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Skin Cancer (T Ito, Section Editor)

The Potential of Targeting P53 and HSP90 Overcoming Acquired MAPKi-Resistant Melanoma

Chi-Che Hsieh, PhD
Che-Hung Shen, PhD[*]

Address
[*]National Institute of Cancer Research, National Health Research Institutes, No.367, Sheng-Li Rd., North Dist., 70456, Tainan, Taiwan
Email: chshen@nhri.org.tw

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Opinion Statement

Melanoma is the deadliest form of skin cancer worldwide. The rising melanoma incidence and mortality, along with its high propensity for metastasis highlights the urgency to identify more effective therapeutic targets. Approximately, one half of advanced melanoma bears a mutation in the BRAF gene that makes BRAF as an important therapeutic target. Significant clinical benefit is associated with BRAF and MEK inhibitors (MAPKi) on targeting patients with BRAF V600 mutations. However, the frequent and rapid development of acquired resistance still is the major challenge facing the melanoma. Several mechanisms by which melanoma passes the inhibitory effects of MAPKi have been characterized and clinically translated, but additional alternations of genetic and epigenetic regulators outside of MAPK and/or AKT networks occurs in a quarter of patients with acquired MAPKi resistance. These studies implicate that targeting signaling networks external MAPK or AKT pathways is critical. In this review, we will focus on two approaches that are under evaluating for targeting melanoma: (1) against genome instability by p53 network restoration and (2) disrupt cancer proteome by chaperone inhibition.

Introduction

Melanoma is the deadliest form of skin cancer with estimated 91,270 newly diagnosed cases in the USA in 2018 [1], and with 3799 cases in Taiwan in 2015 according to Cancer Registry Annual Report, 2018 Taiwan. In Taiwan, the prognosis of metastatic disease remains poor with a median survival of 12 months in 2010 [2]. The rising melanoma incidence and mortality worldwide, along with its high propensity for metastasis highlights the urgency to identify more effective therapeutic targets. One of the most commonly mutated genes in melanoma is BRAF (~ 50%), encoding a member of the RAF protein kinase family and an intermediate in the MAPK signaling cascade. More than 90% of BRAF mutations in cancer involve a single-base substitution in the kinase domain leading to a V600E amino acid change and constitutive activation of its kinase activity [3]. As a major oncogenic driver for melanoma, BRAFV600E has been the No.1 wanted of the targeted therapy in cancer treatment.

Two selective BRAF inhibitors (BRAFi), vemurafenib (also known as PLX4032 or Zelboraf) and dabrafenib (also known as GSK2118436 or Tafinlar), are the standard of care option for metastatic melanomas with BRAF V600 mutations [4]. Vemurafenib was the first FDA-approved BRAFi for treating patients with unresectable or metastatic melanoma with the BRAF V600E mutation on August 17, 2011; however, the resistance which associated with MAPK pathway reactivation was observed in the patients treated with vemurafenib shortly. The acquired BRAFi resistance which mainly driven by the reactivation of MAPK signaling can be overcome by trametinib, an inhibitor of MEK. Trametinib was approved by FDA on May 2013. Based on the promising results of clinical trial studies [5–7], FDA approved the combination of trametinib with dabrafenib for the treatment of patients with BRAF V600E/K-mutant metastatic melanoma on January 2014 and as an adjuvant treatment for BRAF V600E-mutated melanoma after surgical resection on May 2018. Although, the combination of MEKi and BRAFi leads to a longer duration of responses, higher rate of tumor responses, and decreasing the paradoxical side effects. The experienced distant relapse was reported in 40% of patients in the dabrafenib plus trametinib arm (59% in the placebo) [8]. Therefore, significant works have been directed at understanding the factors contribute to BRAFi responses. The major resistance mechanisms have been described, including RAS mutations, MEK1/2 mutations, BRAF splicing variants, amplification of BRAF, and upregulation of the PI3K/AKT pathway as well as multiple receptor tyrosine kinases [9••]. It is noteworthy that a large scale sequencing study showed that about 74% of BRAFi-resistant progressive melanoma tumors had genetic alterations in these two-core pathways (RAF-MEK-ERK and PI3K-AKT), but the resistance mechanisms in the remaining 26% still remain unknown [10]. The frequent and rapid development of secondary resistance remains the most striking problem in targeting unresectable or metastatic melanoma [11].

