Editorial

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Melanoma Management

Histone deacetylase inhibitors: a promising partner for MEK inhibitors in uveal melanoma?

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> "uveal melanoma has unique vulnerabilities that convey sensitivity to drugs that regulate the epigenome"

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Uveal melanoma is a highly aggressive tumor derived from the melanocytes of the eye. More than 90% of uveal melanomas harbor activating mutations in the small G-proteins GNAQ/GNA11 and have constitutive activity in the MAPK pathway [1]. In uveal melanoma, GNAQ/GNA11 activates phospholipase C β, which cleaves phosphatidylinositol-4,5-biphosphate to diacyl glycerol and inositol triphosphate. Both of these products activate protein kinase C, which in turn activates the MAPK pathway. Constitutive signaling in other signal transduction cascades including the PI3K/AKT/mTOR, WNT/β-catenin and the YAP-signaling pathways have also been reported.

Although approximately 4% of patients with uveal melanoma show signs of disseminated disease at diagnosis, approximately 4%, half eventually succumb to metastases [2]. The major site for uveal melanoma metastasis is the liver. For many uveal melanoma patients, development of metastases occurs many years after the successful treatment of the primary tumor. Patients can be stratified into low versus high risk of metastasis development (class 1 or class 2 uveal melanoma) on the basis of a 15-gene expression signature [3]. Class 1 tumors show greater melanocyte differentiation. Class 1 tumors can be further subdivided into class 1a and 1b categories with a 5-year metastasis risk of 2 and 21%, respectively [4]. Class 2 tumors typically lose melanocyte morphology and express genes associated with the primitive neuroectoderm. A class 2 gene signature is associated with a 5-year risk of metastasis equivalent to 70–80% [4].

One of the major genetic drivers of a class 2 phenotype is loss or inactivating mutations in the H2A ubiquitin hydrolase BAP1 [5]. Knockdown of BAP1 in uveal melanoma cell lines causes dedifferentiation and the adoption of a phenotype that confers metastatic behavior. BAP1 is the catalytic subunit of the poly comb repressive deubiquitinase (PR-DUB) complex that deubiquitinates histone H2A, and thus plays a key role in histone modification [6]. Recent work on the role of BAP1 in a *Xenopus laevis* development model has implicated it in the regulation of the epigenetic switch required for lineage commitment [7]. In this model, BAP1 loss was associated with transcriptional silencing and a failure of H3K27ac to accumulate at the promoters of key genes involved in lineage commitment including *Sox2*, *Foxd3* and *Sox10* [7]. Acetylation of histone H3 at lysine 27 (H3K27) is found at active and poised enhancer regions of genes. These data suggest that BAP1 loss leads to repression of lineage-specific gene expression, dedifferentiating the uveal melanoma cells to a primitive, embryonic-like state that favors metastasis.

Once established in the liver, uveal melanomas respond very poorly to therapy options currently available, including targeted therapies, immunotherapies and chemotherapies [8]. There has been some suggestion that the relatively low mutational burden of uveal melanoma compared with cutaneous melanoma – resulting in a lower expression of tumor neoantigens – may underlie the lack of efficacy seen in immunotherapy. To date, most work in the targeted therapy arena has centered upon the inhibition of kinases downstream of GNAQ/GNA11. The

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major focus so far has been upon MEK, for which several US FDA-approved small molecule MEK inhibitors exist. There is preclinical evidence that targeting MEK has some efficacy against uveal melanoma cells *in vitro*, leading to the inhibition of cell growth, cell cycle arrest and the induction of apoptosis [9]. The response of uveal melanoma cell lines to MEK inhibition fits with observations in other cancers, in which pathway inhibition leads to a rapid rewiring of the signaling network, increased receptor tyrosine kinase signaling and recovery of MAPK and other parallel signaling pathways. There is already some evidence that MEK inhibitor monotherapy leads to an increase in signaling through the PI3K/AKT/mTOR pathway [10] and increased RAS protein expression mediated by the RNA helicase DDX43 [11]. Other studies have proposed that host liver cells may also contribute to resistance, with work suggesting that hepatic stellate cells drive the escape from targeted therapies such as MEK inhibitors (via HGF secretion) and BRD inhibitors (via FGF-2 signaling) [12,13].

In the clinical setting, the most extensively explored MEK inhibitor for advanced uveal melanoma is selumetinib (AZD6244). In a Phase II open-label clinical trial of advanced uveal melanoma, selumetinib treatment yielded an improved progression-free survival compared with either dacarbazine or temozolomide [14]. Despite these initially encouraging results, a subsequent Phase III double-blinded trial of selumetinib plus dacarbazine showed no improvement in progression-free survival compared with dacarbazine alone [15]. At this time, it is clear that MEK inhibitor monotherapy is ineffective as a systemic strategy for uveal melanoma, and that combination therapies are needed.

