Targeting the AKT protein kinase for cancer chemoprevention

James A. Crowell,1 Vernon E. Steele,1 and Judith R. Fay2

1Division of Cancer Prevention, National Cancer Institute, NIH, Bethesda, Maryland and 2CCS Associates, Mountain View, California

Abstract

The AKT protein kinase transduces signals from growth factors and oncogenes to downstream targets that control crucial elements in tumor development. The AKT pathway is one of the most frequently hyperactivated signaling pathways in human cancers. Available data are reviewed herein to support targeting the AKT kinase for cancer prevention. This review will present data to show that AKT is up-regulated in preneoplastic lesions across a broad range of target tissues, briefly describe drug development efforts in this area, and present evidence that down-regulation of AKT signaling may be a viable strategy to prevent cancer. [Mol Cancer Ther 2007;6(8):2139–48]

Background

AKT (also known as protein kinase B) is an evolutionarily conserved serine/threonine kinase. Three isoforms, AKT1, AKT2, and AKT3, are expressed in mammals. AKT signaling has been extensively reviewed elsewhere (see refs. 1–7) and is only briefly outlined here. AKT is activated by phosphatidylinositol 3-kinase (PI3K), which transmits signals from cytokines, growth factors, and oncoproteins (e.g., RAS) to multiple targets, including AKT. Activation of PI3K localizes AKT to the plasma membrane via the pleckstrin homology domain of AKT, where AKT is activated by phosphorylation at Thr308 and Ser473. The most important negative regulator of PI3K/AKT signaling is the tumor suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN). Of the numerous PI3K/PTEN effector proteins, AKT1 is critical for development of murine tumors driven by PTEN haploinsufficiency (8).

Once activated, AKT regulates multiple cellular functions, including survival, proliferation (increased cell number), growth (increased cell size), and various aspects of intermediary metabolism. The consequences of AKT activation are mediated by a plethora of AKT effectors (Fig. 1; refs. 2–5, 7). Although the AKT substrates crucial for tumor development have not been clearly defined under all circumstances (9), mounting evidence points prominently to mammalian target of rapamycin complex 1 (mTORC1; refs. 4, 10–12). AKT activates mTORC1 by several mechanisms, including phosphorylation and inhibition of the tumor suppressor tuberous sclerosis complex 2 (TSC2; refs. 4, 7).

As with most intracellular signaling cascades, cross-talk and feedback interactions contribute to the overall regulation of PI3K/AKT signaling. For example, a negative feedback loop exists in which S6 kinase-1, a downstream effector of mTORC1, can phosphorylate and inhibit upstream insulin receptor substrate proteins, which in turn diminishes signaling through PI3K/AKT. This results in activation of AKT when mTORC1 is inhibited (6). The importance of this feedback loop can be realized from the results of clinical trials with mTORC1 inhibitors. Despite findings in model systems, where tumors with activated PI3K/AKT signaling are hypersensitive to mTORC1 blockade, patients bearing tumors with high AKT activity have responded only minimally to treatment with mTORC1 inhibitors (13).

Dysregulation of AKT in Cancer; Isoform Specificities

Mutations in AKT genes are rarely found in human cancers (14); however, aberrant AKT activation can occur via numerous mechanisms that affect elements upstream of AKT. These include mutation or amplification of PI3K, loss of PTEN function, and activation/mutation of receptor kinases and oncogenes (2). Activation of AKT acts as a survival/proliferative signal (6, 12); however, activation of AKT alone is generally insufficient to induce cancer unless combined with a transforming lesion in a second pathway (1, 15). For example, overexpression of constitutively active human AKT1 in the mouse prostate induces precancerous intraepithelial lesions. However, these lesions do not progress even after 78 months (10). Overexpression of AKT1 alone in mouse mammary epithelium causes defective mammary gland involution, but when expressed with a mutant polyoma virus middle T antigen unable to signal through PI3K, the combination produces a marked...
increase in mammary neoplasia (16). Hyperactivation of AKT signaling occurs in a wide variety of human precancerous (see below, Table 1) and cancerous lesions (2). Overexpression and/or activation of AKT in tumor cells causes resistance to traditional chemotherapeutics and molecularly targeted drugs, including trastuzumab, gefitinib, retinoic acid, and tamoxifen (17, 18).