Recently, additional alternations of genetic and epigenetic regulators outside of MAPK and/or AKT networks, which lead to acquired MAPKi resistance had been extended study. The genome-wide chromatin interactions and epigenetic regulators had been reported to mediate MAPKi resistance development. STAG2 and STAG3, the cohesion complex components, were identified and characterized to involve in the development of MAPKi resistance. This study suggested the loss of STAG proteins inhibited CCCTC-binding factor (CTCF)-mediated expression of dual specificity phosphatase 6 (DUSP6), leading to ERK signaling reactivation and tumor relapse [12•]. In addition, the epigenetic regulators mediated the reactivation of IGF-1 receptor (IGF-1R) and its downstream AKT signaling had been characterized in MAPKi resistance melanoma [13•]. The SIRT6 haploinsufficiency decreased the sensitivity to MAPKi through deacetylating H3K56ac at the IGFBP2 locus and promoting the expression of IGFBP2, leading to IGF-1R/AKT activation in melanoma with BRAFV600E mutation. These studies implicate that targeting the signal molecules external, the two-core MAPK or AKT pathways is a considerable strategy for overcoming MAPKi resistance development in melanoma. In this review, we will focus on two attractive approaches (1) against genome instability

by p53 network restoration and (2) disrupt cancer proteome by HSP90 chaperone inhibition, that is under active investigation and translating into clinical applicability for human cancers, including melanoma.

The therapeutic potential of p53 restoration in melanoma

p53, a potent tumor suppressor is the best-known signaling node that coverts diverse upstream stress cues into downstream cellular responses. Classically, p53 plays a role as guardian of the genome to limit the cell propagation, such as inducing cell cycle arrest, senescence, DNA repair, and apoptosis with corrupted genomes [14]. Stress signals principally through two p53-interacting proteins, MDM2 and MDMX, to promote p53 degradation, and thereby inhibit p53 activity [15, 16]. MDM2 and MDMX function as a p53 inhibitor. Evidence from mouse models showed that the gene knockout of MDM2 or MDMX induced embryonic lethality, which can be rescued by p53 ablation [17–20]. Additionally, the knockout of MDM2 or MDMX led to cell apoptosis or suppresses cell proliferation and had been reported in a variety of different tissues, cell lines, and stem/progenitor cells [16, 21, 22]. Importantly, the expression of MDM2 or MDMX was upregulated in many types of human cancers and correlated with constitutively p53 inactivation [22, 23]. Moreover, elevated expression of TA-p63 and/or △ Np63 isoforms, and iASPP (inhibitor of an apoptosis-stimulating protein of p53) were found in the majority of malignant melanoma [24, 25]. These suggest that p53 inactivation-induced tumor progression and malignancy can be attenuated by destroying MDMX/MDM2/p53 complex [26••].

Several small molecules had been designed to restore p53 function. Nutlin-3, the first cis-imidazoline compound had been shown to prevent the formation of MDM2-p53 complex and restore the tumor suppressor function of wild-type p53 preclinically. However, Nutlin-3 did not reach the clinical trials because of the poor bioavailability [27]. The optimized cis-imidazoline derivatives, RG7112 and RG7388 (idasanutlin), had been testing in clinical trials and shown partially encouraging clinical activities in MDM2-amplified liposarcoma [28], advanced solid tumors [29], and leukemia [30, 31], but not in melanoma yet. Despite the modest therapeutic efficacy, the clinical adverse events associated with RG7112 and RG7388 including hematological toxicity, limiting the dose and duration of the treatments.

In the last few years, a potent, selective, and orally bioavailable MDM2-p53 inhibitor, AMG232 had entered in clinical trials. AMG232 is a piperidinone-derived compound from Amgen. The preclinical pharmacological studies showed that similar to that of other MDM2 inhibitors including SAR299155, RG7112, and RG7388, the AMG232 suppressed cell growth and triggered cell apoptosis in a large panel of different cell lines in a p53-dependent manner, which associated with stabilization of p53 and upregulation the expression of MDM2 and p53 downstream effectors, P21 and PUMA. Importantly, AMG232 showed antitumor activity in p53 wild-type tumor cell lines bearing the mutations in the MAPK signaling pathway (e.g., KRAS-mutant HCT116 colorectal cells and BRAF-mutant A375 melanoma cells) [32]. AMG232 is more specific and effective on the inhibition of the MDM2-p53 interaction than RG7112,

which makes AMG232 a potent effect on targeting the stemness of patient-derived glioblastoma cells [33]. These shows AMG232-restored tumor-suppressive function of p53 is broadly effective on human cancers. Notably, AMG232 is currently under clinical evaluation for the treatment of cancers including melanoma. The phase Ib/IIa studies evaluated the toxicity (CTCAE 4.03), maximum tolerated dose (MTD), pharmacokinetics (PK), and preliminary antitumor activity (RECIST 1.1) of AMG232 together with trametinib (MEK inhibitor) and dabrafenib (BRAF inhibitor) in patients with p53 wild-type metastatic cutaneous melanoma, and showed an acceptable PK profile with early evidence of antitumor activity (NCT02110355).

