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It takes two to tango: dual inhibition of PI3K & MAPK in rhabdomyosarcoma

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Summary

The PI3K/AKT/mTOR and RAS/RAF/MAPK pathways play essential roles in rhabdomyosarcoma. Singular targeting of each pathway is ineffective due to extensive crosstalk and compensatory feedback between these two pathways. Dual blockade with inhibitors of PI3K and MAPK in combination synergistically inhibit growth of rhabdomyosarcoma both *in vitro* and *in vivo*.

Keywords

Combination therapy; Rhabdomyosarcoma; MAPK pathway inhibitors; PI3K pathway inhibitors

Commentary

In this issue of *Clinical Cancer Research*, Renshaw and colleagues evaluate the role of PI3K/AKT/mTOR and RAS/RAF/MAPK pathway inhibitors in treating rhabdomysarcoma (RMS) to better account for the shortcomings of singular therapy, while showcasing the benefits of combination therapy (1). They demonstrate the combination of AZD6244, a 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 RMS.

RMS are the most common subtype of sarcomas in the children, and are generally classified either into embroynal RMS (ERMS) or alveolar RMS (ARMS) based on genetic and histological findings. The 5-year survival rate for patients with metastatic disease is at 42% for ERMS versus 18% for ARMS (2). Activation of the PI3K/AKT pathway, as demonstrated by AKT phosphorylation in RMS, 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 with 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, while inhibitors of PI3K, AKT, or mTOR signaling, likely acting through RTKs, can activate both PI3K and MAPK signaling (5). A growing number of crosstalk, feedback and feed forward loops link the PI3K/Akt/mTOR and Ras/MEK/ERK signaling pathways, that provide insights into the compensatory responses observed with targeting either pathway in isolation (Fig. 1). Combination therapy through inhibition of MEK/ERK simultaneously with PI3K/

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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 RMS, 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 either mTORC1/2, AKT, or insulin-like growth factor I receptor (IGFIR), resulting in improved efficacy with the combination therapy (9).

Utilizing immunohistochemical staining of 79 primary rhabdomysarcoma patients (25 ARMS, 54 ERMS), Renshaw and colleagues determined the prevalence of PI3K and MAPK activation, based on staining for p-AKT and p-ERK. 82.5% of their cohort stained positive for PI3K activation with co-activation of MAPK in 46% of the ERMS and 36% of the ARMS subtypes. While 59% of their ARMS patients stained positively for only p-AKT and not p-ERK, this population was much smaller in the ERMS group at 29%. They concluded that ERMS patients might be less responsive to single agent PI3K pathway inhibitors as compared to ARMS patients.

Since the catalytic PI3K isoform p110α is found to be mutated in cancer and is involved in IGF1R signaling seen in RMS, they next used shRNA to target this PIK3CA. Knock-down of p110 α failed to inhibit growth in the majority of their cell lines, due to compensation from other p110 subtypes. While this data might suggest a need for pan-selective PI3K inhibitors to effectively inhibit grown in RMS, 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, while 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 KD 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. ERMS tumors harboring a *NRAS* mutation are typically unresponsive to PI3K inhibitors (11). These tumors were unresponsive *in vivo* to AZD8055 (TORC1/TORC2 inhibitor) or AZD6244 (MEK inhibitor), while NVP-BEZ235 (dual PI3K/mTOR inhibitor) had some impact. The combination of AZD8055 with AZD6244 however led to a significant inhibition of tumor growth while combining NVP-BEZ235 with AZD6244 had no additional benefit when compared to NVP-BEZ235 as the sole treatment (1). Renshaw *et al* recommend three phosphorylated biomarkers for gauging the synergistic action of the PI3K and MEK inhibitors; AKT, S6, ERK, and the 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 1 clinical trial in patients with advanced cancer, PI3K and MEK pathway were dually targeted. While an improvement in efficacy was witnessed, the combined therapy also led to increased toxicity (12). Although Renshaw *et al* 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 development of novel therapeutics for RMS. 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 RMS, these come at a potential cost of increasing toxicity, and may not be tolerated by patients.

