Chapter

Ubiquitination and Deubiquitination in Melanoma Research and Clinically Relevant Outcomes

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Abstract

Malignant melanoma is one of the most invasive tumors with increasing mortality, low overall survival rates and limited effective therapeutic strategies. Ubiquitination is a post-translational protein modification, which is regulated by a series of ubiquitination-associated enzymes. Ubiquitination plays a critical role in diverse pathophysiological activities of cellular and participates in the pathogenesis of various cancers, including melanoma. This study aims to provide a conclusive of ubiquitination and deubiquitination, and their potential clinical application value in melanoma in the following aspects: melanoma pathogenesis-related components and processes in the ubuiquitin-proteasome system (UPS), ubiquitination in melanoma immunological microenvironment modulation, ubiquitination of key transcription factors in melanoma and melanoma therapeutic strategy via targeting the UPS.

Keywords: ubiquitination, deubiquitinating enzymes, melanoma, pathogenesis, application

1. Introduction

Malignant melanoma is one of the most invasive tumors with increasing mortality, low overall survival rates, and limited effective therapeutic strategies [1]. Although melanoma is the third most prevalent skin cancer, the two other skin malignancies, basal cell and squamous cells, are the malignant [2]. A variety of factors, including genetic mutations, sun exposure, and poor lifestyle habits, are involved in the development of melanoma [3].

There is a dynamic protein balance in cells to maintain homeostasis for cell and organism. Intracellular protein degradation by two pathways, autophagy and lysosomal degradation pathway and the ubiquitin-proteasome pathway, is primarily involved in tumor growth. Ubiquitination is one of post-translational modifications of most vital proteins. Ubiquitin, a closely conserved small protein composed of 76 amino acids, is link with the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin ligase (E3) [4]. Specifically, mono-ubiquitination was considered as only one single ubiquitin bond to the lysine, while poly-ubiquitination was considered as ubiquitin chains attached to the lysine [5]. Then the ubiquitinated proteins are transported to the 26S proteasome for degradation. Ubiquitination is involved in the development of different tumors

by regulation important genes or signaling pathways. However, the ubiquitination process can be reversed by the deubiquitinating enzymes (DUBs) via cleaving ubiquitin chains from substrates to prevent protein degradation, which participates in a wide range of cellular signaling pathways, such as the apoptosis, cell cycle, autophagy, DNA damage, inflammation signaling, and protein downregulation [6]. Up to date, there were reported over 600 E3 ubiquitin ligase and 100 DUBs [7].

A significant number of studies have confirmed that ubiquitination and de-ubiquitination play a critical role in melanoma pathogenesis, and have indicated that the key molecular goal of the mechanism may be the therapeutic strategies for the treatment of melanoma. Here, we provide a conclusive introduction about protein ubiquitin modification in relative genes, signaling pathways, and in immune system in melanoma pathogenesis, which concludes the latest DUB studies in melanoma. Besides, we summarize potential therapeutic targets of ubiquitination and de-ubiquitination in melanoma.

2. Melanoma pathogenesis related components and processes of the UPS

2.1 Fbxw7

The F-box/WD repeat-containing protein 7 (Fbxw7) belongs to the F-box protein family, which is the component of an SCF E3 ubiquitin ligase [8]. Fbxw7 is considered to be a tumor suppressor gene [9]. The degradation of Fbxw7 results in accumulation of its substrates, leading to oncogenesis. In a study, the mutation prevalence was found to be 8.1% FBXW7 in melanoma through exome sequencing in a cohort of 103 melanomas. A potential therapeutic approach for melanoma could be the loss and mutation of FBXW7 in melanoma contributing to prolonged activation of NOTCH and targeting NOTCH signaling [10]. FBXW7 deficiency can also unleash heat shock factor 1 (HSF1) and then result in melanoma invasion and metastasis [11]. Meanwhile, FBXW7 can regulate the melanoma metastasis through activating the MAPK/ERK signaling [12]. The microphthalmia-associated transcription factor (MITF) is a key regulator of melanocyte development, differentiation, and melanoma biology [13, 14]. FBXW7 is recognized as a regulator of MITF via post-transcriptional mechanisms [15].