In addition, MDMX, another potent p53 inhibitor, is amplified or upregulated expression in 10–20% of human cancers and is overexpressed in about 65% of human melanomas, irrespective of BRAF or NRAS status. Thus, MDMX has been highly implicated as a therapeutic target in cancers. Small antagonists of MDM2 had recently been tested in MDMX assay. Several lines of evidence suggest that selective MDM2 inhibitors are not effective on tumors with highly expressed MDMX, which suggest that the optimal p53 reactivation can only be achieved by targeting both MDM2 and MDMX simultaneously [23, 34–36]. New efforts, therefore, had been directed to identify the molecules able to target both MDM2 and MDMX. Peptidomimetics have been disclosed in the last decade as MDM2/MDMX inhibitors, including stapled peptides, SAH-p53-8, ATSP-7041, and ALRN-6924. SAH-p53-8, which was the only one MDM2/MDMX inhibitor, showed the inhibitory effect on the cell growth of BRAFi resistance melanoma cells, and synergistically increases the sensitivity to the cytotoxic chemotherapy and BRAF inhibitors preclinically [22]. However, due to high serum-binding affinity, SAH-p53-8 was unsuitable for clinical translation. ATSP-7041, another stapled α-helical peptide, had been shown potent effects on inhibition of MDM2 and MDMX, and induction of p53-dependent apoptosis, leading to suppression of cell proliferation and tumor growth in vitro cell lines and in vivo tumor xenograft mouse models [37, 38]. It was shown that ALRN-6924, an inhibitor for MDM2 and MDMX, activated p53-dependent transcription and displayed robust biochemical and molecular biological effects on targeting leukemia cells in vitro and in vivo AML xenograft models [39]. ALRN-6924 had entered phase I trial for evaluating the safety, PK, PD, and antitumor effects in patients with advanced solid tumors or lymphomas. Fifty-five patients (69 patients in total) evaluable for efficacy, disease control rate was 45%, including 2 patients with complete remissions (peripheral T cell lymphoma and Merkel cell carcinoma), 2 partial remissions (colorectal cancer and liposarcoma), and 21 patients with stable diseases. A phase IIa cohort in peripheral T cell lymphoma was opened in August 2016 and is currently enrolling (NCT02264613). These translational studies showed that ALRN-6924 is well tolerated and has potent clinical antitumor activity. ALRN-6924 should be future evaluated to be as a therapeutic approach in melanoma.

Casein kinase 1 (CK1) is another important mechanism which inactivates p53 networks. CK1 contains seven isoforms including CK1α, CK1β, CK1γ1, CK1γ2, CK1γ3, CK1δ, and CK1ε. CK1α negatively regulates WNT/β-catenin and p53 signaling [40]. The interaction of CK1α between MDM2 and MDMX had been suggested to inactivating p53 [41, 42]. The inhibition of CK1α induced robust p53 activation and cell senescence had been demonstrated in vitro cell models using RNAi approaches and CK1α inhibitors [42, 43], as

well as in *Csnk1a1*$^{\Delta gut}$ genetic engineering mouse models [44]. Recently, A-series CK1α inhibitors had been developed and shown to effectively activate p53 networks with inhibitory effects on AML cells and mouse models [45]. More, lenalidomide, a derivative of thalidomide had been approved in the USA in 2005. Lenalidomide had been characterized to target CK1α and induce CK1α degradation through ubiquitination, leading to p53 network reactivation [46]. More, the effects of lenalidomide on skin cancers had been evaluated in Merkel cell carcinoma (MCC). It showed that the cell viability of MCC was synergistically inhibited in response to the combination of lenalidomide with Nutlin-3, RG7388, or AMG232. Furthermore, the adding of lenalidomide greatly enhanced the efficacy of HDM201 (MDM2 inhibitor) on suppressing MCC xenograft tumor growth [46]. These suggest that targeting CK1α is another approach to restoring p53 network and CK1α inhibitors have potential clinical advantages for melanoma. It is critical to characterize the molecular, cellular, and biochemical mechanisms of action, as well as therapeutic potential of these MDM2, MDMX, and CK1α inhibitors in melanoma.

