Melanoma Management

EDITORIAL

Pin1–FOXM1 inhibitors: a potential therapeutic for metastatic melanoma?

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"Since transcription factors are largely unstructured when unbound to DNA and often lack enzymatic pockets as ATP sites, drugging them is a challenge."

Metastatic melanoma has long been considered an incurable disease with few options for treatment and a rapid progression. Inhibitors of the BRAF–MEK pathway and T-cell checkpoint-blocking immunotherapies have impressively increased tumor response rates and prolonged the overall survival outcome of metastatic melanoma patients [1]. Even long-term survival is now frequently obtained for patients receiving either therapy [2,3]. Although these strategies clearly hold great promise, many patients do not respond and those that do often develop resistance. The next goal is therefore to investigate how the onset of resistance can be delayed. To achieve this it is important to obtain a better understanding biology underlying resistance. Once known, molecular weaknesses that cause resistance can then be more effectively targeted directly, or, as we have focused on, may even entirely be bypassed [4].

An encouraging therapy against metastatic melanoma is BRAF–MEK inhibitor therapy. Designed around the observation that BRAF is the chronic driver in the majority of melanomas, inhibition of mutated BRAF, or its substrate MEK, proved very efficient against melanoma progression [5,6]. The effectiveness of the therapy is eventually stalled due to new mutations in the same pathway, such as the (N)RAS kinase upstream of BRAF, BRAF itself, the BRAF heterodimerization partner CRAF or in distinct MEK-activating kinases as COT-1 [7]. Combined BRAF/MEK inhibition can counter these escape-routes, but also these cocktails are eventually rendered ineffective due to development of resistance [2]. Several attempts have been made to further improve treatment success. Examples include the development of better RAF/MEK inhibitors that do not allow paradoxical MEK activation by CRAF, inhibitors of the kinases downstream of MEK, such as ERK and drug holidays that the temporary influence of nongenetic causes of resistance [8]. Importantly, the majority of these efforts are focused around achieving a similar objective, in other words, reducing

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MEK–ERK activity. Much less attention has gone to targeting signaling downstream of MEK–ERK. Since there are no obvious kinases downstream of ERK this is understandable, but it leaves a broad area of potential targets ignored. Being able to successfully hit the pathway downstream of ERK would in principle be effective independent of upstream mutations keeping MEK–ERK high and could be additive to each of these individual strategies. These targets are therefore worth exploring further.

In this regard, we found the transcription factor FOXM1 to be an interesting candidate for intervention. FOXM1 is a MEK target and we observed FOXM1 to be elevated in melanoma, especially in the metastatic phase [4]. Both FOXM1 levels itself, and that of transcriptional targets as *CCNB1* (Cyclin B1) and *CENPF*, predict a poor disease outcome. We therefore reasoned that targeting FOXM1 activity could be complementary to inhibition of BRAF/MEK inhibition, independent of any potential mutations that cause resistance within this part of the pathway. Since transcription factors are largely unstructured when unbound to DNA and often lack enzymatic pockets as ATP sites, drugging them is a challenge. We reasoned that targeting their interaction domains with important regulating enzymes may provide a solution. We found FOXM1 activity to be physically regulated by the peptidyl-prolyl isomerase Pin1. As this was stimulated by oncogenic BRAF in a MEK-dependent fashion and inhibition of Pin1 through shRNA reduced melanoma progression, we therefore chose to target the Pin1–FOXM1 interaction domain. No classical inhibitors existed that could be employed for this purpose. To overcome such an apparent pharmacological dead-end, we therefore turned to a class of therapeutics that have been relatively understudied in cancer treatment: cell-penetrating peptides (CPPs).

CPPs typically consist of a small stretch of amino acids, targeting a protein structure of choice and linked to an agent or structure that facilitates passage through the plasma membrane, such as the HIV-TAT sequence (reviewed in [9]). While the efficacy and safety has to be addressed for each peptide individually, CPPs have already been applied with success in disease models *in vitro* and *in vivo* [10] and they were found to be well tolerated in patients [11].

To assess whether FOXM1–Pin1-interfering CPPs may eventually be suitable for clinical translation, we employed two novel systems that better mimic the patient situation than conventional cell lines: *ex vivo* cultured metastatic melanoma slices and 3D melanoma cultures, dubbed melanoids. Especially a D-Retro-Inversed peptide mimicking a Pin1-interaction site in FOXM1 potently neutralized progression of a patient-derived melanoid. Moreover, this CPP enhanced the potency of the BRAF-inhibitor vemurafenib, together inducing a near-complete loss of melanoid viability.