All three AKT isoforms have been associated with tumorigenesis (2). Overexpression of AKT2 transforms NIH3T3 fibroblasts (19) and increases the invasive and/or metastatic capacity of human cancer cells in vitro and in vivo (20, 21). Ablation of AKT2 in either HER-2/NEU or polyoma middle T transgenic mice decreases metastatic spread (22). AKT3 activity and expression are up-regulated in estrogen receptor-negative breast carcinomas and androgen-insensitive prostate cancer cell lines (23). Selective knockdown of AKT3, but not of other isoforms, inhibits melanoma development driven by PTEN loss (24). In transgenic mice, constitutively active AKT1 induces precancerous prostatic lesions (10) and accelerates oncogene-dependent mammary tumor formation (16, 25). AKT1 plays a prominent role in tumor angiogenesis. Normal endothelial cells with sustained activation of AKT1 develop the complex structural and functional abnormalities characteristic of tumor blood vessels (26). AKT1 is also critical for the development of murine tumors driven by PTEN haploinsufficiency (8). On the contrary, some studies suggest that AKT1 and AKT2 may inhibit various stages of breast neoplasia (22, 25, 27), as discussed in more detail later.

**Targeting AKT for Cancer Prevention**

Changes in AKT activity or expression observed in human precancerous tissues that might be targeted for chemoprevention are described below (Table 1). For the most part, studies have not reported isoform-specific changes in transformed human tissue; data on isoforms are described, where available. AKT hyperactivation likely results from primary molecular lesions in signaling pathways that impinge on AKT signaling rather than in the AKT gene itself.

**Lung**

Both in vitro and in vivo studies implicate AKT in the early stages of human lung tumorigenesis. AKT is activated by tobacco-specific carcinogens in lung epithelial cells (28, 29) and is constitutively active in premalignant and malignant bronchial cell lines but not in their normal counterparts.
In clinical studies, levels of phosphorylated AKT (pAKT) are elevated in cancers as well as in bronchial metaplasias and dysplasias (21, 31–33). For example, expression of pAKT increased from 27% (12 of 44) in normal bronchial epithelium of former smokers to 44% (4 of 9) in reactive epithelium to 88% (22 to 25) in dysplastic specimens, but decreased to 33% (25 of 76) in non-small cell lung cancers (31). AKT activation also increased with the degree of severity in dysplasias (32, 33). In these investigations, no association was found between AKT activation and tumor stage or patient survival (31–33). One interpretation of these results is that AKT is permissive for early transforming events in the lung but is not required for the more advanced stages of disease.

In mice, AKT is also activated during the early stages of lung transformation. pAKT levels are significantly increased in preneoplastic type II cell hyperplasias and atypical adenomatous hyperplasias induced by tobacco-specific carcinogens (29). Furthermore, pharmacologic and genetic blockade of PI3K/AKT suppresses the growth of premalignant and malignant bronchial epithelial cells in vitro (29, 30, 34). Diminution of PI3K/AKT signaling has also been associated with the prevention of murine lung cancer by the retinoid deguelin (ref. 35; see also below).