2.2 SKP2

S-phase kinase-associated protein 2 (Skp2), also be called FBXL1 or p45, is also a member of the F-box proteins [16]. Skp2 is characterized as a cancer-related protein. In general, in primary melanoma and metastasis melanoma, SKP2 is significantly up-regulated, which is related to the prognosis, as it is reported that nuclear Skp2 expression is strongly associated with a lower survival rate during melanoma [17, 18]. In tumorigenesis, Skp2 stabilizes the MTH1 expression via K63-linked polyubiquitination, and then promotes melanoma cell survival by protecting DNA integrity upon pharmacologic oxidative stress [19]. Meanwhile, skp2 has a direct interaction with melanoma antigen-A11 (MAGE-A11), which may boost Skp2-mediated degradation of cyclin A [20].

2.3 HACE1

HECT domain and ankyrin repeat-containing E3 ubiquitin protein ligase 1 (HACE1), has been showed to act as a tumour suppressor gene in various kinds of cancers [21]. There is a significantly downregulation of HACE1 in colorectal cancer (CRC), and the decreased expression is highly associated with poor clinical features of

patients. HACE1 inhibits YAY1 signaling and then can reverse EMT in CRC [22]. Loss of HACE1 activates RAC-family GTPases to mediate oxidative stress that increases genotoxic cellular ROS generation and then results in lung tumor formation [23]. Even though HACE1 behaves as an anti-oncogene in most reports, its function in melanoma may be cell-specific tumorigenesis. HACE1 plays a pro-oncogenic role in melanoma by regulating fibronectin (FN) secretion and K27 ubiquitination of FN [24].

2.4 ITCH

ITCH, a member of HECT-type ubiquitin E3 ligases, plays a significantly role in regulating cell growth and apoptosis [25]. In melanoma, ITCH mediates BRAF polyubiquitination through the K27-linkage result in sustained activation of BRAF/MEK/ERK signaling, which leads to the survival of melanoma cells [26]. Moreover, ITCH can be regulated by microRNAs (miRNAs), such as miR-10b and miR-520f, and then be involved in the melanoma proliferation and metastasis [27, 28].

2.5 UBE2C

UBE2C belonging to the E2 family is operating in combination with the anaphase-promoting complex/cyclosome (APC/C) E3 ligase. It regulates the cell cycle through mitosis via destructing mitotic cyclin B1 [29]. Silence of UBE2C induces G2/M phase arrest of melanoma cells by suppressing both the level and the activity of M-phase-promoting factor (MPF), a complex consisting of CDK1 and cyclin B1 [30, 31].

2.6 UBE2S

Ubiquitin-conjugating enzyme E2S (UBE2S) belongs to the E2 protein family, and is involved in development of various cancers. Recently, it has been shown that UBE2S plays a vital role in regulating DNA damage-induced transcriptional silencing, by catalyzing Lys11-linkage ubiquitination [32]. Another recent research showed that UBE2S is overexpressed in melanoma, and the expression was significantly related to the cancer staging and grading, with a higher magnitude found for tumor node metastasis staging T4. Moreover, silence of UBE2S may cause melanoma cell proliferation inhibition via inducing cell cycle G1/S phase arrest, and cell apoptosis. In BALB/C nude mice, shUBE2S can suppress tumor growth and inhibit epithelial-mesenchymal transition (EMT) [33].

2.7 MKRN2

Makorin ring finger protein 2 (MKRN2) is known as a novel ubiquitin E3 ligase, and is capable of targeting the p65 subunit of NF-κB [34]. Research indicates that there is a greater expression of MKRN2 in melanoma cell lines relative to normal skin cell lines. The silence of MKRN2 can inhibit melanoma cell growth in a P53-dependent manner. Moreover, MKRN2 can interact with ubiquitylated P53 [35]. This study suggests that MKRN2 may be a potential therapeutic target for melanoma.

2.8 Ub-like proteins

There are also several ubiquitin-like proteins (UBLs) in addition to Ub, such as NEDD8 (neural precursor cell expressed, developmentally down-regulated8), SUMO (small ubiquitin-like modifiers), and ISG15 (interferon-stimulated gene 15).

