THERAPEUTICS

Delving deeper into resistance

> highlighting the heterogeneous nature of RAF inhibitor resistance

Five recent papers have looked more closely at mechanisms that underlie resistance to inhibitors of the ERK MAPK pathway in melanomas that have the BRAF-V600E mutation.

Johannessen *et al.* conducted a screen for genes that induced resistance to inhibitors targeting RAF, MEK or ERK, or RAF and MEK combined in a BRAF-V600E melanoma cell line. Many of the hits were involved in G protein-coupled receptor (GPCR) signalling. GPCRs induce production of cyclic AMP (cAMP) from ATP by adenyl cyclase, and cAMP activates protein kinase A (PKA). Both cAMP and an adenyl cyclase activator (forskolin) induced PKA-dependent resistance to ERK pathway inhibitors. Downstream of PKA, the transcription factor cAMP response element-binding protein (CREB) was also required for ERK pathway inhibitor resistance. CREB activation was high in pretreatment biopsy samples from patients with BRAF-V600E melanoma, was suppressed during RAF inhibitor therapy and then increased to pretreatment levels following relapse. One of the genes activated by CREB in cell lines was microphthalmia-associated transcription factor (*MITF*), a lineage-survival oncogene in melanoma, and MITF knockdown blocked ERK pathway inhibitor resistance mediated by forskolin. Finally, histone deacetylase (HDAC) inhibitors can reduce MITF expression, and, although HDAC inhibitors have other effects, they reversed forskolin-mediated resistance to ERK pathway inhibitors.

Two other studies from this group also looked at resistance to RAF or combined RAF–MEK inhibitors in BRAF-V600E melanoma. Van Allen *et al.* conducted whole-exome sequencing (WES) of tumours with early or acquired resistance to RAF inhibition from 45 patients pretreatment and post-treatment. Nearly 50% of the resistant tumours had mutations in ERK pathway genes or downstream effectors, including previously unobserved mutations in *MEK2*, which were verified to cause resistance in cell lines. *MITF* amplification was observed in one patient at relapse, and consistent with the results from Johannessen *et al.* MITF overexpression induced resistance to ERK pathway inhibitors in BRAF-V600E melanoma cell lines. Some patients had PI3K pathway mutations, although how these contributed to resistance was unclear from this study. Importantly, three patients had several independent resistance mechanisms within the same tumour, highlighting the heterogeneous nature of RAF inhibitor resistance.

Although RAF–MEK combination therapy improves progression-free survival, resistance still occurs in most patients. Wagle *et al.* looked specifically at the mechanisms of this resistance using WES and transcriptome sequencing (RNA-seq) in five patients. They also found a *MEK2* mutation (which was different from those found by Van Allen *et al.*), as well as expression of a BRAF splice isoform and BRAF amplification. Other possible, but unconfirmed, mechanisms of resistance were mutation of the transcription factor *ETS2* and amplification of the putative transcriptional repressor *SAMD4B*.

Two papers by Shi *et al.* also looked at resistance to RAF inhibitors. WES of 100 pretreatment and post-treatment tumour samples from 44 patients with melanoma identified activating alterations in the ERK pathway in 70% of post-treatment tumours, and gain-of-function mutations in PI3K–AKT pathway genes in 22%. These often co-occurred with ERK pathway alterations, again suggesting heterogeneity in resistance mechanisms. The availability of many post-treatment samples from several patients allowed these authors to determine that melanomas underwent extensive branched evolution during progression or relapse (one patient had at least five distinct resistance mechanisms at relapse). In addition, altered mutational spectra (non-ultraviolet light-related mutations) were observed during disease progression. Further analysis of AKT activation in melanomas treated with RAF inhibitors indicated that early in treatment these tumours have increased levels of phosphorylated AKT relative to pretreatment. Experiments in cell lines showed that this can occur via adaptive upregulation of receptor tyrosine kinases, leading to increased recruitment of AKT to the cell surface. PI3K–AKT signalling can also be increased via selection for activating mutations following longer term inhibitor treatment, which increases the dependency of cells on this pathway for growth. Inhibition of both RAF and AKT repressed cell growth more than either agent alone. The observed early or late activation of AKT, as well as the pattern of branched evolution, suggest that initial therapy with ERK pathway and AKT inhibitors might be more efficacious than ERK pathway inhibition alone.

These papers indicate that several mechanisms probably contribute to resistance following ERK pathway inhibition in melanoma, even within the same patient. They also argue that a deeper understanding of resistance mechanisms and evolutionary adaptation to ERK pathway suppression is needed to improve therapy.

Sarah Seton-Rogers

ORIGINAL RESEARCH PAPERS Johannessen, C. M. *et al.* A melanocyte lineage program confers resistance to MAP kinase pathway inhibition. *Nature* **504**, 138–142 (2013) | Shi, H. *et al.* Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0642 (2013) | Shi, H. *et al.* A novel AKT1 mutant amplifies an adaptive melanoma response to BRAF inhibition. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0279 (2013) | Van Allen, E. M. *et al.* The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0617 (2013) | Wagle, N. *et al.* MAP kinase pathway alterations in *BRAF*-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. *Cancer Discov.* http://dx.doi.org/10.1158/2159-8290.CD-13-0631 (2013)