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Beyond Genomics: Multidimensional Analysis of Cancer Therapy Resistance

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Abstract

Cancer resistance to therapy occurs through a selection process generally thought to be driven by mutations. In a recent study, Hugo *et al.* use multidimensional analysis of the dynamic genetic, transcriptional, epigenetic, and immune landscape alterations in baseline and MAPK inhibitor-resistant melanoma tumors, demonstrating a role for ‘non-genomic’ drivers in cancer evolution.

Since the breakthrough therapeutic success of the BCR-ABL kinase inhibitor in chronic myelogenous leukemia, numerous targeted inhibitors have been used to treat cancers. Cancer therapies lead to Darwinian evolutionary selection of cancer cell variants with pre-existing or acquired mutations [1]. To prevent therapy resistance in cancer, combination drug therapy with multimodal mechanisms is essential. Activation of the MAPK pathway through mutation of BRAF (V600E) occurs in 50% of melanomas, and inhibitors targeting mutant BRAF and downstream MEK (MAPKi) in combination have been developed. While this combination therapy is initially efficacious, melanoma patients frequently relapse, prompting investigators to understand multiresistance mechanisms and determine what other therapies could be added to prevent resistance. One such therapy is immune checkpoint blockade directed against cytotoxic T lymphocyte antigen-4 (CTLA4) or programmed death-1 receptor/ligand (PD-1/PD-L1), which has been a marked breakthrough in cancer immunotherapy, especially for the treatment of melanoma. However, resistance to checkpoint blockade also frequently develops [2]. Furthermore, there is emerging pre-clinical and clinical data suggesting that MAPKi alter the tumor immune microenvironment and/or antitumor T cell responses [3]. Thus it is not clear whether MAPKi and other therapeutic interventions should be combined with immune checkpoint blockade, and if so, what the appropriate sequence of treatments would be. In a recent study, Hugo *et al.* [4] examined the resistance mechanisms driving melanoma relapse after MAPKi by performing a comprehensive analysis of serial biopsies (baseline and acquired resistant tumors) from melanoma patients treated with mono or dual MAPKi: BRAFi or BRAFi + MEKi. Their integrated assessment on the coevolutionary dynamics of genetic, non-genetic, and immune alterations provides new insights into cancer evolution and exemplifies a strategy for multifaceted analyses of cancer evolutionary dynamics that may better guide cancer therapy.

Hugo *et al.* first asked whether accumulation of specific genetic mutations could explain MAPKi acquired resistance. However, although recurrent mutations associated with

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resistance to MAPKi were detected in a subset of patients (including mutations in *BRAF*, *NRAS*, and *MEK*), such genetic events were not identified in 40% of MAPKi-resistant melanoma, pointing to non-genomic mechanisms of resistance. Turning next to the dynamic transcriptional landscape, Hugo *et al.* found that transcriptional upregulation or downregulation events were highly recurrent in MAPKi-resistant tumors: (i) genes with increased expression included *c-MET* and *c-FOS* as well as immune compartment genes such as macrophage markers *CD163/CD163L*, chemokine ligand *CCL8*, and inflammation genes *NFKBIA*; (ii) downregulated genes and pathways included antigen-presentation (*B2M*, *HLA*, *TAP1*), Wnt signaling (*LEF1*, *WNT11*, *FZD6*), and receptor tyrosine kinases (*EGFR*, *AXL*, *FGFR2*). Interestingly, *C-MET*, the most frequently upregulated gene could be used to stratify overall survival of melanoma patients from The Cancer Genome Atlas (TCGA) Melanoma data set. The majority of these transcriptional alterations were not associated with any underlying mutational event, leading the authors to examine genome-wide DNA CpG methylation. This analysis demonstrated that there were recurrent differential CpG methylation changes that significantly correlated with the observed transcriptional alterations, suggesting that epigenetic and transcriptional mechanisms contribute to the development of resistance to MAPKi in melanoma (Figure 1). The key findings that *c-MET* overexpression and β -catenin-LEF1 downregulation contribute to MAPKi resistance were further validated through elegant *in vitro* studies using human MAPKi-resistant melanoma cell lines.

