Molecular Pathways: MERTK Signaling in Cancer

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Abstract

MERTK is a receptor tyrosine kinase of the TAM (Tyro3, Axl, MERTK) family, with a defined spectrum of normal expression. However, MERTK is overexpressed or ectopically expressed in a wide variety of cancers, including leukemia, non–small cell lung cancer, glioblastoma, melanoma, prostate cancer, breast cancer, colon cancer, gastric cancer, pituitary adenomas, and rhabdomyosarcomas, potentially resulting in the activation of several canonical oncogenic signaling pathways. These include the mitogen-activated protein kinase and phosphoinositide 3-kinase pathways, as well as regulation of signal transducer and activator of transcription family members, migration-associated proteins including the focal adhesion kinase and myosin light chain 2, and prosurvival proteins such as survivin and Bcl-2. Each has been implicated in MERTK physiologic and oncogenic functions. In neoplastic cells, these signaling events result in functional phenotypes such as decreased apoptosis, increased migration, chemoresistance, increased colony formation, and increased tumor formation in murine models. Conversely, MERTK inhibition by genetic or pharmacologic means can reverse these pro-oncogenic phenotypes. Multiple therapeutic approaches to MERTK inhibition are currently in development, including ligand "traps", a monoclonal antibody, and small-molecule tyrosine kinase inhibitors. Clin Cancer Res; 19(19); 5275–80. ©2013 AACR.

Background

In recent years, therapeutic agents targeting specific molecular aberrations in cancer cells have been effective at prolonging survival in multiple cancer types; however, for the majority of patients with cancer, the oncogenic drivers are complex and identification of additional therapeutic targets has become a major research focus. One potential target is MERTK, a member of the TAM-family of receptor tyrosine kinases, which also includes Axl and Tyro3 (reviewed in ref. 1). The physiologic functions of MERTK have only recently been defined in platelets and macrophages, but its overexpression and potential activation in a wide variety of cancers indicate that MERTK signaling confers an advantage on the tumor cell (2–26). Target validation studies, discussed below, suggest that MERTK inhibition is a viable strategy for decreasing tumor burden in preclinical models. Clinically relevant agents are under development in an effort to add MERTK to the list of effectively targeted proteins in patients with cancer.

Discovery of MERTK

MERTK has been implicated in cancer pathogenesis since it was first cloned from a human B-lymphoblastoid expression library and from a human glioblastoma library (7, 15). Although ectopically expressed in multiple lymphoid leukemia cell lines and patient samples, MERTK is absent from normal B and T lymphocytes, suggesting that MERTK may be an attractive target for childhood acute lymphoblastic leukemia (ALL) therapeutic development (7, 9, 27). MERTK sequencing from human and mouse revealed it to be the human ortholog of the chicken c-eyk proto-oncogene of v-eyk responsible for the oncogenic properties of the RPL30 avian retrovirus, which induces lymphomas, sarcomas, and other tumor types in chickens in vivo (28, 29). Unbiased gain-of-function retroviral insertion screens have also identified the oncogenic role of MERTK (30).

MERTK chimeric receptor signaling

Following cloning of the MERTK cDNA, multiple groups investigated the signaling pathways downstream of MERTK activation (Fig. 1). As a ligand had not yet been identified, early studies used receptor chimeras with the intracellular domain of MERTK fused to the extracellular domain of proteins with a known ligand or dimerization capability. An early strategy combined the transmembrane and cytoplasmic domains of MERTK with the extracellular domain of human colony-stimulating factor 1 (CSF-1) receptor (Fms; ref. 15). In this model, CSF-1 treatment induced
MERTK autophosphorylation, transformed NIH 3T3 cells, and activated phospholipase Cγ, phosphatidylinositol 3-kinase (PI3K), and p70 S6 kinase. Recruitment of growth factor receptor binding protein 2 (Grb2) and phosphorylation of Src homology and collagen (Shc) led to Raf-1, mitogen-activated protein/extracellular signal–regulated kinase (MEK), and extracellular signal-regulated kinase (Erk) activation.