NEDD8 mediates the stabilization of various proteins, and plays a significant role in the incidence and development of malignant melanoma. NEDD8 is a

ubiquitin-like protein composed of 81 amino acids, with around 60% of the sequence that is the same as ubiquitin [36]. The covalent binding of NEDD8 to substrates is known as neddylation. Similar to the ubiquitination, an enzyme cascade is needed for this progression. Neddylation is involved in protein ubiquitination, and is closely associated with the degradation of certain proteins in the cell cycle and apoptosis-related factors [37]. Cullin is one of the most researched neddylation substrates [38]. Besides, studies have also investigated that NEDD8 substrates are diverse. Some proteins can be modified by NEDD8, including p53 [39], MDM2 [40], and VHL [41]. UBA3, as the subunit of NEDD8-activating enzyme, plays a critical role in the linkage of NEDD8 with cullin proteins. Previous studies have shown that in highly proliferative cell lines, NEDD8 conjugation is up-regulated and increased in melanoma cell lines [42]. After knockdown of UBA3, the proliferation of M14 melanoma cells was suppressed both in vitro and in vivo. Hence, interference of the neddylation might offer a hopeful method for melanoma therapy [43].

SUMO has been described to alter protein interactions rather than directly involving in protein degradation [44]. Sumoylation involves a 3-step pathway analogous to the ubiquitination pathway. Dysregulation of sumoylation has been implicated in multiple cancers, including melanomas. Ubc9, the single SUMO E2 conjugating enzyme, is overexpressed in advanced-stage melanomas where it protects melanoma cells from chemotherapy-induced apoptosis [45]. Moreover, SUMOylation-defective MITF germline mutation may be more susceptible to melanoma [46].

ISG15, a ubiquitin-like modifier, is implicated in both tumor oncogenic and suppressive programs [47]. It is activated by a three steps enzymatic cascade consisting of a specific E1-activating enzyme (UBE1L), E2 conjugating enzyme (typically UBCH8) and E3 ligase (commonly HERC5A), which promotes ISG15 transfer to protein substrates [48]. Previous study shows that ISG15 can be removed from its target proteins by USP18 and then the effects of ISGylation was reversed [49, 50]. A study identifies PTEN as a new substrate of the ISGylation post-translational modification pathway and USP18 can regulate PTEN stability. Inhibition of ISGylation may be a therapeutically relevant in melanoma [47].

2.9 Deubiquitinating enzymes (DUBs)

To date, several DUBs have confirmed to be consistent with melanoma tumorigenesis and metastasis. USP54 is overexpressed in intestinal stem cells, and is defined to promote cancer progression and regulate embryonic development and normal growth of adult mice. USP54 upregulates in melanoma, the loss of USP54 is dispensable for metastasis of melanoma cells [51]. An IFN stimulated to regulate type-I IFN signaling in the anti-viral immune response has been reported to be USP18 [52]. It is also reported that IFN-γ can stimulate USP18 protein expression in melanoma cells. Through IFN-γ-induced USP18 expression in melanoma cells and -regulated CTL CD8 + immune cell activity in the tumor microenvironment, endogenous IFN-γ signaling influences melanoma tumorigenesis [53]. In 2014, Harish Potu et al. reported that USP5 mediates the change in ubiquitinylated protein content and unanchors Ub chains in BRAF mutant cells treated with vemurafenib. BRAF can activate USP5, contributing by suppressing p53 and FAS induction, to inhibit cell cycle checkpoint regulation and apoptosis [54]. In 2018, USP4 upregulation in melanoma, especially in metastatic melanoma, was discovered by Weinan Guo et al. The archive of TCGA skin cutaneous melanoma (SKCM) confirms this finding. USP4 can protect melanoma cells from cisplatin-induced apoptosis in a p53-dependent manner. Moreover, USP4 up-regulation plays an important role in melanoma invasion and migration by promoting EMT [55]. The USP15 knockdown lowers the expression of MDM2 in melanoma cells, and then leads to upregulation

of p53 and MDM2 target genes p21 and Puma. Moreover, Usp15−/− melanoma mice models have an increased frequency of CD8+ effector T cells tumor-infiltrating [56].