Whether FOXM1–Pin1 inhibition can overcome vemurafenib resistance remains to be further investigated. However, since resistance-causing mutations are likely to require several cell cycling events to allow them to develop, the more pronounced the initial reduction in tumor burden, the more likely the occurrence of resistance is to be delayed. Irrespective of the outcome, the observed synergy with RAF inhibitors therefore already makes Pin1–FOXM1 blocking CPPs of interest for complementing the current lines of RAF/MEK inhibition solely for the potential of delaying resistance onset.

Curiously, the effects of the CPPs were more pronounced in the patient-derived melanoids and *ex vivo* cultured melanomas than in long established 2D cultured cell lines. It is therefore well possible that part of the mechanism through which Pin1–FOXM1 inhibition reduces melanoma growth is mediated by specific alterations in 3D growth characteristics. FOXM1 has been associated with stimulation of a more invasive phenotype in spheroids [12] and with the secretion of factors that add to tumor invasiveness by altering the tumor microenvironment, for example, matrixmetalloproteases (MMPs) [13] and interleukins [14]. Though more difficult to mimic in cell lines, these are clearly relevant to tumor progression in patients and warrant further investigation. Next to influencing tumor growth and migration, the microenvironment also plays a role in the success or failure of cancer treatment. This is also for instance the case for immunotherapy.

Using the patient's own immune system has long been considered an attractive approach for eliminating cancer cells, irrespective of their mutation status. A milestone in the development of more effective immunotherapies was the discovery and exploitation of T-cell checkpoint inhibitors. Activated T cells upregulate immune checkpoint molecules, such as CTLA-4 and PD-1, which abrogate the T-cell response. Inhibitors of CTLA-4 (ipilimumab) or PD-1, (pembrolizumab and nivolumab),

either individually or in combination, proved to be remarkably effective against metastatic melanoma [1]. Unfortunately, however, also to immunotherapy the majority of patients develop resistance. Due to the role of environmental factors, the molecular causes for immunotherapy-resistance are more difficult to study *in vitro*. However, previous research again puts FOXM1 in the spotlight, because of its role in regulating the β-catenin/TCF4 pathway. Chronic activation of β-catenin/TCF signaling was found to abrogate the efficacy of CLTA-4/PD-1 blockade on melanoma progression and survival in mouse models [15]. This can be explained by the fact that CTLA-4 is a downstream target of Wnt/β-catenin signaling [16]. Interestingly, FOXM1 promotes the activity of β-catenin and thereby controls expression of Wnt target genes [17]. As such, whether Pin1–FOXM1 inhibiting CPPs are effective in lowering Wnt/β-catenin signaling and thereby overcome resistance to CTLA4/PD-1 blockade is an attractive line of research to further investigate.

Next to Pin1–FOXM1 signaling, other oncogenic BRAF-stimulated pathways are of interest for targeting by CPPs. Of particular interest are members of the FOXO family, which are involved in a tight regulation with FOXM1 [18]. FOXOs are downstream targets of oncogenic BRAF as a consequence of ROS–JNK signaling [19]. Like FOXM1, FOXOs are under control of Pin1 [20] and relevant to melanoma progression [19]. Targeting defined interaction domains in FOXOs may therefore be also complementary strategy for overcoming therapy resistance to BRAF/MEK-inhibitors. Future research will tell whether this is indeed the case.

There are strong arguments why CPPs deserve further attention in cancer research. First, the choice of targetable domains is vast. While chemical inhibitors frequently rely on enzymatic pockets in their substrates, CPPs can in theory target any surface-exposed interaction domain, thereby greatly increasing the number of potential targets. Second, CPPs can be designed so they are predominantly hydrophilic. Irrespective of their efficacy, many chemical compounds, such as vemurafenib, are hydrophobic. Additional modification, retesting and optimization of pharmacological administration may therefore be necessary. Hydrophilic CPPs are well suited for intravenous injection reducing such need. Third, while CPPs are a theoretical risk for being recognized as antigens, triggering an immune response, their rapid cellular uptake generally limits CPP toxicity *in vivo*. Though this has to be determined for each CPP individually, we have not readily observed immune- or hepatic toxicity in mouse experiments using CPPs and neither has this been reported for other studies in mice or patients [10,11].

A final argument, which strongly underscores the true potency of CPPs as potential anticancer agents, is that unlike inhibitors that tend to target the overall activity of a protein, CPPs can be designed such that they interfere only with specific interaction sites. Therefore, CPPs are not automatically inhibitors of overall protein function, but can in fact be employed to steer a signaling response into a particular direction. Future research will undoubtedly reveal more suitable applications in which such a choice of biological outcome is favorable. For metastatic melanoma, Pin1–FOXM1 CPPs at least are the beginning of an exciting area of research.

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