Another MERTK chimeric protein was made by fusing the extracellular domain of CD8 to the intracellular domain of MERTK creating a constitutively active MERTK chimera (31). This MERTK chimera conferred an interleukin 3 (IL-3)–independent phenotype to Ba/F3 pro-B lymphocytes. The Grb2, MEK1, and Erk pathways as well as PI3K were also activated. NF-κB–mediated transcription was increased as assessed by a luciferase-reporter assay with potential activation of antiapoptotic signaling in tumor cells (reviewed in ref. 32). The p38 pathway was also activated and p38 inhibition decreased proliferation in MERTK chimeric Ba/F3 cells. Similar to NF-κB, p38 has complex effects on proliferation, migration, and survival in cancer cells (reviewed in ref. 33).

Finally, our group constructed a chimeric receptor composed of the extracellular and transmembrane domains of the EGF receptor (EGFR) fused to the intracellular domain of MERTK (34). Ligand activation prevented apoptosis in the myeloblast-like 32D cells promoting IL-3-independence and stimulated Erk, Akt, and p38 activity. In this model, MERTK signaling had a primary role in cell survival and cytoskeletal alterations without a substantial effect on proliferation.

MERTK ligands

Although studies using chimeric receptors provided important information on MERTK signaling, identification of authentic MERTK ligands permitted analysis in a more physiologic context. The first ligand described was Gas6, identified by purification of Axl-activating conditioned media. Gas6 binds MERTK, but with 3- to 10-fold lower affinity (35, 36). Subsequently, protein S was identified as a MERTK ligand that did not activate Axl (37, 38). Both ligands are secreted by multiple cell types and are present in human blood, although total protein S levels are 1,000-fold higher; however, modifications of protein S may be
necessary for it to activate Mer (39–41). More recently, a novel phagocytosis-based functional cloning screening strategy identified 3 new MERTK ligands: tubby, tubby-like protein 1 (Tulp1), and galectin-3 (42, 43). All known MERTK ligands induce MERTK autophosphorylation, although to date, roles for tubby, Tulp1, and galectin-3 have not been studied in MERTK-driven cancers. However, overexpression of galectin-3 has been shown in many cancers and galectin-3 is known to play roles in a wide variety of oncogenic processes, consistent with the possibility that these phenotypes may be mediated by MERTK signaling (44).

**MERTK signaling in leukemia**

The ectopic expression of MERTK in pediatric ALL led our group to investigate full-length MERTK receptor oncogenic signaling in lymphocytes (9, 45). A transgenic model was made expressing MERTK from the Vav promoter; lymphocytes at all stages expressed MERTK. Lymphoblastic leukemia and lymphomas resulted from this ectopic MERTK expression and stimulation of these cells with Gas6 induced MERTK autophosphorylation and downstream activation of Erk1/2 and Akt. In human T-cell lymphoblastic leukemia cell lines, Gas6 also activated MERTK, Erk1/2, Akt, STAT5, and STAT6 (10). STAT activation within tumor cells contributes to prosurvival phenotypes (46–48). MERTK knockdown by short hairpin RNA (shRNA) resulted in decreased levels of phospho-STAT5 and phospho-Erk. In addition, in acute myeloid leukemia cells that did not express Axl or Tyro3, Gas6 activated MERTK and resulted in the phosphorylation and activation of Erk1/2, p38, MSK1, cAMP-responsive element binding protein (CREB), activating transcription factor 1 (ATF1), Akt, and STAT6 (49). shRNA knockdown of MERTK reduced the activation of p38, Erk, and CREB, further strengthening these signaling findings. Taken together, these studies broaden and define endogenous MERTK signaling pathways.

