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It Takes Two to Tango: Dual Inhibition of PI3K and MAPK in Rhabdomyosarcoma

Arman Jahangiri and William A. Weiss

The PI3K/AKT/mTOR and RAS/RAF/MAPK pathways play essential roles in rhabdomyosarcoma. Singular targeting of each pathway is ineffective due to extensive cross-talk and compensatory feedback between these two pathways. Dual blockade with inhibitors of PI3K and MAPK in combination synergistically inhibits growth of rhabdomyosarcoma both *in vitro* and *in vivo*. *Clin Cancer Res; 19(21); 5811–3. ©2013 AACR.*

In this issue of *Clinical Cancer Research*, Renshaw and colleagues evaluate the role of phosphoinositide 3-kinase (PI3K)/AKT/mTOR and RAS/RAF/mitogen-activated protein kinase (MAPK) pathway inhibitors in treating rhabdomyosarcoma to better account for the shortcomings of singular therapy, while showcasing the benefits of combination therapy (1). They demonstrate the combination of AZD6244, a MAP–ERK kinase (MEK) inhibitor in clinical trials, with AZD8055, an inhibitor of mTOR kinase (within the PI3K pathway) that failed clinically, to have a synergistic effect both *in vitro* and *in vivo* for models of rhabdomyosarcoma.

Rhabdomyosarcomas are the most common subtype of sarcomas in children, and are generally classified either into embryonal rhabdomyosarcoma or alveolar rhabdomyosarcoma based on genetic and histologic findings. The 5-year survival rate for patients with metastatic disease is 42% for embryonal rhabdomyosarcoma versus 18% for alveolar rhabdomyosarcoma (2). Activation of the PI3K/AKT pathway, as demonstrated by AKT phosphorylation in rhabdomyosarcoma, has emerged as a potential target for therapeutic inhibition, as high levels of AKT phosphorylation are associated with poor overall and disease-free survival (3). Inhibitors of this pathway, such as tensirolimus, which targets mTOR complex 1 downstream of PI3K, showed limited activity stabilization in a phase II clinical study (4). Preclinical evidence, however, has demonstrated that inhibitors of mTORC1 stabilize IRS-1, leading to activation of PI3K signaling, whereas inhibitors of PI3K, AKT, or mTOR signaling, likely acting through receptor tyrosine kinases, can activate both PI3K and MAPK signaling (5). A growing number of cross-talk, feedback, and feed-forward loops link the PI3K/Akt/mTOR and Ras/MEK/ERK signaling pathways, which provide insights into the compensatory responses observed with targeting either pathway in isolation (Fig. 1; refs. 6 and 7). Combination therapy through inhibition of MEK/ERK simultaneously with PI3K/mTOR led to growth suppression in preclinical models for lung cancer, offering an approach to overcome therapeutic resistance (8).

The RAS/MEK/ERK pathway also plays a major role in rhabdomyosarcoma, as it can lead to uncontrollable proliferation and cancer cell survival. AZD6244 is a selective inhibitor of MEK1/2, leading to decreased phosphorylation of MAPK, a downstream target. In resistant melanoma cell lines, AZD6244 treatment activates the PI3K pathway, as evidenced by AKT phosphorylation leading to resistance. Interestingly, this resistance was overcome with coordinate inhibition of mTORC1/2, AKT, or insulin-like growth factor I receptor (IGFIR), resulting in improved efficacy with the combination therapy (9).

Using immunohistochemical staining of 79 primary patients with rhabdomyosarcoma (25 with alveolar rhabdomyosarcoma and 54 with embryonal rhabdomyosarcoma), Renshaw and colleagues determined the prevalence of PI3K and MAPK activation, based on staining for p-AKT and p-ERK. Of note, 82.5% of their cohort stained positive for PI3K activation with coactivation of MAPK in 46% of the embryonal rhabdomyosarcoma and 36% of the alveolar rhabdomyosarcoma subtypes, respectively. Although 59% of patients with alveolar rhabdomyosarcoma stained positively for only p-AKT and not p-ERK, this population was much smaller in the embryonal rhabdomyosarcoma group at 29%. The authors concluded that patients with embryonal rhabdomyosarcoma might be less responsive to single-agent PI3K pathway inhibitors than patients with alveolar rhabdomyosarcoma.