Given the recurrent transcriptional changes found in multiple immune pathways, Hugo *et al.* next focused on understanding the coevolutionary dynamics of immune responses in MAPKi-resistant melanoma. Interestingly, about half of the tumors showed transcriptional evidence of increased inflammation, monocyte/macrophage infiltration, and M2 macrophage polarization. In a subset of patients, the macrophage/M2 transcriptional signature correlated with lower amounts of transcripts encoding for proteins associated with antigen processing and presentation, and lower numbers of tumor-infiltrating CD8 T cells. Importantly, the subgroup of resistant melanomas containing significantly lower numbers of infiltrating CD8 T cells displayed a high ratio of *EOMES/CD8A* expression, suggesting an 'exhausted' state similar to that described for terminally differentiated, exhausted T cells during chronic viral infections [5]. Indeed, reduced intratumoral *CD8A* expression was associated with increased amounts of transcripts encoding for proteins associated with T cell exhaustion, including the inhibitory receptors *PDI*, *LAG3*, *HAVCR2*, *CD 244*, *CD160*, and the transcription factors *PRDM1* and *EOMES*. These findings suggest that at least in a subset of patients, immune evasion and T cell exhaustion coevolve with the acquisition of MAPKi resistance (Figure 1).

The study from Hugo *et al.* along with several recent preclinical and clinical studies highlights the importance of integrating studies of intratumoral immune dynamics into our understanding of the signaling pathways that drive tumor development and the impact of different therapies on the cancer cells and the tumor microenvironment. For example, *BRAF*^{V600E} melanoma cells express immunosuppressive cytokines (IL-6, IL-10, VEGF), which promote the recruitment of myeloid derived suppressor cells and regulatory T cells [6]. In a mouse model of melanoma, constitutively active β -catenin signaling was shown to result in T cell exclusion and resistance to checkpoint blockade through defective dendritic cell recruitment and T cell priming [7]. The findings of Hugo *et al.* suggest that for patients

afflicted with melanoma tumors that are resistant to MAPKi and exhibit decreased mRNA levels of antigen presentation genes and *CD8A*, subsequent salvage therapy with immune checkpoint blockade may not be efficacious. This raises the question of whether underlying characteristics of the baseline tumor prior to MAPKi treatment can be used to predict which tumors will develop an immunosuppressive microenvironment with few infiltrating CD8 T cells along with MAPKi resistance.

These findings also emphasize the need to understand cancer evolution along multiple dimensions: genomic, non-genomic, and environmental (Figure 1). While genomic, epigenomic, and transcriptional analyses can be performed on small amounts of fixed tumor samples, direct analysis of the immune cell populations in these samples (e.g., flow cytometry) is challenging. The approach taken by Hugo *et al.* of extracting information regarding immune cell infiltration and function from bulk tumor transcriptional data presents a valuable alternative. These analyses will be aided by bioinformatics tools developed to deconvolute immune cell composition and functional states from complex tumor and tissue transcriptomic datasets [8]. Furthermore, two recent studies demonstrate that metabolic alterations in tumors also drive immune suppression and cancer progression [9,10]. Cancer cells and T cells compete for metabolites in the tumor microenvironment, and nutrient deprivation may not only diminish T cell responses, but also increase cancer cell resistance to therapy.

Cancer development, therapy-induced remission, and drug resistance/relapse result from the complex interplay of coevolutionary genetic, transcriptional, epigenetic, immune and metabolic events (Figure 1). A surprising finding of Hugo *et al.* is that the genomic and non-genomic alterations found in MAPKi-resistant melanomas was quite diverse; thus there are likely many pathways leading to resistance. Whether this diversity is due to intrinsic properties of the tumors, patient characteristics, environmental factors, or all three, is a critical question for future studies. While genome sequencing and other ‘omics’ technologies rapidly advance, we need to understand the interplay of these multidimensional factors determining cancer evolution to develop effective truly personalized therapies.

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Figure 1. Genomic and Non-Genomic Alterations in Cancer Evolution

Genetic, epigenetic, immune, and metabolic alterations coevolve during cancer development, therapy-induced cancer regression/remission, and pre-existing or acquired cancer therapy resistance/relapse. (Top left) Technologies that can be used for multidimensional data acquisition and analyses for cancer diagnostics. (Top right) Multimodal cancer therapies targeting one or multiple events of cancer evolution and progression.