Abstract. Gastric cancer is a highly lethal malignancy with more than 700,000 deaths every year worldwide. Despite significant improvements in our understanding of disease biology, the 5-year survival rates remain low. With the exception of trastuzumab, targeted agents have failed to add any meaningful benefit for this patient population, despite promising pre-clinical data. Protein kinase B (AKT) is essential for cell growth, proliferation, and survival. Aberrant activation of AKT is one of the most common molecular findings in human malignancies including gastric cancer, and it is believed to play an important role in cancer cell survival and chemotherapy resistance. Combining phosphatidylinositol 3-kinase (PI3K)/AKT pathway inhibitors with chemotherapy has successfully attenuated chemotherapeutic resistance in gastric cancer cell lines. Drugs designed to specifically target AKT are now being developed for clinical use. In this article, we will review the current knowledge on AKT signaling in the pathogenesis of gastric cancer and the evolving therapeutic implications of targeting this pathway.

Gastric cancer is the 13th most common type of cancer in the United States. In 2009, an estimated 21,130 new cases of gastric cancer were diagnosed in the US and 10,620 died from the disease (1). Survival for patients with gastric cancer has improved only modestly over the last 50 years. In the United States, where endoscopic screening is not routinely performed, patients are often presented with advanced stage. Overall five-year survival rates are less than 10% (2).

Cytotoxic agents have been the mainstay of systemic treatment for metastatic disease. Median survival for patients with distant metastases remains approximately 8-10 months. Combination chemotherapy produces higher response rates than single agents at the expense of a higher toxicity and modest prolongation of disease control and survival (3-5). During recent years, multiple molecular abnormalities underlying gastric cancer carcinogenesis and progression have been identified. This has improved our understanding over the biology of the disease and stimulated the search for novel therapeutic approaches (6). The phosphatidylinositol 3-kinase 3-kinase (PI3K) pathway has been implicated in gastric cancer, although its precise function remains to be determined. In this article, we discuss the role of PI3K/AKT pathway in gastric carcinogenesis and chemotherapy resistance.

The PI3K/AKT Pathway

The PI3K enzymes are involved in the phosphorylation of membrane inositol lipids (7). The activation of PI3K generates the second messenger phosphatidylinositol (3,5)-trisphosphate (PIP3) from phosphatidylinositol 4,5-bisphosphate (PIP2). This recruits proteins to the cell membrane, including the AKT/PKB kinases, resulting in their phosphorylation by phosphoinositide-dependent kinase 1 (PDK1) (8), and by PDK2 (9). AKT is a serine-threonine protein kinase. Activated AKT translocates to the cytoplasm and nucleus and activates downstream targets involved in survival, proliferation, cell cycle progression, growth, migration, and angiogenesis (10, 11) (Figure 1).

The AKT family consists of three isoforms (AKT1, 2, and 3) that have overlapping functions. AKT1 has a role in overall growth (12, 13), AKT 2 in insulin signaling (14, 15), and AKT 3 in brain development (16, 17).

AKT is negatively regulated by the phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a tumor suppressor gene that dephosphorylates PIP3.

AKT mediates the phosphorylation of mammalian target of rapamycin complex 2 (mTORC2) which, when activated,
plays a role in protein translation and synthesis, and angiogenesis. Dysregulation of the PI3K/AKT/mTOR pathway can occur secondary to oncogenic mutations of phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA) (18), loss of PTEN function (19, 20), mutation of AKT/PKB isoforms (11) or upstream activation through other pathways such as IGF1R.

Rationale for AKT Inhibition in Gastric Cancer

Somatic mutation of the kinase domain of AKT2 has been commonly reported in human cancer, including gastric cancer (19). Hyperactivation of AKT is one of the most common molecular findings in human malignancies (21). The correlation between AKT activation and prognosis in human malignancies remains to be fully elucidated; AKT activation is reported to correlate with poor outcome in subgroups of breast and pancreatic cancer (22-24), while neither PIK3CA gene copy number nor activated AKT has prognostic significance in lung cancer (25).

In gastric cancer, expression of AKT and phosphorelated AKT (p-AKT) are detected in 74% and 78% of tumors respectively (26). In a report of 50 advanced gastric carcinomas (27), there was a statistically significant correlation between p-AKT expression and depth of tumor, number of involved lymph nodes and poor outcome. Nuclear p-AKT expression was also found to inversely correlate with the apoptotic index. Due to the pivotal role this pathway plays in the gastric cancer biology, inhibition of AKT might play a role in the treatment of gastric cancer and the reversal of chemotherapy resistance.