As most uveal melanoma metastases show loss of BAP1 function/expression there has been considerable interest in uncovering novel therapeutic vulnerabilities conferred by the loss of BAP1. Although recent studies have proposed a link between BAP1 loss and sensitivity to EZH2 inhibitors in some cancers, this does not seem to hold true for uveal melanoma [16,17]. Instead, there is evidence that histone deacetylases (HDACs) are required to maintain the phenotype conferred by BAP1 loss. In preclinical studies, gene expression analyses have demonstrated that pan-HDAC inhibitors can shift the expression profile of class 2 uveal melanoma cell lines to that of class 1 [18]. Mechanistically, it seems that HDAC inhibition counteracts the effects of BAP1 loss by transcriptionally repressing the polycomb repressive complex component BMI1, leading to decreased histone H2A ubiquitination [19]. Further work showed that pan-HDAC inhibitors, such as valproic acid, induced the morphological differentiation of class 2 uveal melanoma cells, an effect accompanied by an inhibition of growth and survival *in vitro* and in *in vivo* uveal melanoma xenograft models [18]. Multiple isoforms of HDACs exist, and it is not yet clear which HDAC or combination of HDACs regulate the BAP1 loss phenotype. Some recent evidence from both *X. laevis* and human uveal melanoma cell line models have demonstrated that the BAP1 loss phenotype can be rescued in part through the silencing of HDAC4. Here, it was found that HDAC4 preferentially localized to the nucleus of BAP1 mutant uveal melanoma cells, and that shRNA-mediated silencing of HDAC4 significantly decreased uveal melanoma cell growth [7]. At this time, there are a number of clinical trials exploring the efficacy of the pan-HDAC inhibitors, valproic acid and vorinostat, in patients with metastatic uveal melanoma inhibition in both the monotherapy and combination therapy settings. The development of more specific HDAC inhibitors is still ongoing.

There has been some suggestion that MAPK inhibition in cancer cells may lead to a unique epigenetic state that could present novel therapeutic vulnerabilities and open up the possibility of combined epigenetic–MEK inhibitor combinations. Recent work from our group, which focused on *BRAF*-mutant melanoma, identified an increased dependency on HDAC8 activity following MAPK pathway inhibition [20]. In this instance, HDAC8 conferred BRAF inhibitor resistance through a mechanism involving the direct modulation of c-JUN acetylation, which led in turn to enhanced c-JUN transcriptional activity and the increased expression of EGFR. As HDACs are required to maintain the phenotype of aggressive uveal melanoma, we performed a multi-omics (proteomics and RNA sequencing) analysis of primary and uveal melanoma cell lines treated with the MEK inhibitor trametinib [21]. It was noted that although the cell lines initially responded to trametinib these effects were quite weak, and the cells rapidly evaded therapy. To understand more about the underlying mechanism, we interrogated our RNA sequencing and affinity-based protein profiling data and found that MAPK inhibition led to adaptive AKT signaling that was mediated through the receptor tyrosine kinases, ROR1/2 and IGF1R (Figure 1). Although follow-up experiments demonstrated that co-inhibition of MEK and AKT improved the durability of the response, it did not prevent resistance. We next turned our attention to the role of adaptive G-protein coupled receptor signaling and identified MEK inhibition to be associated with increased expression of multiple G-protein coupled receptors. Among these, the endothelin-B receptor was found to be upregulated upon MEK inhibition. Further mechanistic studies demonstrated a role for an endothelin-B–endothelin-3 signaling loop that activated YAP and contributed to therapeutic escape [21]. As dual inhibitors of YAP and AKT have yet to be identified, we performed a screen

