MERTK: upstream from BRAF

By Michael J. Haas, Senior Writer

U.S. researchers have shown that inhibiting c-Mer proto-oncogene tyrosine kinase, a protein that sits upstream from BRAF, reduced melanoma growth in mice.¹ Future studies will need to determine whether inhibitors of this kinase are effective in BRAF-resistant tumors.

c-Mer proto-oncogene tyrosine kinase (MERTK) is a member of the TAM receptor family of transmembrane proteins that includes TYRO3 protein tyrosine kinase (TYRO3; Sky) and AXL receptor tyrosine kinase

(AXL; UFO). *MERTK* is expressed on at least three types of normal cells: platelets, for which it aids their aggregation; macrophages, which it enables to engulf target cells; and a range of epithelial cell types, for which it plays a role in survival.

Studies by multiple groups have shown that *MERTK* is overexpressed or hyperactivated—and thus a potential therapeutic target—in leukemia² and in prostate,³ brain⁴ and lung⁵ cancers. At least one study has shown that TAM and other tyrosine kinase receptors are activated in melanoma,⁶ and two more have linked TYRO3, AXL and the TAM receptor ligand, growth arrest–specific 6 (GAS6), to melanoma tumorigenesis and invasiveness.^{7,8}

Collectively, these findings prompted Douglas Graham and colleagues to hypothesize that MERTK might also be a therapeutic target in melanoma. Graham is assistant professor of pediatrics and immunology at the University of Colorado Denver School of Medicine.

To investigate this hypothesis, Graham's team performed microarray analyses and found that *MERTK* was overexpressed in about 50% of melanomas compared with normal human melanocytes. Moreover, there was higher *MERTK* expression in metastatic melanoma than in primary tumors.

Figure 1. Downstream of MERTK.

According to a study in *The Journal of Clinical Investigation*, inhibiting c-Mer proto-oncogene tyrosine kinase (MERTK)—which lies upstream from BRAF—could help treat melanoma.

In melanoma tumor cells [a], overexpression of *MERTK* activates multiple signaling pathways. Activated Ras [b(1)] signals through BRAF, CRAF (RAF1) and other Raf proteins to activate MAP kinase kinase 1 (MAP2K1; MEK1) and MEK2 (MAP2K2). Activated JAK kinases (JAKs) [b(2)] in turn activate signal transducer and activator of transcription (STAT) proteins. Activated phosphoinositide 3-kinase (PI3K) [b(3)] induces activation of protein kinase B (PKB; PKBA; AKT; AKT1). Upregulation of any or all of these three pathways leads to melanoma growth, proliferation and survival [c].

Additionally, expression of *MERTK* on tumor-infiltrating macrophages [d] may enhance their ability to engulf and clear apoptotic tumor cells [e], thereby leading to reduced immune system responses [f] and enhanced tumor survival.

Multiple companies market or are developing products that inhibit proteins in pathways [b(1)]–[b(3)] to treat melanoma.

CEP-32496, a dual inhibitor of BRAF and epidermal growth factor receptor (EGFR) from **Ambit Biosciences Corp.** and **Teva Pharmaceutical Industries Ltd.**, is in preclinical testing to treat melanoma.

DP-4978, an oral small molecule dual inhibitor of mutant BRAF and CRAF from **Deciphera Pharmaceuticals LLC** and **Eli Lilly and Co.**, is in preclinical testing to treat melanoma.

Nanolipolee 007, an i.v. nanoparticle formulation containing leelamine derived from the bark of a pine tree that inhibits protein kinase Bγ (PKBγ; AKT3) and STAT3, is in preclinical testing from **Melanovus Oncology Inc.** to treat melanoma.

MERTK overexpression did not correlate with mutations in *BRAF* or *neuroblastoma Ras viral (v-Ras) oncogene (NRAS)*, which are found in about 50% and 20% of melanomas, respectively.

In human melanoma cell lines, a small molecule inhibitor of *MERTK* decreased proliferation and invasion and increased apoptosis compared with vehicle.

Mice injected with human melanoma cell lines pretreated with *MERTK* small hairpin RNA developed smaller tumors than mice injected with cells pretreated with a scrambled control shRNA. Graham said the team did not test the small molecule in mouse models because it was not suitable for *in vivo* work.

Additional *in vitro* studies revealed that *MERTK* promoted growth, proliferation and survival in melanoma cells by activating at least three signaling pathways, including the Ras/Raf/MEK/MAPK signaling cascade (see Figure 1, “Downstream of *MERTK*”).

Data were reported in *The Journal of Clinical Investigation*.

The team included researchers from **The University of North Carolina at Chapel Hill**, the **University of Pittsburgh School of Medicine**, **Harvard Medical School** and **Massachusetts General Hospital**.

“It is particularly noteworthy that the *JCI* study showed 28% of brain metastases of melanoma had elevated *MERTK* expression” because so little is known about how melanoma metastasizes to the brain, said Keiran Smalley. He is assistant professor in the molecular oncology program and

scientific director of the Melanoma Research Center of Excellence at the **H. Lee Moffitt Cancer Center & Research Institute**.