Because the catalytic PI3K isoform p110α is found to be mutated in cancer and is involved in IGFIR signaling seen in rhabdomyosarcoma, Renshaw and colleagues next used short hairpin RNA (shRNA) to target this PIK3CA. Knockdown of p110α failed to inhibit growth in the majority of their cell lines due to compensation from other p110

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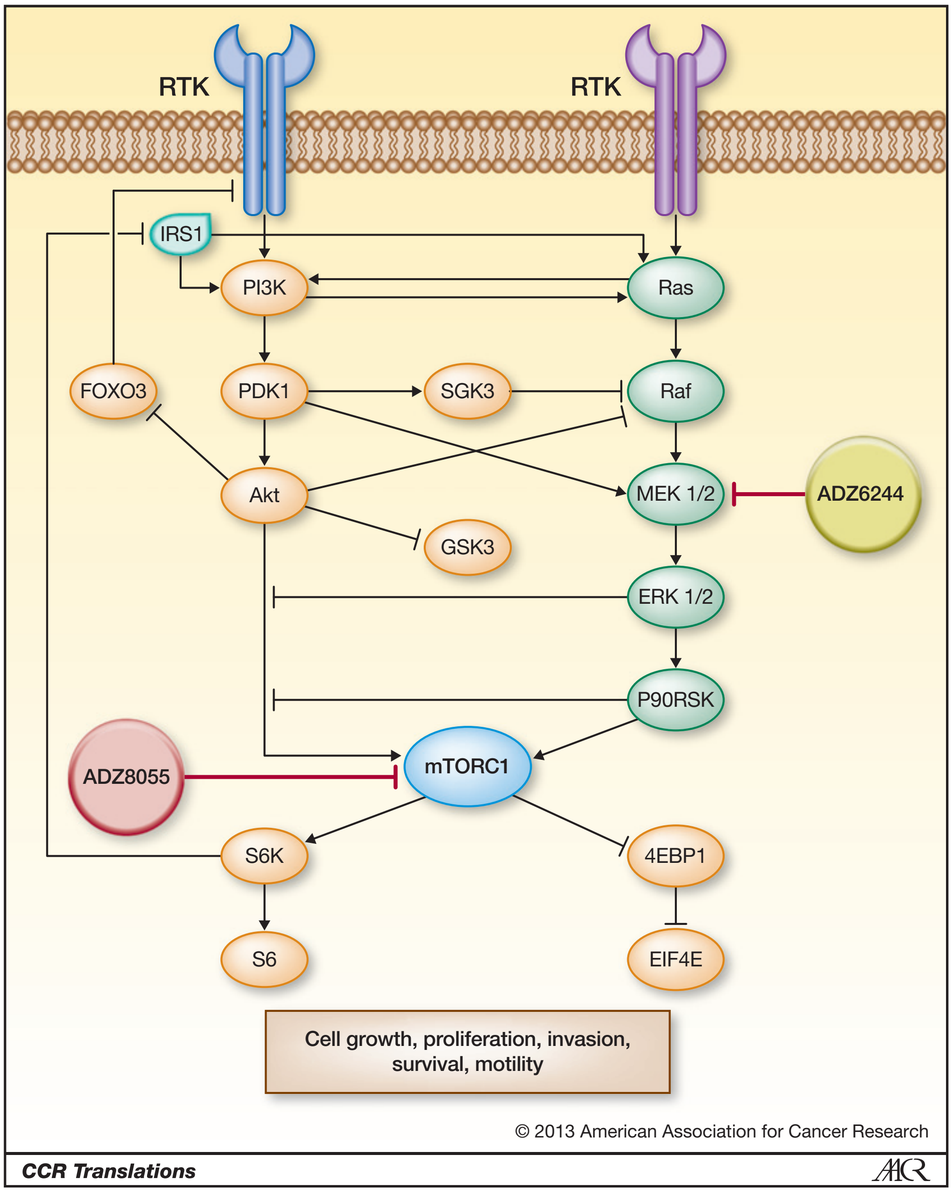