### Prostate

Several studies show that AKT is activated in precancerous prostatic intraepithelial neoplasias (PIN; refs. 36–38). The percentage of PIN lesions expressing pAKT varied in these studies from 10% to 100%, perhaps due to differences in the grade of PIN examined or other methodologic variables. For example, pAKT was not detected in normal prostatic tissues but was found in almost half of low-grade PIN (n = 23) and in all high-grade PIN (n = 36) and invasive carcinomas (n = 86; ref. 38). Moreover, at the transition from histologically normal epithelium to PIN, a surge in AKT activity was observed (36). As noted above, PIN lesions that do not progress are induced by prostate-restricted expression of human AKT1 in mice; pharmacologic inhibition of mTORC1 completely reversed the PIN phenotype in AKT1 transgenic mice (10). Other studies showed that introduction of constitutively active AKT1 into dissociated mouse prostate epithelial cells or putative murine prostate cancer stem cells is sufficient to induce PIN lesions but not carcinoma (39). In accord with the necessity for a transforming lesion in a second molecular pathway, simultaneous expression of AKT and the androgen receptor in prostate cells produced adenocarcinomas competent to override the effects of androgen ablation (15).

It is noteworthy that PTEN alterations have been highly implicated in both human and murine prostate carcinogenesis. PTEN mutations occur in ~15% of primary human prostate cancers, with homozygous inactivation frequently found in metastatic lesions. It is unknown if primary tumors harboring PTEN mutations are at increased risk for progression (40), but PTEN loss exerts a potent transforming effect in the mouse prostate. For example, prostate-specific PTEN deletion leads to PIN lesions by 6 weeks of age, with invasive/metastatic lesions presenting as early as 9 weeks. PTEN deletion correlates with AKT activation in tumors (41). Furthermore, in PTEN+/– mice, AKT1 deficiency markedly inhibits prostate tumor development; even AKT1 haploinsufficiency is sufficient to significantly diminish PIN formation induced by PTEN deficiency (8).

### Skin Cancer

AKT activation increases during melanoma formation. Strong pAKT expression was observed in 2 of 12 (17%) normal nevi, 25 of 58 (43%) dysplastic nevi, 84 of 170 (49%) primary melanomas, and 40 of 52 (77%) metastases. Although more pAKT is expressed in dysplastic nevi compared with normal nevi, this difference did not reach significance, possibly due to the small number of normal nevi examined. However, significantly more pAKT was expressed in primary melanomas compared with normal nevi and in metastatic lesions compared with primary melanomas (42). In thin tumors (≤1.5 mm), strong pAKT expression was an independent prognostic factor of poor 5-year overall survival. AKT3, but not AKT1 or AKT2, has specifically been associated with sporadic melanoma development. Activity and expression of AKT3 are increased in sporadic human melanomas; AKT3 is also the predominant isoform activated in an in vitro model of human melanoma progression (24). Moreover, selective knockdown of AKT3, but not other isoforms, inhibits melanoma formation driven by PTEN loss in a murine model (24).

The mechanisms involved in the transforming actions of AKT in melanocytes have been investigated. Constitutively active AKT transforms melanocytes in a mildly hypoxic environment; this level of hypoxia simulates conditions found in normal skin. Hypoxia-inducible factor-1α is not only required for, but can further enhance, AKT-driven transformation. Activation of mTORC1 is necessary to

### Table 1. Alteration in the PI3K/PTEN/AKT pathway in human precancerous lesions

<table>
<thead>
<tr>
<th>Target</th>
<th>PI3K/AKT pathway alteration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>↑ AKT activity</td>
<td>(21, 31–33)</td>
</tr>
<tr>
<td></td>
<td>PI3K catalytic subunit amplification</td>
<td>(36–38, 85)</td>
</tr>
<tr>
<td>Prostate</td>
<td>↑ AKT activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ AKT expression</td>
<td>(24, 86)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>↑ AKT activity</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>↑ AKT expression</td>
<td>(49)</td>
</tr>
<tr>
<td>Endometrium</td>
<td>PTEN mutation</td>
<td>(55, 87)</td>
</tr>
<tr>
<td>Cervix</td>
<td>↑ AKT activity</td>
<td>(56, 58, 88)</td>
</tr>
<tr>
<td></td>
<td>↑ PTEN expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ PTEN expression</td>
<td>(56, 58, 88)</td>
</tr>
<tr>
<td></td>
<td>PI3K catalytic subunit amplification</td>
<td>(59)</td>
</tr>
<tr>
<td>Breast</td>
<td>↑ AKT activity</td>
<td>(59)</td>
</tr>
<tr>
<td>Stomach</td>
<td>↓ PTEN expression</td>
<td>(69)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>↑ AKT activity</td>
<td>(90)</td>
</tr>
</tbody>
</table>
maintain AKT-dependent transformation and fosters sustained hypoxia-inducible factor-1α activity (11). Cross-talk also exists between the AKT and BRAF signaling pathways (43); BRAF mutations are very common in human melanomas and melanocytic nevi (44).

