Current Topics

Target Therapy for Cancer:
Anti-cancer Drugs Targeting Growth-Factor Signaling Molecules

Targeting the Extracellular Signal-Regulated Kinase Pathway in Cancer Therapy

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The extracellular signal-regulated kinase (ERK) pathway is a major determinant in the control of diverse cellular processes such as proliferation, survival, and motility. This pathway is often upregulated in human cancers and as such represents an attractive target for mechanism-based approaches to cancer treatment. However, specific blockade of the ERK pathway alone induces mostly cytostatic rather than proapoptotic effects, resulting in limited therapeutic efficacy. Blockade of the constitutively activated ERK pathway by an ERK kinase (MEK) inhibitor sensitizes tumor cells to apoptotic cell death induced by several cytotoxic anticancer agents including microtubule-destabilizing agents and histone deacetylase inhibitors, not only in vitro but also in tumor xenografts in vivo. Thus, low concentrations of these anticancer drugs that by themselves show little cytotoxicity effectively kill tumor cells in which the ERK pathway is constitutively activated when co-administrated with a MEK inhibitor. The combination of a cytostatic signaling pathway inhibitor (MEK inhibitors) and conventional anticancer drugs (microtubule-destabilizing agents or histone deacetylase inhibitors) provides an excellent basis for the development of safer anticancer chemotherapies with enhanced efficacy through lowering the required dose of the latter cytotoxic drugs.

Key words anticancer drug; ERK pathway; MEK inhibitor; molecular targeted therapy; combination therapy

1. INTRODUCTION

The most fundamental characteristic of individual cells that constitute multicellular organisms is communicating with each other to regulate the behavior of respective cells strictly in a coordinated manner, which is essential for the maintenance of homeostasis in the organisms; such behaviors include cell division, differentiation, motility, and survival. Cell-to-cell communication is initiated by the binding of an extracellular signaling molecule, which is synthesized and released by cells that emit signals, to the corresponding receptor specifically expressed in the target cells. The signals are then processed and integrated by complex circuits to induce the expression of several genes, which finally culminates in the induction of appropriate responses in the cells.

The extracellular signal-regulated kinase (ERK) pathway is activated in a variety of cell types by diverse extracellular stimuli and is among the most extensively studied of signaling pathways that connect various membrane receptors to the nucleus.1 Activation of the ERK pathway is triggered by guanosine 5’-triphosphate (GTP) loading of Ras at the plasma membrane, which is followed by sequential activation of a series of protein kinases including a member of the Raf family (such as Raf-1), mitogen-activated protein (MAP) kinase or ERK kinase (MEK) 1 and 2, and ERK1 and ERK2. Activated ERK1/2 then phosphorylates various downstream substrates that contribute to the regulation of a wide range of cellular processes such as proliferation, survival, and motility (Fig. 1).

Mechanisms for precise spatiotemporal control of intracellular signaling pathways have evolved to ensure homeostasis in multicellular organisms. Inappropriate activation of these pathways underlies several refractory diseases, with aberrant activation of the ERK pathway having been shown to be a key contributing factor to many types of human cancer.2,3 In particular, overexpression or activating mutation of the epidermal growth factor receptor (EGFR) gene,4 activating mutation of RAS,5 and activating mutation of RAF6 are associated with cancer and found to result in the activation of MEK1/2 and ERK1/2 in many cases. Inhibition of the ERK

Fig. 1. The ERK Pathway and PI3K-Akt Pathway
Abnormal signaling molecules (active mutation: RTK, Ras, Raf, PI3K, and Akt; deletion: PTEN) found in human cancer cells. Several pathway inhibitors are shown.
pathway thus represents a promising strategy for cancer treatment. Accordingly, interest in the components of the ERK pathway as attractive targets for cancer chemotherapy has exploded in the past few years. Recently, multiple small-molecule inhibitors have been developed to target different components throughout the ERK pathway, and several of them are currently in clinical oncology trials: these include EGFR tyrosine kinase inhibitors (gefitinib, erlotinib, lapatinib, afatinib, icotinib, varlitinib, etc.), Raf inhibitors (RG7204 [ve-murafenib], GS-K2118436, BMS-908662, etc.), and MEK inhibitors (AZD6244 [selumetinib], GSK1120212, RDEA119, MSC1936369, GDC-0973, RO4987655, PD0325901, etc.). Here, we focus on the potential application of small-molecule inhibitors of MEK as anticancer drugs.