Targeting molecular chaperones in melanoma

Emerging evidence suggests that cancer is a proteomic disease and is controlled by the onco-proteome directed the tumor growth and malignancy, the interaction with surrounding cells, and the response to therapy [47]. Heat shock protein 90 (HSP90) chaperone complex has been suggested to play an essential role in coordinating cancer-specific proteome and alternated signaling pathways [48••].

HSP90 consists five family members, HSP90α and HSP90β (both are cytoplasmic form), GRP94 (glucose-regulated protein 94 locates at ER), TRAP1 (tumor necrosis factor receptor-associated protein-1 locates at mitochondria), and isoform HSP90N. HSP90 works together with other co-chaperones to form HSP90 chaperone machine which functions in protein folding and maturation, intracellular disposition, as well as proteolytic turnover of several crucial regulators for cellular metabolic functions, including cell proliferation, differentiation, and survival [49].

The interaction surface between HSP90 and its clients is not entirely characterized. The electron microscopy reconstruction and crystal structure showed that the N-terminal and middle domains of HSP90 provided a large surface area for binding to the client proteins, including cyclin-dependent kinase 4 (CDK4) [50], microtubule-binding protein Tau [51], and the ligand binding domain of glucocorticoid receptor (GR) [52]. The atomic structure revealed that HSP90 together with co-chaperone CDC37 trapped CDK4 in an open and partially unfolded state which was required for the maturation of CDK4 [50].

The binding and releasing of client proteins with/from HSP90 machine is mediated by co-chaperones, such as HSP70, CDC37, HOP, AHA1, and p23. HSP70 has high binding affinity for short and five-residue-long stretches with three or more large hydrophobic or aromatic residues which only present within unfold clients [53]. Once HSP70 brings unfold clients into HSP90 complex, the ATPase activity of HSP90 mediates the unfold clients transferring from HSP70 to HSP90 through ATP hydrolysis [52]. This shows HSP90 chaperone machine is tightly regulated by the ATP-binding hydrolysis cycles [54].

The role of ATPase cycle in regulating HSP90 machine is another active area of investigation. The ATP-binding and -hydrolyzing pocket is located at the N-terminal domain of HSP90. The ATPase cycle is controlled by two known co-chaperones, p23 and AHA1. p23 mediates the interaction of both N-terminal domains in the HSP90 dimer and keeps HSP90 dimer in an ATP-binding state [55]. The ATP hydrolysis is stimulated by AHA1 [56]. The ATPase activity of HSP90 is essential for folding and stability of HSP90 clients, which is evident by the inhibition of HSP90 ATPase by HSP90 inhibitors [57, 58].

Many oncogenic proteins, such as tyrosine-kinase receptors (i.e., EGFR, HER2, and VEGFR); signal-transduction proteins (i.e., BRAF and AKT); transcription factors (i.e., HIF1α and p53); cell cycle regulatory proteins (i.e., CDK4 and cyclin D); antiapoptotic proteins (i.e., BCL2 and survivin); and telomerase (hTERT) are client proteins of HSP90 complex [59]. The positive correlation between the overexpression of oncoproteins and the accumulation of HSPs in cancer cells highly suggests that (1) the accumulated expression of HSPs is essential for stabilizing the altered expression of oncoproteins, by which promotes cancer progression; (2) the cancers with upregulated HSPs could be more sensitive to HSP inhibitors [49]. Thus, the development of small molecular compounds aims at disrupting HSP90 machine, destroys the cancer proteome, and suppresses cancer cell progression, becomes an attractive therapeutic concept for anticancers.