Dysregulation of AKT signaling may also be an important event in nonmelanoma skin photocarcinogenesis. UVB light, a major cause of nonmelanoma skin cancer, induces PI3K/AKT activity in cultured human keratinocytes. This leads to activation of the FOS and COX-2 promoters, both of which have been associated with the tumorigenic actions of UVB (see ref. 45). In healthy human keratinocytes, the activation status of AKT determines sensitivity to the early-activated apoptotic pathway induced by UVB (46). In SKH-1 hairless mice, a good model for human photocarcinogenesis (45), AKT is activated following either acute or chronic exposure to UVB irradiation (47). Treatment with the flavonoid silibinin strongly inhibits UVB-induced AKT phosphorylation (among other signaling pathways; ref. 47), suggesting that down-regulation of AKT signaling may contribute to the chemopreventive actions of silibinin in this context (48).

**Colon**

Normal colonic mucosa and hyperplastic polyps express very low levels of AKT. Immunoreactivity to a pan-AKT antibody was detected in 57% (17 of 30) of adenomas and in an equal percentage (21 of 37) of sporadic colorectal cancers, with increased expression less common in cancers with microsatellite instability (8 of 26, 31%). Staining with an AKT2-specific antibody duplicated findings with the pan-antibody, implicating AKT2 as the predominant isoform. In addition to increased expression, AKT was hyperactivated in neoplastic colonic epithelium (49). The importance of the PI3K pathway in colon cancer can be appreciated from the finding that ~40% of the 146 human colorectal tumors examined had alterations in one of eight PI3K pathway genes examined (50).

Studies in chemical and genetic models confirm that AKT is up-regulated in the early stages of intestinal tumorigenesis. In chemical carcinogenesis experiments, AKT was overexpressed in rat premalignant colonocytes and in 42% of the tumors that developed (49). In Apcmin/+ mice, a model of human colon cancer, total AKT protein levels were significantly elevated in tumors compared with normal cells; levels of pAKT were also increased >35-fold in tumors (51). AKT1 is a critical effector of PTEN loss in the small intestine. AKT1 deficiency suppressed formation of intestinal polyps in PTEN+/− mice, with AKT1 haploinsufficiency sufficient to inhibit both tumor incidence and multiplicity almost to the extent seen with complete AKT1 loss (8).

Aberrant activation of the canonical Wnt pathway is a key step for initiation of colorectal cancer. This leads to constitutive transcription by the β-catenin/T-cell factor complex (52). Expression of AKT1 is activated by the β-catenin/T-cell factor complex in human colon cancer cells, and overexpression of AKT1 colocalizes with overexpression/nuclear localization of β-catenin in sporadic colorectal carcinoma biopsies (53). New studies show that...
AKT phosphorylates β-catenin at Ser552, resulting in its nuclear localization (54). Thus, β-catenin activates AKT, which can phosphorylate and activate β-catenin, suggesting that a positive feedback loop exists. Furthermore, PTEN-deficient intestinal stem cells that show increased AKT activity are competent to initiate intestinal polyposis. These findings imply that aberrant activation of the PTEN/AKT pathway may be directly involved in colon tumor formation at the level of the cancer stem cell (54).

Endometrium

Mutations and deletions of PTEN are the most common genetic changes found in human endometrial carcinoma. PTEN mutations are prevalent in both precancerous and cancerous endometrial tissues; one study found PTEN mutations in 55% (16 of 29) of premalignant lesions (55). As in the prostate and intestine, model studies showed that AKT1 is a critical effector of PTEN loss in the endometrium. AKT1 deficiency suppressed formation of both precancerous and malignant endometrial lesions and carcinomas in PTEN+−/− mice (8).

