Published OnlineFirst May 5, 2016; DOI: 10.1158/2159-8290.CD-RW2016-085



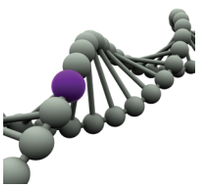
## Melanoma

**Major finding:** A mutation in the *CYCLTR2* G-protein–coupled receptor promotes tumorigenesis in uveal melanomas.

**Concept:** *CYCLTR2* mutations constitutively activate $G_{\alpha q}$ in tumors lacking *GNAQ*, *GNA11*, and *PLCB4* mutations.

**Impact:** *CYCLTR2* is an uveal melanoma oncogene, and *CYCLTR2* mutations may be actionable targets.

### UVEAL MELANOMAS HARBOR RECURRENT ACTIVATING MUTATIONS IN *CYSLTR2*



Uveal melanomas are eye tumors arising from melanocytes in the uveal tract. These tumors harbor frequent activating mutations in two subunits of $G_{\alpha q}$ heterotrimeric G proteins (*GNAQ* and *GNA11*), and in *PLCB4*, a downstream effector of $G_{\alpha q}$ signaling. To identify additional mutations driving uveal melanoma, Moore and colleagues analyzed whole-genome and whole-exome sequencing data from 136 patients with uveal melanoma from multiple cohorts. Using a mutation analysis algorithm to detect hotspot mutations in oncogenes, a previously undescribed mutation in cysteinyl leukotriene receptor 2 (*CYSLTR2*) was identified only in samples lacking mutations in *GNAQ*, *GNA11*, and *PLCB4* (4 of 9 samples), suggesting that these mutations activate the same pathway. *CYSLTR2* encodes a G-protein–coupled receptor, $CysLT_2R$, involved in leukotriene-mediated signaling in inflammation and fibrosis. The identified mutation results in a Leu129Gln substitution in transmembrane helix 3, which associates with the extracellular ligand, intracellular Gα subunit, and other transmembrane helices. Leu129Gln $CysLT_2R$ was coupled to $G_{\alpha q}$ and was constitutively active, as evidenced by increased calcium mobilization and insensitivity to the $CysLT_2R$ agonist leukotriene $D_4$. Leu129Gln $CysLT_2R$ had melanocyte lineage–specific effects, as it allowed melan-a melanocytes to grow in the absence of 12-O-tetradecanoylphorbol-13-acetate (TPA), which is usually required for melanosome growth and pigmentation. Moreover, Leu129Gln $CysLT_2R$ rescued pigmentation of Melan-a cells withdrawn from TPA, and enhanced expression of melanocyte lineage–specific genes. Subcutaneously engrafted melan-a cells expressing Leu129Gln $CysLT_2R$ were able to form tumors more rapidly than wild-type cells, indicating that the mutation enhances melanoma tumorigenesis in *vivo*. Further, CYSLTR2 knockdown suppressed the growth of Leu129Gln $CysLT_2R$ cells, and reduced expression of melanocyte-lineage genes. Taken together, these findings reveal an oncogenic role for *CYSLTR2* in in uveal melanoma through activation of $G_{\alpha q}$ signaling, and further suggest that Leu129Gln $CysLT_2R$ may be a potential therapeutic target in uveal melanoma. ■

*Moore AR, Ceraudo E, Sher JJ, Guan Y, Shoushtari AN, Chang MT, et al. Recurrent activating mutations of G-protein-coupled receptor* CYSLTR2 *in uveal melanoma. Nat Genet 2016 April 18 [Epub ahead of print].*

## Targeted Therapy

**Major finding:** Rigosertib acts as a RAS mimetic, binding to the RAS-binding domain of multiple RAS effector proteins.

**Concept:** Rigosertib inhibits RAS-mediated activation of MEK–ERK and PI3K pathway signaling.

**Impact:** RAS signaling can be effectively targeted by disruption of RAS binding to downstream effectors.

### BLOCKADE OF RAS-BINDING DOMAIN INTERACTIONS INHIBITS RAS SIGNALING

Aberrant RAS signaling is present in the majority of human tumors and promotes proliferation and survival, but development of RAS inhibitors has been largely unsuccessful due to the lack of well-defined druggable pockets on the surface of RAS. Rigosertib is a non-ATP competitive inhibitor that suppresses the growth of various types of cancer cells and is currently in clinical development, but its mechanism of action is not well understood. In a mass spectrometry––based approach to identify direct targets of rigosertib, Athuluri-Divakar and colleagues identified three proteins that contain a RAS binding domain (RBD) as the primary rigosertib-binding partners: ARAF, BRAF, and CRAF. Determination of the BRAF RBD–rigosertib complex structure with nuclear magnetic resonance (NMR) revealed that rigosertib bound to the same region of the RBD as RAS, suggesting that rigosertib would prevent RAS and RAF from interacting. These findings were supported by RAF mutagenesis studies, which indicated that mutations in the RAF RBD that disrupted RAS binding also disrupted rigosertib binding. As predicted by NMR, rigosertib blocked the association between RAS and the RAF RBD. Rigosertib inhibited RAF heterodimerization and activation, and suppressed phosphorylation of the downstream effectors MEK and ERK, indicating that blocking the RAS–RAF interaction inhibits MEK–ERK pathway signaling. Rigosertib also bound to the RBD of PI3Ks and reduced AKT phosphorylation, suggesting that it also inhibited PI3K/AKT signaling by disrupting the RAS–PI3K interaction. In mouse models of mutant KRAS–driven colorectal, lung, and pancreatic cancers, rigosertib reduced tumor growth and reduced the phosphorylation of MEK, ERK, and AKT, indicating that rigosertib can inhibit RAS-driven MAPK and PI3K signaling *in vivo*. Taken together, these results suggest that rigosertib suppresses tumors by acting as a RAS mimetic, and illustrate that RAS can be effectively targeted by preventing its interaction with downstream effectors. ■

*Athuluri-Divakar SK, Vasquez-Del Carpio R, Dutta K, Baker SJ, Cosenza SC, Basu I, et al. A small molecule RAS-mimetic disrupts RAS association with effector proteins to block signaling. Cell 2016;165:643–55.*

**Note:** *Research Watch* is written by *Cancer Discovery* editorial staff. Readers are encouraged to consult the original articles for full details. For more Research Watch, visit *Cancer Discovery* online at http://cancerdiscovery.aacrjournals.org/content/early/by/section.



Downloaded from cancerdiscovery.aacrjournals.org on October 31, 2020. © 2016 American Association for Cancer Research.

Published OnlineFirst May 5, 2016; DOI: 10.1158/2159-8290.CD-RW2016-085

AACR American Association for Cancer Research

CANCER DISCOVERY

# Blockade of RAS-Binding Domain Interactions Inhibits RAS Signaling

*Cancer Discov* 2016;6:573. Published OnlineFirst May 5, 2016.

**Updated version** Access the most recent version of this article at: doi:10.1158/2159-8290.CD-RW2016-085

**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, use this link http://cancerdiscovery.aacrjournals.org/content/6/6/573.2. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerdiscovery.aacrjournals.org on October 31, 2020. © 2016 American Association for Cancer Research.