**MERTK signaling in solid tumors**

MERTK signaling observed in leukemia cells is also seen in solid tumor cells; additional novel downstream effectors have also been identified. In non–small cell lung cancer, p38, Erk1/2, GSK3α/β, MEK1/2, Akt, mTOR, CREB, and ATF1 phosphorylation were all induced following Gas6 addition (23). shRNA-mediated MERTK inhibition resulted in decreased levels of CREB, Bcl-xl, survivin, and phospho-Akt, and increased levels of Bcl-2, in response to serum starvation. These results suggest additional mechanisms by which MERTK may impact tumor cell survival. In glioblastoma cells, shRNA mediated reduction of MERTK protein expression and also decreased levels of phosphorylated Erk and Akt (16). In addition, MERTK expression correlated with Nestin and Sox2 expression in a glioblastoma spheroid culture model, indicating possible roles for MERTK maintaining cells in an undifferentiated state (18). In melanoma, Gas6-induced MERTK activation resulted in p38, ERK1/2, GSK3α/β, Akt, AMPK, STAT5, CHK-2, focal adhesion kinase (FAK), and STAT6 phosphorylation, whereas overexpression of MERTK increased the levels of phospho-Akt (21, 22). Inhibition of MERTK by shRNA prevented the Gas6-induced increase of pAkt, pERK1/2, and pSTAT6, decreased basal phosphorylation of Akt, mTOR, and p70S6 kinase, increased PARP cleavage, and decreased CDC42 activity. In prostate cancer cells, MERTK associates with and facilitates the activation of Ack1, a non–receptor tyrosine kinase. This process results in an Ack activity-dependent degradation of the Wwox tumor suppressor, suggesting yet another oncogenic mechanism, that is, control of a tumor suppressor (2). An FMS-MERTK chimera expressed in a prostate cancer cell line induced Raf, MEK1/2, p90RSK, Erk1/2, and Akt phosphorylation and increased c-Fos and c-Jun transcription factor expression (25).

**MERTK migration and cellular traffic**

MERTK signaling has been implicated in tumor cell migration and invasion. In non–small cell lung cancer cells, FAK is phosphorylated in response to Gas6 (23). Both total and phosphorylated FAK and RhoA increase, whereas total and phosphorylated myosin light chain 2 are decreased in glioblastoma cells in response to shRNA-mediated MERTK inhibition, with impaired migration (17, 18). Melanoma cell migration and invasion are also decreased by shRNA MERTK expression (21, 22). MERTK’s physiologic role in ingesting apoptotic material in macrophages is an action that is in part dependent on a MERTK-Vav activation process that stimulates Rac CDC42 and Rho GDP to GTP exchange (50). With respect to cellular trafficking, a recent report links MERTK action to EGFR surface levels (51). In the absence of MERTK, EGF treatment induced a higher rate of EGFR internalization and degradation, resulting in decreased levels of EGFR on the cell surface and reducing downstream signaling. Related to the concept of receptor trafficking, a recent study found MERTK to be located not only at the cell surface, but also in the nucleus. Consensus nuclear localization sequences have also been identified in the MERTK gene (52). This raises the possibility that MERTK may have effects on gene transcription.

**Clinical-Translational Advances**

**Tumor biology and target validation**

Many studies have used shRNA to show critical oncogenic roles for MERTK in a variety of tumor types. In T-cell acute lymphoblastic leukemia, shRNA against MERTK resulted in increased apoptosis, decreased methyllumellose colony formation, increased sensitivity to cytotoxic chemotherapies, and delayed disease onset with increased survival in a murine leukemia model (9, 10). Similarly, in acute myeloid leukemia, MERTK inhibition resulted in increased apoptosis, decreased colony formation, and increased survival in a mouse model (49). In glioblastoma, MERTK shRNA increased apoptosis and autophagy, decreased colony formation, increased chemosensitivity, altered morphology, and decreased migration (16–18). In non–small cell lung cancer, MERTK inhibition increased apoptosis, decreased colony formation, increased STAT3 expression, and reduced cell growth.
formation, increased chemosensitivity, and decreased tumor formation in a mouse model (23). shRNA-mediated inhibition of MERTK in melanoma cell lines resulted in increased cell death, decreased proliferation, decreased colony formation in soft agar, decreased migration, and decreased tumor formation in xenografts (21, 22).