Cervix

A link has been found between AKT and the human papillomavirus-16 (56), a prominent cause of cervical cancer in women (57). The E7 protein is one of the major transforming proteins expressed by human papillomavirus-16. E7 acts by inhibiting the function of retinoblastoma (RB) tumor suppressor protein (56, 58). Expression of pAKT was increased in precancerous cervical lesions and correlated with RB loss (56). In model studies, expression of human papillomavirus-16 E7 protein in primary human foreskin keratinocytes increased AKT activity, inhibited differentiation, and induced hyperproliferation. Knocking down RB alone was sufficient to enhance AKT activity in differentiated keratinocytes. Together, these data suggest a mechanistic link between inactivation of RB by E7 and increased AKT activity during cervical transformation.

Breast

As in other tissues, AKT is activated in a subset of premalignant breast lesions. In a small study, pAKT was absent or weakly expressed in normal breast tissue and fibroadenomas, but was markedly increased in intraductal hyperplasias, ductal carcinomas in situ, and invasive cancers (59). In a larger study, pAKT was overexpressed in 33% (38 of 114) of ductal carcinomas in situ and in a similar percentage (38%, 52 of 136) of invasive breast cancers. In contrast to AKT activation, which was not associated with tumor progression, functional PTEN decreased as the disease progressed. PTEN expression

---

**Figure 3.** Broadly targeted inhibitors of AKT. SR13668 is a synthetic analogue of diindolylmethane.
was lost in 12% of ductal carcinomas in situ (13 of 113) and in 25% (34 of 134) of invasive lesions (60). In ductal carcinomas in situ, loss of PTEN expression correlated with high nuclear grade and necrosis (60).

Animal studies suggest that AKT isoforms may play differential and complex roles during breast cancer development. AKT1 deletion markedly delayed murine mammary tumor formation driven by activated RAS (12). Although expression of activated AKT1 accelerated HER-2/NEU-driven mammary tumor formation, the tumors that developed were highly differentiated, poorly invasive, and rarely metastasized (25). In human breast cancer cell lines, AKT1 activation blocked invasion and migration (27). Consistent with these findings, knocking out AKT1 inhibited the development of mammary adenocarcinomas and increased the invasive potential of HER-2/NEU and polyoma middle T-driven tumors in transgenic mice. On the contrary, ablation of AKT2 enhanced tumor formation in these mice (22). In apparent contrast to the in vitro studies described above (27), metastatic spread was decreased in both AKT1 and AKT2 knockout animals. Notably, AKT wild-type mice express AKT1 in both mammary epithelial and stromal cells, but express AKT2 primarily in the stroma (22). This may contribute to the differential effects noted between in vitro and in vivo studies.

The status of the TSC2 tumor suppressor may be critical for the invasion-inhibiting actions of AKT1. TSC2 diminished the anti-invasive actions of AKT1 in breast cancer cells, apparently acting as a tumor promoter under these conditions (61). Thus, the cellular milieu in which AKT is activated determines cell fate.

**AKT Inhibitors**

Classes of AKT-specific inhibitors that have been developed include agents that target the pleckstrin homology domain or the ATP-binding pocket, allosteric inhibitors, and pseudosubstrates (Fig. 2). These inhibitors diminish the growth of tumor cell lines with constitutively active AKT and induce regression of AKT-dependent tumors in vivo. In some cases, efficacy can be increased in combination with mTORC1 inhibitors (62). Among the toxicities observed with AKT inhibitors are effects on glucose homeostasis (62). This likely stems from inhibition of AKT2, which regulates normal insulin signaling (63). It is hoped that the development of isoform-specific inhibitors may mitigate these effects (64).