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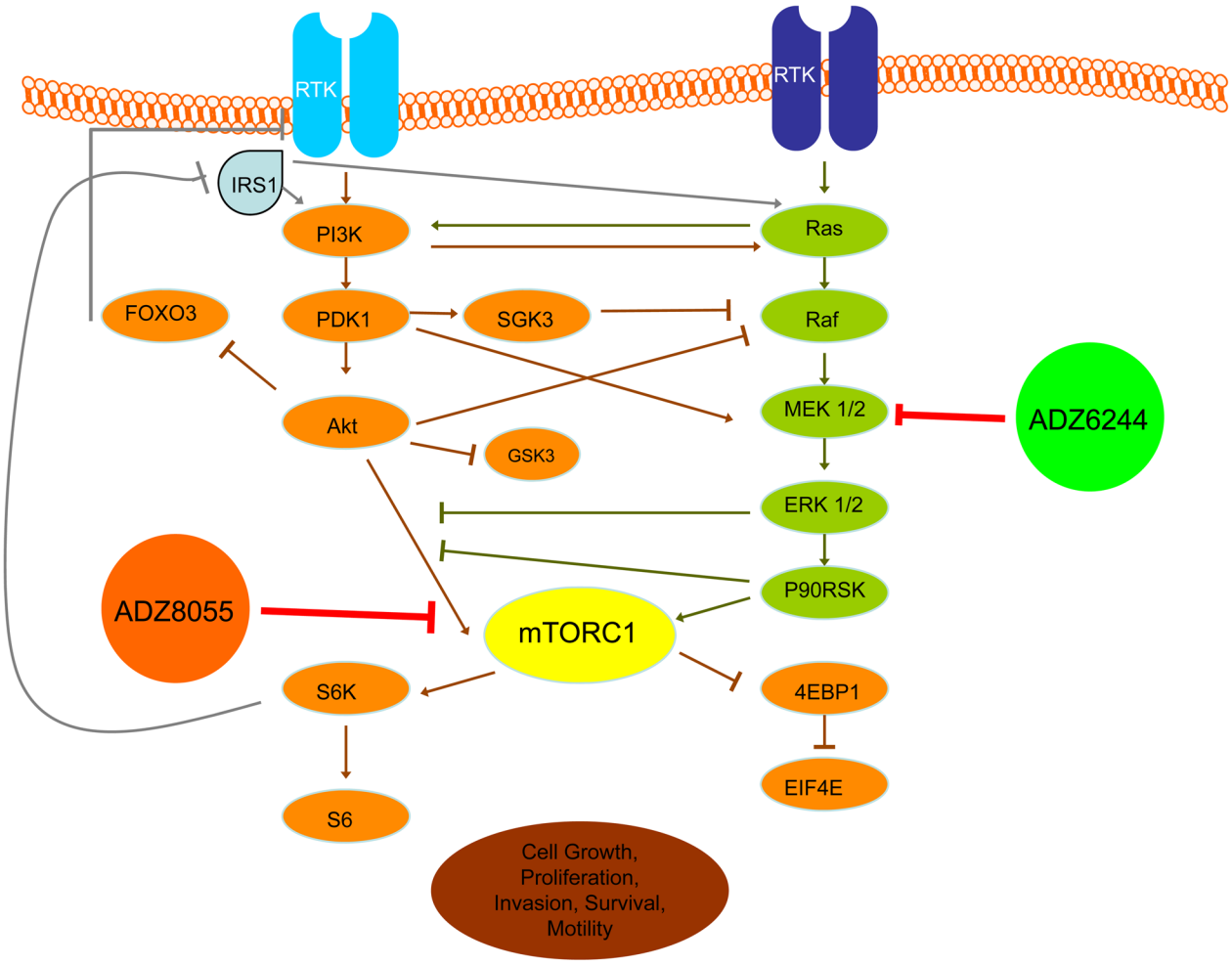

Fig. 1.

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