Drug Resistance

Major finding: ERBB3 upregulation mediates adaptive resistance to RAF/MEK inhibition in *BRAF*-mutant melanoma.

Mechanism: RAF/MEK blockade induces FOXD3-mediated *ERBB3* transcription and enhances ERBB3/ERBB2 signaling.

Impact: ERBB3 inhibition may improve the therapeutic efficacy and duration of vemurafenib treatment.

ENHANCED ERBB3 SIGNALING PROMOTES RESISTANCE IN MELANOMA

Inhibitors targeting oncogenic BRAF or downstream MAP/ERK kinase (MEK) signaling suppress tumor growth and prolong survival in patients with *BRAF*-mutant melanoma. However, the response is heterogeneous and most patients who respond ultimately develop acquired resistance, emphasizing the need to identify the pathways by which *BRAF*-mutant tumors adapt to RAF inhibition. Recent studies have defined several mechanisms of resistance to the BRAF inhibitor vemurafenib, including upregulation of the forkhead box D3 (FOXD3) transcription factor. Abel and colleagues found that FOXD3 induced the transcription of *ERBB3* in a panel of melanoma cell lines via direct binding to the *ERBB3* enhancer region. FOXD3-mediated *ERBB3* expression enhanced neuregulin 1β (NRG1β) ligand-driven activation of ERBB3 signaling, resulting in enhanced AKT phosphorylation. Treatment with RAF/MEK inhibitors also positively regulated ERBB3 signaling and was associated with elevated ERBB3 phosphorylation in xenograft tumors and in a subset of on-treatment and relapsed patient tumor samples, suggesting that ERBB3 upregulation may promote resistance. In support of this idea, NRG1β stimulation increased cell viability following RAF/MEK blockade, whereas depletion of ERBB3 decreased AKT activation and impaired the growth of vemurafenib-resistant tumors. Enhanced ERBB3 pathway activity was specifically dependent on activation of the ERBB2 coreceptor but not the EGF receptor or ERBB4, as knockdown of ERBB2 or treatment with lapatinib eliminated NRG1β-induced ERBB3 signaling. Moreover, combined treatment with lapatinib and vemurafenib suppressed ERBB3-driven growth *in vitro* and more effectively reduced tumor burden *in vivo* compared with vemurafenib single-agent treatment. These results define an adaptive response that can mediate RAF/MEK inhibitor resistance in melanoma and suggest that targeted inhibition of ERBB3 may overcome this resistance and improve the therapeutic efficacy of these drugs in patients with *BRAF*-mutant tumors. ■

Abel EV, Basile KJ, Kugel CH 3rd, Witkiewicz A, Le K, Amaravadi RK, et al. Melanoma adapts to RAF/MEK inhibitors through FOXD3-mediated upregulation of ERBB3. *J Clin Invest 2013 Apr 1 [Epub ahead of print].*

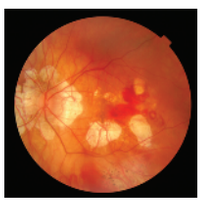
Retinoblastoma

Major finding: *MYCN* amplification, not *RB1* mutation, may be a driving event in a subset of retinoblastomas.

Concept: *MYCN*-amplified, $RB1^{+/+}$ retinoblastomas have distinct clinical, molecular, and histologic features.

Impact: Treatment decisions may be different in patients with *MYCN*-amplified, $RB1^{+/+}$ retinoblastomas.

MYCN IS AMPLIFIED IN RETINOBLASTOMAS WITHOUT *RB1* MUTATION



Biallelic loss of *RB1* is thought to underlie all cases of retinoblastoma, a childhood cancer of the developing retina. However, Rushlow and colleagues found in the course of clinical work that approximately 3% of unilateral retinoblastomas do not have any evidence of *RB1* mutations, promoter hypermethylation, or loss of heterozygosity. Strikingly, *MYCN* copy number was elevated in 90% of these $RB1^{+/+}$ retinoblastomas, compared with 65% of *RB1*-deficient retinoblastomas, and 53% of the $RB1^{+/+}$ tumors had acquired 28 or more copies of *MYCN*, whereas high-level *MYCN* amplification was not observed in any $RB1^{-/-}$ tumor. *MYCN*-amplified, $RB1^{+/+}$ retinoblastomas expressed full-length, nuclear, functional RB1 protein that was normally hypophosphorylated and hyperphosphorylated and capable of binding E2F1. These tumors expressed embryonic retinal markers and arose in the retina, suggesting they were retinoblastomas, but they were molecularly, histologically, and clinically distinct from $RB1^{-/-}$ retinoblastomas. Copy number abnormalities characteristic of $RB1^{-/-}$ retinoblastomas were significantly less common in *MYCN*-amplified, $RB1^{+/+}$ retinoblastomas, and *MYCN*-amplified, $RB1^{+/+}$ tumors were generally more genomically stable than $RB1^{-/-}$ tumors, providing support for a driving role of *MYCN* amplification in the initiation of these retinoblastomas. Histologic hallmarks of $RB1^{-/-}$ retinoblastomas were absent in *MYCN*-amplified, $RB1^{+/+}$ retinoblastomas, which instead had cellular features similar to those of other *MYCN*-amplified tumors. Clinically, *MYCN*-amplified, $RB1^{+/+}$ retinoblastomas were larger and more invasive than $RB1^{-/-}$ retinoblastomas and were diagnosed at a significantly younger median age (4.5 months vs. 24 months). These findings show that not all retinoblastomas are caused by *RB1* loss and identify a retinoblastoma patient population that may especially benefit from aggressive treatment, such as eye removal, and potentially respond to therapies that exploit MYCN dependency. ■

Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Thériault BL, et al. Characterisation of retinoblastomas with RB1 *mutations: genomic, gene expression, and clinical studies. Lancet Oncol 2013;14: 327–34.*