No prevention studies per se have been conducted with specific AKT-targeted agents, and their biological properties are covered only briefly here. More details can be found in recent reviews (17, 63–65). The pleckstrin homology domain inhibitor perifosine (Keryx Biopharmaceuticals) is the most clinically advanced AKT inhibitor. It is currently in phase II trials alone and in combination to treat multiple forms of cancer (66). Clinical studies found no evidence of perifosine-induced hyperglycemia, and gastrointestinal side effects were dose limiting. As perifosine interacts with additional signaling pathways downstream of growth factors (e.g., extracellular signal-regulated kinase 1/2), it will be important to correlate clinical effects with modulation of AKT signaling. Indeed, this measurement will be an essential aspect of the clinical development of all AKT inhibitors. Other pleckstrin homology domain, lipid-based AKT inhibitors have been examined preclinically. These include a series of phosphatidylinositol ether lipid analogues (67), such as PX-316 (ProX Pharmaceuticals). Potential issues with pleckstrin homology domain inhibitors include selectivity for AKT versus other pleckstrin homology domain-containing proteins, limited bioavailability and solubility, and the potential for causing hemolysis (17, 65).

Among the most well-characterized inhibitors that target the ATP-binding pocket are a series of indazole-pyridines synthesized by researchers from Abbott Laboratories. The most potent of these, A-443654, was highly selective for AKT, suppressed growth of AKT-dependent tumors in vivo, and displayed increased efficacy in combination with the mTORC1 inhibitor rapamycin. However, efficacy was achieved only at doses ~2-fold lower than the maximally tolerated dose. Increased insulin secretion was noted together with side effects associated with abnormal glucose metabolism. The mechanism of action of these indazoles is complex and incompletely understood. Unlike most AKT inhibitors, they induce an apparent feedback loop, which increases phosphorylation of AKT at both Ser473 and Thr308; this is observed despite concomitant decreases in activation of AKT downstream targets (62).

Researchers at Merck & Co. have identified a series of isoform-selective AKT inhibitors. The most potent are the pyrazinones AKTI-1 and AKTI-2, which are selective for AKT1 and AKT2, respectively, and the tricyclic quinoxaline AKTI-1/2, which inhibits both AKT1 and AKT2. These compounds are inactive toward related kinases, are not competitive with ATP, and require the presence of the pleckstrin homology domain, although they do not bind to the latter. In combination with tumor necrosis factor-related apoptosis-inducing ligand or standard chemotherapeutics, maximal apoptotic activity is achieved in cell lines when both AKT1 and AKT2 are inhibited either by virtue of combined administration of AKTI-1 and AKTI-2 or by using the dual inhibitor (64). In in vivo studies, AKTI-1/2 suppressed basal and growth factor-stimulated phosphorylation of AKT1 and AKT2 in the lungs of treated animals without affecting AKT3 phosphorylation. However, poor solubility and pharmacokinetic characteristics precluded further in vivo experimentation (64).

**Modulating AKT Signaling to Prevent Cancer**

Inhibition of AKT signaling has been associated with the biological actions of numerous chemopreventive compounds (Fig. 3). A few examples include curcumin (68), selenium (69), and the flavonoids quercetin (70), genistein (71), apigenin (72), and silibinin (73). Most of these effects

Mol Cancer Ther 2007;6(8). August 2007

Downloaded from mct.aacrjournals.org on August 4, 2017. © 2007 American Association for Cancer Research.
have been shown in vitro, in some cases using doses far above those that are physiologically achievable. However, several in vivo studies have correlated chemopreventive actions with diminution of AKT signaling. Suppression of intestinal tumor formation by sulforaphane (74) and epigallocatechin gallate (51) in Apc<sup>min/+</sup> mice was associated with decreased activation of AKT. In the latter study, oral administration of epigallocatechin gallate markedly suppressed levels of pAKT in intestinal tumors without significantly altering total AKT levels. Epigallocatechin gallate is known to affect many signaling pathways; indeed, extracellular signal-regulated kinase 1/2 phosphorylation was also dramatically decreased in the intestines of these animals. Thus, the contribution of AKT diminution to the chemopreventive actions of epigallocatechin gallate in this model remains unknown.