AKT and Chemotherapy Resistance

Chemoresistance is a major obstacle to treatment of all malignancies including gastric cancer. AKT is activated in advanced gastric cancer (28, 29) and it is believed that induction of AKT activity by chemotherapy leads to cell resistance; therefore, inhibition of AKT activation might overcome resistance of cancer cells to anti-cancer therapy. The exact mechanism of AKT activation leading to chemoresistance remains unclear. Different mechanisms have been suggested (Table I).

AKT and PTEN. Several studies have suggested that loss of function of the tumor suppressor gene PTEN might underly AKT activation in gastric cancer and chemoresistance (29, 30). In vitro, ectopic PTEN expression down-regulates basal AKT activity and significantly sensitizes gastric cancer cells to anticancer drugs. The role of PTEN was evaluated in resected gastric tumors. Primary gastric carcinoma tissues and corresponding normal mucosa were obtained from 119 gastric cancer patients and were further analyzed. Methylation of PTEN was rarely recognized. However, patients who had a higher activated AKT appeared to have loss of heterozygosity of PTEN (p<0.0008), and the prognosis of those patients was significantly poorer. When chemotherapeutic sensitivities of these tumors were studied in an 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, activated AKT was associated with increased resistance to multiple chemotherapeutic agents including 5-fluorouracil, doxorubicin, mitomycin C, and cisplatin (31).

AKT and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). Another theory of the AKT role in chemoresistance is through the activation of the transcription factor NFκB. Chemotherapy promotes the activation of NFκB which can be blocked by inhibition of PI3K/AKT. NFκB regulates various genes involved in angiogenesis, metastasis and suppression of apoptosis (32); it is believed to be a substrate of AKT (33, 34) and to play a role in chemoresistance in gastric cancer (35-37).

PI3K activation. Treating gastric cancer cells with etoposide and doxorubicin increased AKT activity in a time- and concentration- dependent manner independent of baseline activity but dependent on PI3K. PI3K blockade increased the sensitivity of gastric cancer cells to anticancer drugs. The precise mechanism whereby chemotherapy induces PI3K/AKT activities is yet to be defined (38).

Cellular prion protein (PrPC). PrPC is a glycosyl-phosphatidylinositol-anchored membrane protein and its expression is up-regulated in an adriamycin (ADR)-resistant gastric carcinoma cell line. Overexpression of PrPC in gastric cancer up-regulates P-glycoprotein, leading to tumor cell resistance to chemotherapy. In human gastric cancer cell lines, immunohistochemistry revealed a positive correlation between PrPC and p-AKT expression; the level of p-AKT increased in PrPC-transfected cells and inhibition of PrPC expression resulted in a decrease in p-AKT expression. On the other hand, inhibition of the PI3K/Akt pathway results in a reduction of multidrug resistance of gastric cancer cells through down-regulation of P-glycoprotein induced by PrPC (39).

B-cell lymphoma-2 (BCL2) and BCL2-associated X protein (BAX). p-AKT significantly up-regulates expression of BCL2 and down-regulates that of BAX, promoting growth factor-mediated cell survival. Inhibition of p-AKT reversed these effects, leading to p53 up-regulation and down-regulation of the expression of P-glycoprotein and the transcripation of the multidrug resistance gene (40). This hypothesis was further evaluated by transfecting siRNA eukaryotic expression vectors of AKT1 into gastric cells. After transfection, the expression of AKT1 was decreased as expected in the
transfected cells and down-regulation of AKT1 significantly enhanced the sensitivity of these cells to chemotherapeutic agents by down-regulating the expression of BCL2, and up-regulating the expression of PTEN (41).

**Y-Box-binding protein-1 (YB-1).** Another proposed mechanism of AKT mediated chemotherapy resistance is through YB-1, a broad-specificity RNA-binding protein that is involved in the regulation of mRNA transcription, splicing, translation, and stability (42). In 81 gastric cancer samples and corresponding normal mucosa, AKT activation was again found to be associated with an increased resistance to multiple chemotherapeutic agents including 5-fluorouracil, doxorubicin, mitomycin and cisplatin. Nuclear expression of YB-1 correlated with a high expression of p-AKT, as well as LOH of PTEN, leading to an aggressive phenotype with poor prognosis. The data suggest that phosphorylation by AKT disables the inhibitory activity of YB-1 and thereby enhances the translation of transcripts that are necessary for oncogenesis and chemoresistance (43).

**Role of other transcript functions.** Other mechanisms thought to be involved in PI3K/AKT induction of chemoresistance in gastric cancer include: phosphorylation of forkhead transcription factors (44); phosphorylation of murine double minute oncogene (MDM2) which enhances its nuclear localization leading to an increase in p53 degradation (45); modulation of caspase-3 and caspase-9 activities (46, 47).