HSP90 is the most widely investigated HSPs in targeting cancers and several HSP90 inhibitors (HSP90i) are in advanced stages of clinical development. Originally, the HSP90i is a natural compound including geldanamycin (GA) and radicicol (RD). GA was derived from *Streptomyces hygroscopicus* in 1970 and was characterized to bind with HSP90 in 1994 [60]. GA binds to the ATP-binding pocket of HSP90, preventing ATP binding and hydrolysis, leading to the ubiquitin-mediated proteasomal degradation of oncoproteins [61, 62]. The antitumor activity of GA had been shown preclinically but not been approved clinically. The structural instability and poor solubility of GA result in the hepatotoxicity at therapeutic doses [63, 64]. To improve the pharmacological properties and toxicity profiles, GA was chemical modified. 17-AAG (17-allyl-17-demethoxygeldanamycin), a GA analogue was developed by substituting C_{17} at GA with an allylamino group [65]. 17-AAG was the first HSP90i to progress into phase I/II clinical trials [66]. Unfortunately, the initial phase II clinical trials which recruited unselected patients with melanoma [67], breast, prostate, or renal cancer were noted with no objective responses. The combination of 17-AAG with other anticancer drugs, such as sorafenib in melanoma [68] and other human cancers showed limited efficacy in phase I trials [69].

RD was isolated from *Monosporium bonorden* in 1953. RD is structurally unrelated to GA but inhibits HSP90 by blocking the ATP pocket, same as GA. The anticancer activity of RD only showed in v-SRC-transformed cells and did not prove in vivo animal studies [61] due to the serum instability. To improve the stability for subsequently clinical development, several RD analogues, such as AT-13387, STA-9090 were developed [69]. STA-9090 is the one that had been proven for phase I/II clinical trials in melanomas but terminated due to weak accrual (NCT01551693 and NCT01200238).

Based on the availability of X-ray crystallographic data for the interaction between HSP90 and ATP/ADP, the development of HSP90i had been progressed from the first generation of natural derivatives to the second generation of fully synthetic small molecules in the past decade [70]. These synthetic HSP90i cover purines and resorcinols, as well as pyrimidines, aminopyridines, azoles, and other chemotypes. Among them, only N-aryltropane XL888 had been evaluated in an open-label, single-center phase I trial of escalating doses in the combination with vemurafenib for patients with unresectable or metastatic melanoma. Seventy-five percent of 20 evaluable patients (21 patients in total) had a response to the combination treatment with 3 complete and 12 partial responses. The patients had a median progression-free survival for 9.2 months and a median overall survival for 34.6 months. This trail shows the combination of XL888 with vemurafenib has clinical activity with manageable toxicities in patients with advanced BRAFV600-mutant melanoma [71].

Additional agents for targeting HSP90 chaperone are under active investigation. The small molecules that bind to the C-terminal/middle domain of HSP90-Coumarin derivatives including Nonobiocin, A4, DHN1, and DHN2; the agents targeting co-chaperone/HSP90 interactions such as celastrol; the inhibitory peptides targeting TPR co-chaperones/HSP90 and client/HSP90 associations; and the cell surface HSP90 inhibitors. The pioneering development of HSP90i with the natural origin compounds was critical for the biological evaluation of HSP90 as a drug target and establishing the technical drugability of the ATP-binding N-terminal domain, as well as development other potential approaches for targeting HSP90 chaperone. However, none of these HSP90i have yet successfully reached the clinic market. The inherent toxicity and the heat shock responses of HSP90i induce severe side effects, by which limit the clinically applicable dosages and result in the discontinuation of the clinical trials [69, 72]. Although the mechanisms underlying the function of HSP90 in malignant transformation and the translation of this knowledge into anticancer drug development are rapidly progressing and actively investigating, it is a significant issue to discover the pathogenic clients and pathways in response to HSP90i-induced cytotoxicity effects, such as ocular toxicity. In fact, a study suggested that the HSP90 clients, G protein receptor kinase 1 and phosphodiesterase in the phototransduction pathway were involved in the visual impairment induced by Hsp90i [73].

Conclusion

Targeting MAPK is a standard treatment in melanoma with BRAF V600 mutations. However, the genetic and epigenetic mutations causing global changes in cancer proteome makes melanoma bypass this standard-of-care targeted therapy and develop acquired MAPKi resistance. It is critical to evaluate the small molecules that target the signaling knots external MAPK or AKT pathways, such as p53 networks and HSP90 chaperone.

Although much progress is being made, no MDM2, MDMX, or HSP90 inhibitor has yet been approved for clinical application in the treatment of melanoma. Despite a comprehensive understanding the molecular mechanisms and protein-inhibitor interactions lead to novel inhibitor development as

well as therapeutic strategy improvement, it is important to explore the molecules in response to inhibitor-induced cytotoxicity, which will largely benefit the clinical management for patients receiving treatments.

Compliance with Ethical Standards

Conflict of Interest
Chi-Che Hsieh and Che-Hung Shen declare they have no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note

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Papers of particular interest, published recently, have been highlighted as:
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