Another chemopreventive agent whose effects on AKT signaling have been studied in some detail is the rotenoid deguelin. In AKT-inducible transgenic mice, deguelin was competent to suppress AKT activation in the lung (35). At doses achievable in vivo, it reduced pAKT levels, induced apoptosis, and suppressed proliferation of premalignant and malignant human bronchial epithelial cells. At similar doses, only minimal effects were observed in normal bronchial cells. Importantly, overexpression of a constitutively active AKT diminished the actions of deguelin, showing that AKT mediates, at least in part, these effects in lung cells (30). Blockade of AKT activation also likely contributes to the proapoptotic actions of the agent in breast cancer cell lines (75) and antiangiogenic effects in in vitro models (76). In in vivo studies, deguelin inhibited formation of murine lung tumors in conjunction with suppression of AKT activation (35).

Suppressing phosphoinositide-dependent kinase-1 (PDK-1)/AKT signaling may play a role in the antitumor activity of a subset of selective cyclooxygenase-2 inhibitors. PDK-1 is the kinase upstream of AKT responsible for phosphorylating AKT at Thr<sup>308</sup> (63). For example, the cyclooxygenase-2 inhibitor celecoxib, which decreases colon cancer risk in humans and animals (77), diminishes PDK-1/AKT signaling (78). Effects on PDK-1/AKT are noted in conjunction with induction of apoptosis/cell cycle arrest and inhibition of angiogenesis and metastasis (78). The direct target of celecoxib remains unknown, with evidence pointing to AKT and/or PDK-1 (78). The importance of blunting AKT activity for the chemopreventive actions of celecoxib has recently been examined. Clinically relevant doses of celecoxib in combination with another chemopreventive, N-(4-hydroxyphenyl)retinamide, inhibited growth and induced apoptosis in premalignant human bronchial cell lines. These effects were linked with decreased activity of AKT and its molecular targets. Overexpression of constitutively active AKT partially protected bronchial epithelial cells from the apoptotic effects of the drug combination. Thus, the growth-inhibitory effects of celecoxib and N-(4-hydroxyphenyl)retinamide were, in part, attributable to attenuation of AKT signaling (79).

The cruciferous vegetable component indole-3-carbinol has shown extensive chemopreventive activity, which may also be associated with down-regulation of AKT signaling (80). The drug SR13668 was designed to optimize the anticancer activities of indole-3-carbinol and minimize its undesirable metabolic, estrogenic, and toxicologic characteristics. The growth-inhibitory effects of SR13668 in breast, ovarian, and prostate xenografts in vivo and cell lines in vitro correlate with decreased pAKT expression (81). SR13668 is orally available and shows no adverse effects on the fasting glucose levels in mice at 10-fold higher doses than needed for antitumor activity (81). Subsequent to phase 0 studies to define the best formulation, the Division of Cancer Prevention is planning to initiate studies with SR13668, most likely in a lung dysplasia setting.

Finally, a 2-week treatment with mTORC1 inhibitor RAD-001 was competent to completely reverse the PIN phenotype in the ventral prostate of mice expressing human AKT1. These effects were associated with induction of apoptosis and inactivation of hypoxia-inducible factor-1α target genes, including genes encoding most glycolytic enzymes (10). The prospects for using mTORC1 inhibitors for chemoprevention have recently been reviewed.3