**AKT and Metastasis in Gastric Cancer**

More than 50% of patients with gastric cancer have distant metastases upon diagnosis. No molecular indicator is yet available to predict metastasis in this disease. In a recent study of 53 gastric carcinoma patients, p-AKT was not expressed in normal gastric mucosa, but was highly expressed in lymph node metastases and distant metastases compared to the primary tumor, suggesting that phosphorylation of AKT plays a role in promoting invasion and metastasis in gastric cancer patients (48).

In another study, combretastatin A4 (CA4), an antiangiogenic compound, inhibited growth of a cell line with high AKT expression and down-regulated AKT in vitro, supressing tumor formation and metastasis by reducing cell attachment, migration, and invasiveness. P-Akt expression correlated well with response to CA4 (49).

**AKT Inhibitors Currently in Development**

Several classes of AKT inhibitors are currently in development (Table II), including isoform selective AKT catalytic-domain and Pleckstrin homology (PH) domain inhibitors. Targeting all AKT isoforms has been shown to be superior to the inhibition of a single isozyme although toxicity may be a potential issue. GS690693 (GlaxoSmithKline, Inc.) is an ATP-competitive, low-nanomolar pan-AKT kinase inhibitor (50). Several other small-molecule AKT inhibitors are now in early clinical trials, including MK2206 (Merck, Inc.) a potent highly selective pan-AKT allosteric inhibitor that is currently in phase I/II clinical trials, both as a single agent and in combination with other agents.

Triciribine phosphate (TCN-PM) is a potent, small-molecule inhibitor of all three isoforms of AKT in vitro. In a phase I study of tumors with increased AKT phosphorylation as measured by immunohistochemical analysis (IHC), treatment with TCN-PM inhibited tumor p-AKT at doses that were tolerable, yet single agent activity was not observed (51).

**Can We Select Patients Who Are Likely to Benefit from AKT Inhibitors?**

Gastric cancer is clinically and epidemiologically a heterogeneous disease. Molecular intrapatient and even intratumoral heterogeneity has been reported, suggesting the presence of multiple cell clones in the same patient (52). Although not studied in human gastric cancer, a significant degree of mutational heterogeneity in PTEN was found among different metastatic sites within the same patient in other malignancies (19), which complicates patient selection; in addition baseline p-AKT might not correlate with the p-AKT level following chemotherapy. The success of agents targeting the AKT pathway could be maximized by identifying those patients who may have a higher likelihood of responding by testing pre- and post-treatment tumor samples. Another important factor is to determine if the inhibitor maintains target inhibition. In a phase I study of MK2206 in patients with advanced solid tumors, a
A decrease in blood p-AKT concentration was evident at all dose levels (53).

In a phase I study of TCN-PM in patients with solid tumors (51), TCN-PM was administered to patients whose tumors displayed evidence of increased p-AKT as measured by IHC. Tumor specimens collected before and after treatment were assessed for p-AKT by IHC and western blot analyses. Modest decreases in tumor p-AKT following therapy were observed, however, single agent activity was not observed, possibly due to the small sample size.

Combination of AKT Inhibitors with Conventional Chemotherapy and Other Targeted Therapies

Since an activated AKT pathway contributes to tumor resistance over chemotherapy, targeting AKT activation has emerged as a promising approach in combination with chemotherapy and targeted therapy. The combination of conventional chemotherapy and AKT inhibitors has been shown, *in vitro*, to decrease AKT activity which is thought to play a role in chemoresistance. A second strategy is to target different components of a single oncogenic pathway (*e.g.* combine PI3K and AKT inhibitors). A third possibility

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<th>Compound</th>
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<td>Merck</td>
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<tr>
<td>TCN(PM)</td>
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*Studies terminated; **Akt anti-sense.
is to combine inhibitors of parallel signaling pathways that crosstalk or regulate each other. For example, the activation of the RAS pathway overcomes resistance to PI3K inhibition (54), which suggests that targeting this pathway along with AKT inhibition may be necessary. Confirmation in preclinical models is crucial and overlapping toxicity needs to be addressed.

Conclusion

Cytotoxic agents are effective in advanced gastric cancer, but overall survival does not exceed 12 months in phase III studies. AKT plays an important part in cell cycle regulation, is critical to gastric cancer cell survival and proliferation, and plays a significant role in chemotherapy resistance which makes it an attractive target for anticancer therapy. Although the preliminary data are promising, several questions have yet to be answered. For example, it is unclear whether inhibition of AKT is sufficient, in itself, to improve patient outcomes, or whether combination therapy with other cytotoxic or targeted agents is necessary. We have also yet to identify validated tests to predict which tumors will prove sensitive to AKT inhibition. Biomarker-driven clinical trials are necessary to answer these questions.

References


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