Better with BRAF?

The immediate questions are whether a *MERTK* inhibitor can show efficacy in primary melanoma tumors that have developed resistance to BRAF inhibitors—such as Zelboraf vemurafenib—and whether blocking *MERTK* will have additive effects in combination with inhibitors of BRAF and MEK.

“One aspect that is not yet clear is the importance of *MERTK* signaling relative to that of other driver oncogenes in melanoma,” such as mutant *BRAF* and *NRAS*, Smalley said.

He noted that the *JCI* team showed that *MERTK* knockdown “only delayed melanoma growth, making it likely that other drivers are also involved in tumor progression.”

Jennifer Low, group medical director at the **Genentech Inc.** unit of **Roche**, agreed that “studies specifically looking at the prevalence of *MERTK* activation in the general melanoma population and in patients with BRAF mutations would be of interest.”

Smalley also wanted to know whether *MERTK* expression was elevated in tumors from patients with melanoma in whom BRAF inhibitors failed. “This possibility could be explored further by determining whether *MERTK* expression mediates the increased MEK signaling in melanoma cells that are resistant to BRAF inhibition,” he said.

Graham said previous studies by his group have shown that *MERTK* expression plays a role in resistance to chemotherapy in brain cancer⁴,

lung cancer⁵ and acute lymphoblastic leukemia (ALL),^{9,10} “and we are investigating whether this is also the case in melanoma. We think that *MERTK* inhibition might help treat melanoma that has developed resistance to BRAF inhibitors” and other therapies.

However, Low pointed out that the *JCI* study did not establish “whether inhibiting *MERTK* would reverse or delay progression in melanoma if combined with, or used following, a BRAF inhibitor.” Thus, she wanted to see *MERTK* inhibition tested in cell lines and animal models of BRAF mutation–positive melanoma that had developed resistance to BRAF inhibitors.

Smalley agreed it would be important “to determine whether the combination of *MERTK* and BRAF inhibitors gives a more durable response to treatment than a BRAF inhibitor alone,” as has been observed with combined BRAF/MEK inhibition.

“It would also be wise to test *MERTK* inhibitors in combination with inhibitors of the MEK/ERK and PI3K/AKT [phosphoinositide 3-kinase/protein kinase B] pathways,” he added.

Graham said the team has developed a next-generation *MERTK* inhibitor with better bioavailability than the molecule used in the published study and is now testing it in combination with Zelboraf and an undisclosed MEK inhibitor.

“We have found these combinations increased efficacy in melanoma cell lines compared with monotherapy,” he said. “Now we plan to test the combinations in primary melanoma cells and mouse models of melanoma” and expect to publish the results this year.

Daichi Sankyo Co. Ltd., Roche and Roche’s **Chugai Pharmaceutical Co. Ltd.** unit market Zelboraf, an oral small molecule inhibitor of oncogenic BRAF V600E.

Cobimetinib (GDC-0973; RG7421; XL518), a MAP kinase kinase 1 (MAP2K1; MEK1) and MEK2 (MAP2K2) inhibitor from **Exelixis Inc.** and Genentech, is in Phase III testing to treat metastatic melanoma and Phase I trials to treat BRAF V600 mutation–positive melanoma.

Roche and Genentech also have Zelboraf and MPDL3280A (RG7446), a human mAb against programmed cell death 1 ligand 1 (CD274 molecule; PD-L1; B7-H1), in Phase Ib testing in previously untreated patients with BRAF V600 mutation–positive melanoma.

Macrophage futures

The team has conducted follow-on studies to explore the therapeutic implications of another finding from the *JCI* study: results of microarray analyses showing that more than 50% of tumor-infiltrating macrophages highly expressed *MERTK*.

Indeed, Graham said the team has another paper in press showing that *MERTK* inhibition—in addition to its direct effect on tumors—has an immunomodulatory action in melanoma and other *MERTK*-expressing cancers.

“We think that the high levels of *MERTK* expressed on tumor-infiltrating macrophages may increase their efficiency at clearing apoptotic melanoma cells” and thus limit the time during which antigens from those dying cells could trigger an immune response, Graham said. Thus, *MERTK* inhibition could decrease macrophage-aided cell clearance and thereby stimulate immune responses to the tumor, he said.

He added, “The immunomodulatory effect of *MERTK* inhibition on macrophages could also be important in cancer types that themselves do not overexpress *MERTK*.”

“One aspect that is not yet clear is the importance of *MERTK* signaling relative to that of other driver oncogenes in melanoma.”

**—Keiran Smalley,
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The team also is collecting plasma serum from patients with melanoma to determine whether circulating levels of soluble MERTK or GAS6 correlate with disease progression and is planning studies to better understand the role of MERTK in cancer cell migration, invasion and metastasis, Graham said.

The University of North Carolina at Chapel Hill has patented the MERTK inhibitors and their therapeutic uses. The IP is available for licensing.

Haas, M.J. *SciBX* 6(16); doi:10.1038/scibx.2013.380
Published online April 25, 2013

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