**Strategies for Developing AKT Inhibitors for Prevention**

Because chemopreventive agents will likely be administered for extended periods, availability of oral formulations and chronic safety are major factors to be considered during clinical development. The greatest benefit in terms of both efficacy and toxicity may be achieved by combining AKT inhibitors with other chemopreventive drugs. Specifically, overexpression and/or activation of AKT in tumor cells causes resistance to agents that target elements upstream of AKT. For example, activation of AKT causes resistance to the epidermal growth factor receptor inhibitor gefitinib (17), suggesting that increased preventive/therapeutic efficacy can be achieved by administering AKT inhibitors together with epidermal growth factor receptor inhibitors. Combining AKT inhibitors and the chemopreventive drugs tamoxifen or retinoic acid may also have translational implications, given that hyperactivation of AKT contributes to resistance to both drugs in tumor cell lines (17, 18). The efficacy of AKT-specific inhibitors can also be boosted in therapeutic models in combination with mTORC1 inhibitors (62). This is apparently due to the ability of AKT inhibitors to override the negative feedback loop, which leads to activation of AKT when mTORC1 is chronically inhibited (6).

The potential for both on-target toxicities (i.e., effects on glucose homeostasis), as well as off-target toxicities, must be rigorously assessed. The decreased glucose tolerance

---

observed with PI3K inhibitors is precluded when administered with the antidiabetic drug pioglitazone. Importantly, this decrease in toxicity is observed in the absence of effects on antitumor actions (5). Pioglitazone may also have similar effects in combination with AKT inhibitors. Other antidiabetic drugs, such as metformin, might also be used to control hyperglycemia. Both metformin and pioglitazone activate the energy sensor AMP-activated protein kinase, which in turn inhibits signaling through mTORC1 (82). Furthermore, both drugs are chemopreventives in their own right (83, 84). It is possible that antidiabetic drugs will not only relieve the toxicity of AKT inhibitors but also augment their efficacy.

As with all molecularly targeted approaches, pharmacodynamic markers are necessary to direct preventive/therapeutic development of AKT inhibitors. Clinical studies with AKT-specific inhibitors are in their early phases, and thus far, no markers for patients with a high probability of responding to AKT inhibitors, or biomarkers of dose/efficacy, have been validated. Based on the results of preclinical studies, initial studies should use cohorts bearing precancerous lesions in which the AKT pathway is hyper-activated. Outcome would correlate lesion regression with pathway inhibition. Trials should examine effects on AKT targets to determine which is the best predictor of response (Fig. 1). As preclinical studies suggest that mTORC1 is a critical mediator of AKT-dependent tumorigenesis, emphasis should initially be placed on evaluating elements in this branch of the pathway. Additional issues that need to be addressed include the merits of targeting upstream (e.g., PI3K, growth factor receptors, and RAS) versus more selective downstream targeting (e.g., mTORC1). The virtues of using pan-AKT versus isoform(s)-specific AKT inhibitors must also be examined.

Conclusions
Blunting AKT signaling is associated with the biological actions of known preventive agents with acceptable toxicity profiles; however, these agents do not selectively target AKT. To date, no AKT-specific inhibitors are available that are clinically viable as cancer preventives, and the ability to establish an acceptable therapeutic window with such agents remains unknown. Probably, the best evidence that this can be achieved comes from studies in PTEN-deficient mice. Haplodeficiency of AKT1 is competent to markedly diminish tumor formation in these mice in the absence of severe physiologic consequences (8). This finding increases the probability that targeted, partial inhibition of AKT activity using small molecules will prevent cancer.

Understanding the complex circuitry of the AKT pathway and cross-talk between other major cellular circuits will be crucial to pharmacologic manipulation for cancer prevention. Combinations of “natural” or dietary agents/supplements that seem to modulate aspects of this pathway with molecularly targeted agents, such as receptor blockers, may provide synergistic responses with minimal toxicity.

References
Downloaded from mct.aacrjournals.org on August 4, 2017. © 2007 American Association for Cancer Research.


Targeting the AKT protein kinase for cancer chemoprevention

James A. Crowell, Vernon E. Steele and Judith R. Fay


Updated version Access the most recent version of this article at:
http://mct.aacrjournals.org/content/6/8/2139

Cited articles This article cites 88 articles, 36 of which you can access for free at:
http://mct.aacrjournals.org/content/6/8/2139.full#ref-list-1

Citing articles This article has been cited by 20 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/6/8/2139.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.