2. BLOCKADE OF THE ERK PATHWAY ALONE IS INSUFFICIENT TO EFFECTIVELY INDUCE APOPTOTIC CELL DEATH IN TUMOR CELLS WITH ABBERRANT ACTIVATION OF THE PATHWAY

Specific blockade of the ERK pathway by MEK inhibitors totally inhibits the proliferation of a wide variety of tumor cells in which the ERK pathway is constitutively activated as a result of activation mutation of Ras, Raf, or EGFR. In these tumor cells, MEK inhibitors induce the upregulation of p27Kip1 (a cyclin-dependent kinase [CDK] inhibitor), association of p27Kip1 with cyclin E-CDK2 complexes, concomitant inhibition of cyclin E-CDK2 kinase activity, and consequent decrease in the phosphorylation state of the retinoblastoma protein (Rb), which finally leads to the activation of Rb to function as the essential guardian of the restriction-point gate.

In addition to the anti-proliferative effect, specific inhibition of the ERK pathway results in an anti-metastatic effect via several mechanisms, which include the downregulation of matrix metalloproteinase (MMP)-9 and the restoration of SH3P2 function. Expression of MMP-9 is regulated in an ERK pathway-dependent manner, and its activity is required for the induction of cell motility via the degradation of the extracellular matrix. Elevated expression of MMPs is associated with increased metastatic potential in many tumor cells. SH3P2 is a negative regulator of cell motility, and its function is inhibited by ribosomal S6 kinase-mediated phosphorylation in an ERK pathway-dependent manner. All these observations further highlight the notion that specific blockade of the ERK pathway represents an attractive approach for cancer treatment.
therapeutic strategy to treat cancers.

Blockade of the ERK pathway alone, however, is mostly cytostatic rather than cytotoxic, resulting in only a moderate induction of apoptosis in tumor cells with aberrant pathway activation.19 Thus, although treatment of T24 bladder carcinoma cells (with H-Ras activation) with PD184352, a MEK inhibitor, totally suppresses the activation of ERK1/2 as well as cell proliferation (Fig. 2A), it does not increase the proportion of dead cells with a fractional DNA content (cells in sub-G1 phase) (Fig. 2B), which is a characteristic feature of apoptotic cell death. Furthermore, the MEK inhibitor-induced suppression of cell growth is reversible, with cells resuming proliferation after removal of the inhibitor (Fig. 2A)10; the majority of cells are just “resting” but not “dying” in the presence of MEK inhibitors. In accordance with these observations, recent clinical studies of MEK inhibitors in patients with advanced cancers have shown that, although PD184352 or AZD6244 achieves target inhibition at well-tolerated doses, these drugs alone exhibit insufficient antitumor activity.15,16 Efficient induction of apoptotic cell death is essential for the development of effective cancer chemotherapy.

3. BLOCKADE OF CONSTITUTIVELY ACTIVATED ERK PATHWAY ENHANCES THE CYTOTOXICITY OF MICROTUBULE-DESTABILIZING AGENTS OR Histon DEACETYLASE INHIBITORS IN TUMOR CELLS

Combination therapy is becoming the norm in cancer treatment, with combinations of cytotoxic agents with non-overlapping toxicities being driven by safety considerations. In this regard, specific interruption of the cytoprotective ERK pathway by MEK inhibitors has been proposed as a means to enhance the lethal actions of cytotoxic anticancer agents through a shift in the balance between pro- and anti-apoptotic signaling.17 Consistent with this notion, blockade of the ERK pathway by MEK inhibitors markedly enhances the cytotoxicity of several cytotoxic anticancer agents in tumor cells in which the ERK pathway is constitutively activated; these agents include microtubule-destabilizing agents14,18 and histone deacetylase (HDAC) inhibitors.19,20

MEK inhibitors (PD98059, PD184352, PD0325901) markedly and selectively enhance the induction of cell death by microtubule-destabilizing agents (vincristine, vinorelbine, TZT-1027) in a variety of tumor cell lines in which the ERK pathway is constitutively activated (HT-29 and TCO cells with Raf activation; HT1080, A549, and T24 cells with Ras activation; H1650, PC-9, and II-18 cells with EGFR activation), but not in tumor cells in which the ERK pathway is not activated (HeLa-S3, PC3, Colo320 cells) (Fig. 2B, left and 2C).14 Importantly, this effect of MEK inhibitors on cell death induction by microtubule-destabilizing agents is most pronounced at low concentrations of the latter drugs; under such conditions, microtubule-destabilizing agents by themselves exhibit only a small cytotoxic effect in the cells (Fig. 2B, right).