Figure 1. The ERK and PI3K signaling networks are shown, with illustrations of feedback loops and cross-talk. These pathways (PI3K–AKT and RAS/MERK/ERK) mediate cell survival, growth, proliferation, and invasion in rhabdomyosarcoma. Targeted therapeutics that inhibit one pathway typically lead to feedback activation of the second pathway, which serves as the mechanism for unresponsiveness to treatment and/or the development of resistance. AZD8055, an inhibitor of mTOR kinase, blocks both mTORC1 and mTORC2. For simplification, only mTORC1 is shown.

subtypes. Although these data might suggest a need for pan-selective PI3K inhibitors to effectively inhibit growth in rhabdomyosarcoma, the PI3K field contains many examples of disagreement between kinase inhibitors and RNA interference (10). Interestingly, every p110α knockdown cell line in their study demonstrated increased levels of ERK phosphorlyation; however, these cell lines were not resistant to PI3K inhibitors. Following p110α knockdown, only one of the cell lines exhibited increased sensitivity to MEK inhibtion via AZD6244, and this cell line expressed p110β but not p110α or δ. This suggests that following MEK inhibition, p110β does not allow for compensatory activation of PI3K, whereas p110α and δ allow for MEK inhibition (1). Further studies should be conducted to better define the role of each p110 subtype by conducting lentiviral shRNA knockdown for each one.

Renshaw and colleagues then evaluated the impact of dually blocking the MAPK and PI3K pathways *in vitro* and *in vivo*. This combination therapy proved synergistic *in vitro*, through the reciprocal inhibition of feedback activation, which is seen in monotherapy after inhibition of each individual pathway. Embroynal rhabdomyosarcoma tumors harboring an *NRAS* mutation are typically unresponsive to PI3K inhibitors (11). These tumors were unresponsive *in vivo* to AZD8055 (TORC1/TORC2 inhibitor) or AZD6244 (MEK inhibitor), whereas NVP-BEZ235 (dual PI3K/mTOR inhibitor) had some impact. The combination of AZD8055 with AZD6244, however, led to a significant inhibition of tumor growth, whereas combining NVP-BEZ235 with AZD6244 had no additional benefit when compared with NVP-BEZ235 as the sole treatment (1). Renshaw and colleagues recommend three phosphorylated biomarkers for gauging the synergistic action of the PI3K and MEK inhibitors (AKT, S6, and ERK) and a simultaneous reduction of their phosphorylated forms.

Toxicity and drug–drug interactions are often a concern when therapeutics are administered in a combined manner. In a recent phase I clinical trial in patients with advanced cancer, the PI3K and MEK pathway was dually targeted. Although an improvement in efficacy was witnessed, the combined therapy also led to increased toxicity (12). Although Renshaw and colleagues did not witness significant toxicity with their combined regiments *in vivo*, pharmacokinetic analysis demonstrated lower levels of AZD6244 in plasma and tumor, while leading to higher levels of PI3K inhibitors. This interaction continued to escalate with subsequent treatments. The dual inhibition of the PI3K/AKT/mTOR and RAS/RAF/MAPK pathways, as described in this study, might play a key role in the development of novel therapeutics for rhabdomyosarcoma. Renshaw and colleagues have demonstrated the intricate crossover and compensatory mechanisms that exist between these two important pathways, which counteract when one is individually targeted. Although combination therapy may lead to better efficacy in debilitating cancers such as rhabdomyosarcoma, this comes at a potential cost of increasing toxicity, and may not be tolerated by patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Jahangiri
Writing, review, and/or revision of the manuscript: A. Jahangiri, W.A. Weiss
Study supervision: A. Jahangiri

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