu Y, Kovacevic Z, and Richardson DR (2007) Tuning cell cycle regulation with an iron key. *Cell Cycle* **6:**1982–1994.
Yu Y, Wong J, Lovejoy DB, Kalinowski DS, and Richardson DR (2006) Chelators at the cancer coalface: desferrioxamine to triapine and beyond. *Clin Cancer Res* **12:**6876–6883.
Yuan J, Lovejoy DB, and Richardson DR (2004) Novel di-2-pyridyl-derived iron chelators with marked and selective anti-tumor activity: in vitro and in vivo assessment. *Blood* **104:**1450–1458.
Zhang P, Tchou-Wong KM, and Costa M (2007) Egr-1 mediates hypoxia-inducible transcription of the NDRG1 gene through an overlapping Egr-1/Sp1 binding site in the promoter. *Cancer Res* **67:**9125–9133.
Zhu ML, Partin JV, Bruckheimer EM, Strup SE, and Kyprianou N (2008) TGF-β signalling and androgen receptor status determine apoptotic cross-talk in human prostate cancer cells. *Prostate* **68:**287–295.

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