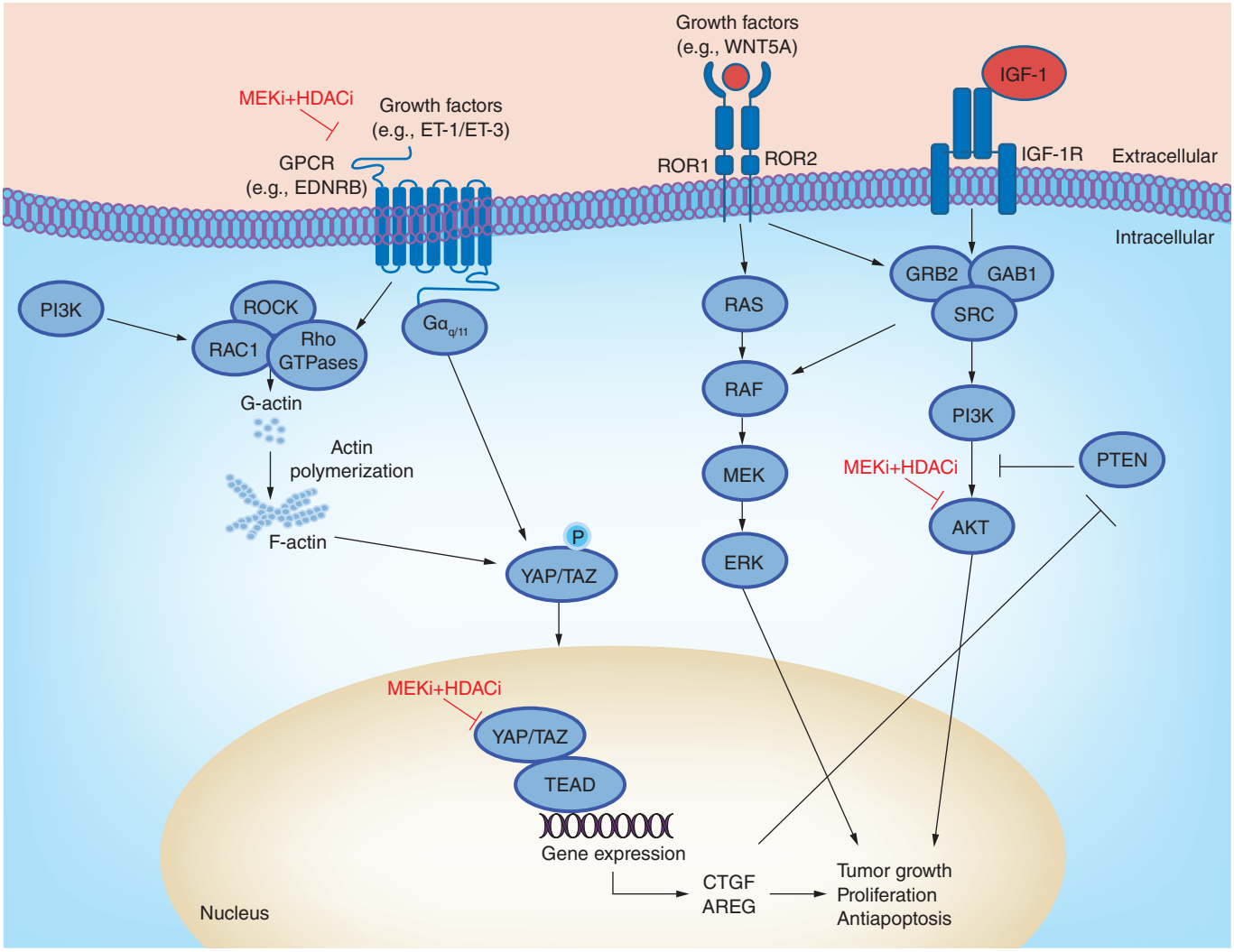

Figure 1. Scheme showing the likely mechanism of action of the MEK inhibitor–histone deacetylase inhibitor combination in uveal melanoma. MEK inhibition leads to increased expression in many RTKs, such as IGF-1R, ROR1 and ROR2, triggering second messengers through MAPK and PI3K/AKT pathways. Moreover, modulation of GPCRs (such as ET_B signaling) regulates cytoskeleton remodeling and actin polymerization through RAC1/ROCK/Rho GTPases and YAP/TAZ translocation to the nucleus. The MEKi + HDACi combination suppresses the adaptive signals that follow MEKi monotherapy in part through inhibition of IGF1R-AKT and ET_B-YAP signaling.
ET_B: Endothelin-B; GPCR: G-protein coupled receptor; HDACi: Histone deacetylase inhibitor; MEKi: MEK inhibitor.

of approximately 280 FDA-approved drugs and identified the pan-HDAC inhibitor panobinostat as a suitable combination partner for trametinib in *in vitro* growth assays. In this instance, pan-HDAC inhibitors were noted to be more effective than specific HDAC inhibitors, including the HDAC1/2/3 inhibitor etinostat, the HDAC6 inhibitor tubastatin and the HDAC8 inhibitor PCI-03451. Intriguingly, panobinostat was found to suppress the recovery of MAPK, as well as blocking the adaptive AKT and YAP signaling that followed trametinib treatment. This impressive level of signaling inhibition translated into improved *in vivo* efficacy, with the trametinib–panobinostat combination found to deliver durable responses in both subcutaneous xenograft and liver metastasis mouse models of uveal melanoma [21]. There is growing evidence across multiple tumor types that the use of targeted therapies such as BRAF and MEK inhibitors lead to epigenetic genetic changes that allow for therapeutic escape. Targeting this epigenetic remodeling in conjunction with the inhibition of a major oncogenic driver could be an excellent strategy to limit therapeutic escape. Our work demonstrates that uveal melanoma has unique vulnerabilities that convey sensitivity to drugs that regulate the epigenome, opening up new areas for further research and drug development. These findings in uveal melanoma mirrored our prior work in cutaneous melanoma and provided the rationale for evaluating the MEK-HDAC inhibitor combination in patients with metastatic uveal melanoma. Our

group is planning to open a clinical trial evaluating dual MEK-HDAC inhibition in patients with either advanced *BRAF*-mutant cutaneous or uveal melanoma.

Author contributions

F Faião-Flores contributed in the writing and figure creation. KSM Smalley contributed in the writing and editing of the manuscript.

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