THERAPEUTIC RESISTANCE

Multiple mechanisms to keep going

Inhibition of the MAPK pathway in melanoma frequently results in acquired resistance. Lo and colleagues characterized the melanoma genome, transcriptome and epigenome to understand the landscape of acquired resistance to MAPK pathway inhibitors (MAPKi).

Hugo, Shi, Sun, Piva *et al.* used a cohort of 67 human melanomas with matched pretreatment samples and samples taken at disease progression while on therapy (either a BRAFi or a BRAFi plus MEKi). Comparison of whole-exome sequencing of the resistant samples with that of matched pretreatment samples revealed mutations in several MAPK and PI3K pathway genes. Mutations in *NRAS*, *KRAS* and *BRAF* were the most common MAPKi resistance-associated genetic alterations. However, 26 of the melanomas had no genetic mechanism of acquired resistance, indicating that non-genetic events could account for MAPKi resistance in these tumours.

Next, the authors undertook gene expression profiling of 48 of the melanoma samples. They found that changes in gene expression were more frequent than mutations in the same gene. There was also evidence of mutant-allele-selective transcriptional upregulation for certain mutant genes, including *BRAF^{mut}*, *NRAS^{mut}* and *KRAS^{mut}* (although wild-type RAS genes were also transcriptionally upregulated). Furthermore, they found that *MET* and interleukin-8 (*IL8*) were overexpressed in 44% and 40% of 48 tumours, respectively — although they were not genetically altered. The mechanism of BRAFi melanoma cell killing is thought to involve the immune system. Therefore, several markers of immune cells and of inflammation were transcriptionally upregulated, and

cytotoxic T lymphocyte-associated protein 4 (*CTLA4*) expression was downregulated. Analysis of transcriptome data for 118 BRAF-mutant melanoma samples revealed that *MET* upregulation and *CTLA4* downregulation were significantly associated with reduced 10-year survival. The authors analysed gene expression data and genomic DNA CpG methylation levels and found that *MET*, lymphoid enhancer-binding factor 1 (*LEF1*) and dual specificity phosphatase 4 (*DUSP4*), among others, had altered expression that correlated with CpG methylation changes in melanoma cells. Because *MET* upregulation was so frequent, the authors examined this further. They showed that *MET* was overexpressed in the melanoma cells. Using a series of isogenic cell lines that were MAPKi sensitive, BRAFi resistant or BRAFi plus MEKi resistant (*MET* was upregulated in the resistant cell lines), they found that resistance was associated with activating phosphorylation of AKT, indicating that reactivation of survival signalling occurred independently of MAPK signalling in these cells. Moreover, treatment of the resistant cell lines — and not the parental line — with crizotinib, a METi, reduced their survival *in vitro*.

The authors then turned to *LEF1* downregulation as another example of acquired MAPKi resistance. They re-expressed *LEF1* in a panel of MAPKi-resistant melanoma cell lines and found that MAPKi sensitivity was restored. Moreover, treatment of these cells with glycogen synthase kinase 3 β inhibitor (GSK3 β i) resensitized cells to MAPKi. Yes-associated protein 1 (YAP1) post-transcriptional upregulation was also identified as a MAPKi resistance mechanism, and treatment with GSK3 β i and YAP1

knockdown had a synergistic effect on the restoration of MAPKi sensitivity by inducing apoptosis signalling.

Finally, the authors explored MAPKi resistance associated with infiltrating immune cells and inflammation. They found that gene expression changes associated with the nuclear factor- κ B (NF- κ B) pathway were correlated with gene expression changes indicative of monocyte activation and the M2 macrophage phenotype, indicating that tumour-infiltrating macrophages contribute to acquired MAPKi resistance. A subset of macrophage-associated MAPKi-resistant melanomas also had reduced expression of markers of CD8⁺ T cell activation and T cell recruitment. Indeed, further analyses revealed that approximately 50% of all MAPKi-resistant melanomas exhibited reduced numbers and activation of CD8⁺ T cells and reduced markers of antigen presentation.

This study has characterized and integrated genetic, transcriptional, epigenetic and stromal mechanisms of acquired MAPKi resistance in melanoma, identifying intriguing stromal mechanisms and potential therapeutic targets for patients with recurrent disease.

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the landscape of acquired resistance to MAPK pathway inhibitors