Endocrine Surgery Review

BRAF Splice Variant Resistance to RAF Inhibitor Requires Enhanced MEK Association

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In Brief

BRAF V600E mutations are a poor prognostic indicator for papillary thyroid cancers and have been previously been implicated as a risk factor for thyroid cancer mortality1,2. Therefore targeted BRAF V600E therapies are of interest to clinicians who treat these malignancies. However, evolved tumor resistance can render these ineffective.

In this study, Vido et al investigated the molecular mechanisms underlying the well-known clinical phenomenon of acquired resistance to RAF inhibitors for BRAF-mutated tumors. The work recognizes that a key hurdle to these RAF inhibitors being effective therapies for papillary thyroid cancer and other BRAF-associated malignancies is the deterrence or avoidance of resistance.

One important means by which BRAF-mutated tumors acquire resistance to RAF or MEK inhibition is via alternatively spliced BRAF V600E isoforms. Utilizing a combination of in vitro and in vivo studies, the investigators demonstrate that these isoforms are enhanced in their ability to associate with their substrate, MEK. This unique ability plays a key role in the ability to acquire resistance.

When exposed to RAF inhibitors such as vemurafenib, the V600E variably-spliced isoforms increasingly become phosphorylated at the serine-729 site. This proves to be clinically significant mechanism of the acquired resistance, as phosphorylation improves dimerization and MEK interactions that are key components of RAF resistance. The phospho-binding site serine-729 on the BRAF isoforms specifically is implicated in this enhanced coupling potential. Switching this serine to a non-phosphorylatable amino acid prevented this, and thereby was demonstrated to increase sensitivity to RAF inhibitors.

In this study, Vido et al used melanoma cell lines (1205LuTR) with known V600E mutations and varied exposure to a non-clinical analog of vemurafenib called PLX4720. Protein quantification of BRAF isoforms and downstream targets such as MEK was performed with Western blot assays. Co-immunoprecipitation was used to analyze and quantify protein-protein interactions. Melanoma cell growth with various BRAF V600E isoforms was compared with 2D growth assays. EdU growth proliferation assays allowed the team to measure in real time DNA replication and cell division under various conditions (for instance, with and without RAF inhibition). For the in vivo studies, nude mice were injected with xenograft LuTR melanoma cells with varied V600E isoforms (such as differences in serine-729 phosphorylation) with luciferase assays and tumor volume measurements to compare growth patterns with or without vemurafenib exposure. Standard statistical analysis was utilized to compare groups, such as Fisher's exact test.

The results of their experiments can be broken down into 6 key findings, (1.) Homodimerization of BRAF V600E is decreased with low dose vemurafenib treatment (a potentially unrecognized therapeutic mechanism of action). (2.) Aberrantly spliced BRAF V600E isoforms had increased associations with MEK compared to full-length mutant BRAF. (3.) The serine-729 site on the BRAF V600E was important for both BRAF homodimerization and BRAF-MEK interaction. (4.) Mutation at this serine-729 site impairs the ability of the BRAF V600E spliced isoforms to interact with MEK in the presence of vemurafenib. (5.) Serine-729 is required for RAF inhibitor acquired resistance. (6.) Disrupting MEK association with BRAF V600E variably spliced isoforms increases susceptibility to vemurafenib.

Critique

It should be noted that the study does not include any human trials, as the in vivo aspects of the study were performed in mice. Further translational work is needed to determine the full clinical applicability of these data. Secondly, the study utilized melanoma cell lines (1205LuTR), and not thyroid cancer cells, for its experiments which may make the results less clinically relevant for thyroid cancer research.

Future Directions

The main directions that the authors suggest, moving forward with this knowledge, is that development of new therapies that work to disrupt the association between MEK and BRAF could disrupt or delay resistance to RAF or MEK inhibitors, thus prolonging their efficacy and leading to better oncological outcomes. The serine-729 site on the BRAF slice variant isoforms may be an ideal potential drug target to increase the sensitivity and therapeutic efficacy of V600E mutation neoplasms. This article helps elucidate the biochemical mechanisms of BRAF acquired resistance at the preclinical level, which will hopefully lead to novel clinical developments in the fight against thyroid cancer in the future.

References

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