MEK inhibitor-mediated blockade of the ERK pathway enhances the induction of apoptosis by microtubule-destabilizing agents as well as markedly potentiates the therapeutic efficacy of microtubule-destabilizing agents in human tumor xenografts in nude mice.14,18 Thus, co-administration of PD184352 markedly sensitizes HT-29 or HT1080 tumor xenografts to TZT-1027- or vinorelbine-induced cytotoxicity. Low doses of TZT-1027 or vinorelbine that by themselves show little or moderate cytotoxicity suppress the growth of HT-29 xenografts almost completely and induce essentially complete regression of HT1080 xenografts when co-administered with PD184352 (Fig. 2D). None of the mice treated with the drug combination show signs of drug toxicity, including weight loss or gastrointestinal toxicity; they instead appear healthier than the vehicle-treated animals. Furthermore, such the enhanced therapeutic efficacy of the drug combinations is achieved by a relatively transient blockade of the ERK pathway. Recent clinical trials of MEK inhibitors in patients with advanced cancers have shown that daily administration of PD184352 or AZD6244 for up to several months is well tolerated, with the most common treatment-related toxicities being mild rash, diarrhea, asthenia, nausea, and vomiting.15,16 However, given the essential role of the ERK pathway in the regulation of a wide range of cellular processes including the immune response,21 shortening of the period during which an administered MEK inhibitor suppresses the ERK pathway might be expected to be beneficial in terms of reducing potential side effects in patients.

MEK inhibitors also enhance the cytotoxicity of HDAC inhibitors (HC-toxin, FK228, valproic acid, trichostatin A, MS-275) in a variety of tumor cells with aberrant ERK pathway activation, including those resistant to gefitinib, not only in vitro19,20 but also in tumor xenografts in vivo (Sakamoto et al., unpublished observations). As in the combination with microtubule-destabilizing agents, the enhancing effect is most prominently observed when tumor cells or tumor xenografts are treated with the combination of a MEK inhibitor and low concentrations of HDAC inhibitors. MEK inhibitors sensitize tumor cells to HDAC inhibitor-induced accumulation of reactive oxygen species (ROS), and the accumulated ROS mediate the induction of enhanced apoptotic cell death by the drug combination.

4. CONCLUDING REMARKS

After initial active interest, the ERK pathway is now becoming a highly promising therapeutic target for the development of mechanism-based anticancer drugs.22 However, specific blockade of the ERK pathway alone is mostly cytostatic rather than cytotoxic, which limits the therapeutic efficacy of MEK inhibitors when administered alone. ERK1/2 phospho-rylate BimEL, a pro-apoptotic B cell lymphoma-2 (Bcl-2) family protein, and thereby trigger its degradation by the proteasome.22 Thus, blockade of the ERK pathway by MEK inhibitors induces the suppression of BimEL phosphorylation, resulting in the stabilization and accumulation of this protein, which alone, however, is insufficient to induce substantial cell death in many tumor cells with aberrant ERK pathway activation. Cell death induction is determined by the balance between pro-apoptotic and anti-apoptotic signals. MEK inhibitor-induced up-regulation of Bim alone can induce substantial cell death in tumor cells in which expression levels of anti-apoptotic Bcl-2 family proteins (Bcl-2, Mcl-1, Bcl-xL, etc.) are very low; such tumor cells appear rather exceptional. In this regard, our recent results have indicated that the combination of a MEK inhibitor and a microtubule-destabilizing agent induces the up-regulation of pro-apo-
ptotic Bim and the down-regulation of several anti-apoptotic Bcl-2 family proteins in the prolonged mitosis following drug treatment (Kawabata et al., unpublished observations).

Constitutive activation of the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, the major cytoplasmic signaling pathway involved in the regulation of a wide variety of cellular processes including survival, motility, and angiogenesis, is associated with the neoplastic phenotype in many human tumor cells. Thus, inhibition of the PI3K-Akt pathway also represents a promising strategy for cancer treatment. Accordingly, multiple small-molecule inhibitors have been developed to target different components throughout this pathway, and several of them are currently in clinical oncology trials. These include PI3K inhibitors (NVP-BEX235, SAR245409, GSK2141795, etc.) and Akt inhibitors (perifosine, enzastaurin, etc.). However, specific blockade of the PI3K-Akt pathway by these inhibitors alone results in only a moderate induction of apoptosis in tumor cells in which the pathway is constitutively activated. All these PI3K-Akt pathway inhibitors are categorized as cytostatic but not cytotoxic agents. Importantly, blockade of the constitutively activated PI3K-Akt pathway by these inhibitors markedly sensitizes tumor cells to the induction of cell death by several anti-cancer drugs including microtubule-destabilizing agents, doxorubicin, and HDAC inhibitors. Thus, low concentrations of these cytotoxic anticancer drugs that by themselves show little cytotoxicity effectively kill tumor cells in which the PI3K-Akt pathway is constitutively activated when co-administered with LY294002, a PI3K inhibitor.

All these observations clearly indicate that combination of a cytostatic signaling pathway inhibitor, such as a MEK inhibitor or a PI3K inhibitor, and conventional anticancer drugs, such as microtubule-destabilizing agents or HDAC inhibitors, provides an excellent basis for the development of safer anticancer chemotherapies with enhanced efficacy by lowering the required dose of the latter cytotoxic drugs. Consistent with this notion, optimal use of molecular targeted therapies has recently been proposed to lie in combination treatment, either with classic cytotoxic agents or with other targeted therapies.

REFERENCES