MERTK knockdown in breast cancer cells resulted in decreased formation of metastases and inhibited endothelial cell recruitment in mouse models (12). In breast cancer, miR-126 and miR-335 negatively regulate MERTK expression; loss of these miRNAs contributes to breast cancer progression and metastasis. Mechanistically, miR-126 loss increased MERTK expression. Increased MERTK was followed by extracellular domain cleavage, and soluble MERTK was postulated to act as a ligand sink, sequestering Gas6 and preventing activation of TAM receptors on migrating endothelial cells. Decreased Gas6-induced signaling in endothelial cells increased their migration, resulting in tumor-associated angiogenesis.

Inappropriate or overexpression of MERTK, one of its ligands, or both, has been the most studied mechanism linking MERTK and cancer. However, recent studies in multiple myeloma, melanoma, renal cancer, and head and neck cancer have also identified mutation of MERTK as another possible mechanism (21, 53, 54). Mutation has been recognized as a common method of activating multiple other receptor tyrosine kinases, and it will be interesting to determine the frequency of and functional effects of these recently discovered MERTK mutations.

**Therapeutics in development**

One strategy to inhibit MERTK is to use a ligand trap, consisting of the extracellular domain of MERTK or an antibody against Gas6, to prevent ligand-dependent MERTK activation (3). A monoclonal antibody against MERTK has been used to decrease the levels of MERTK protein on the surface of glioblastoma cells, resulting in decreased tumor cell migration in vitro (17). In addition, several multi kinase small-molecule inhibitors have been shown to have inhibitory effects against MERTK, including sunitinib, BMS-777607, R-428, and Compound-52 (55–58). Recently, several MERTK-selective small-molecule inhibitors have been developed using structure-aided design algorithms. The first of these, UNC569, is a pyrazolopyrimidine sulfonamide derivative of UNC569, which has reduced human ether-a-go-go-related gene potassium channel activity and therefore a more favorable toxicity profile (60). This compound has shown promising preclinical activity in a melanoma model, blocking MERTK activation and downstream signaling via STAT6, Erk, and Akt, increasing apoptosis, decreasing colony formation in soft agar, and decreasing collagen matrix invasion (22).

**Summary**

The MERTK tyrosine kinase has been linked to the pathogenesis of cancer since it was first discovered by expression cloning from a neoplastic cell nearly two decades ago. It has since been shown to activate a wide variety of pro-oncogenic signaling pathways in an ever-expanding list of human cancer types. Signaling pathways, including those involving MAPK and p38, PI3K, Janus-activated kinase (Jak)/STAT, FAK/RhoA/MLC2, and Bcl-2 family members, contribute to increased proliferation and migration, and decreased apoptosis and chemosensitivity. Several types of MERTK inhibitors are currently in development, including ligand traps, a monoclonal antibody, and small-molecule tyrosine kinase inhibitors. Such agents are designed to exploit the selective requirement for MERTK in tumor cells. Although MERTK was infrequently studied in the first decade after its discovery due to a lack of understanding about ligand and cellular biology, this is changing due to recent evidence linking MERTK to oncogenic progression. The hope is that these efforts will result in clinically available MERTK-targeted therapeutics in the near future, as well as a further understanding of how best to deploy them.

**Disclosure of Potential Conflicts of Interest**

D. DeRyckere, H.S. Earp, and D.K. Graham hold equity in Meryx Incorporated. No potential conflicts of interest were disclosed by the other author.

**Authors’ Contributions**

Conception and design: C.T. Cummings, H.S. Earp, D.K. Graham

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.T. Cummings

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.T. Cummings, D.K. Graham

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