Ubiquitin specific peptidase 9, X-linked (Usp9x), a member of the USP family, is upregulated in many cancers, which has a positive and negative impact on tumorigenicity depending on the various forms of cancer [57–59]. A study shows that the growth of melanoma cells can be inhibited by Usp9x loss. The Ets-1 proteasomal, abased site-specific de-ubiquitination, is inhibited by Usp9x, which leads to Ets-1 aggregation and increases tumorigenicity of melanoma [60]. Moreover, in malignant melanoma, about 15–20% of NRAS mutations have been identified [61]. Harish Potu et al. also revealed that inhibition of BRAF and/or MEK kinase pathway can increase Ets-1 expression. The increased Ets-1 expression upregulates NRAS levels by activating the NRAS promoter. In all, Usp9x plays a critical role in Ets-1 regulation and melanoma tumorigenicity through mediating NRAS transcription [60]. UCHL1 (ubiquitin C-terminal hydrolase 1) belongs to the ubiquitin carboxy terminal hydrolase family of DUBs. It catalyzes hydrolysis of C-terminal ubiquitin esters to regulate protein degradation [62]. Eun Young Seo et al. have investigated that UCHL1 influences melanogenesis by regulating stability of MITF in human melanocytes, which provides a framework for the further researches to evaluate potent therapeutic approaches for melanoma and other dyspigmentation disorders [63]. BAP1 (BRCA1-associated protein-1) belongs to the UCH subfamily of DUBs, and is known as a tumor suppressor gene [64, 65]. BAP1 mutations were first identified in a small number of lung and breast cancer samples, and have recently been described as leading to the pathogenesis of melanoma [66, 67]. The germline mutations in BAP1 are more prone to malignant melanoma [68]. In 2010, a study reported that 84% of inactivating somatic BAP1 mutations were identified in metastasizing uveal melanomas, including 15 premature protein termination mutations, and six affecting their ubiquitin UCH domains, which were associated with a decrease in BAP1 mRNA level [69]. However, in cutaneous melanoma, the germline mutations in BAP1 were less than 1% and its effect was unknown [70]. A recent study reported that low BAP1 mRNA predicted a better OS in older than 50 years cutaneous melanoma patients after adjusting for ulceration or Breslow depth [71]. The different function of BAP1 in cutaneous melanoma and uveal melanoma needs to be studied further.

3. Ubiquitination in melanoma immunological microenvironment modulation

Tumor microenvironment (TME) is consisted of cancer cells, cancer-associated fibroblasts, immune cells, and stromal cells. TME emerges as a key mechanism that mediate tumor progression [72]. A previous study reported that protein ubiquitylation plays a critical role in modulating immune responses and TME [73].

The Cbl proteins are a family of ubiquitin ligases (E3s). Cbl-b, a member of the family, functions as a negative regulator that regulates CD8 T cells costimulatory pathway and natural killer cell function [74]. In recent years, Cbl-b prone to be one of the hotspot targets of tumor immunotherapy because Cbl-b deletion can cause spontaneous or induced autoimmune call, and Cbl-b overexpression can result in the tumor immune tolerance in infiltrated lymphocytes in TME [75]. A study shows that NK cells knocking down of Cbl-b, or targeting its E3 catalytic activity, inhibit the progression of melanomas and distant melanoma metastases. Moreover, compared with WT T cells, Cbl-b−/− CD8+ and CD4+ T cell proliferation are highly suppressed by a recombinant PD-L1 Ig, and IFN-γ production is significantly less suppressed. Cbl-b deficiency in mice seems to cause a functional resistance of NK cells and T cells to PD-L1/PD-1-mediated immune suppression [76]. Adoptive cell therapy (ACT) with

autologous T cells can enforce the immune-mediated tumor cell killing, and show a promising result in various types of cancer treatments [77, 78]. However, the therapeutic efficacy of ACT is still limited because of the tumor-bearing host immune-evasion mechanisms, such as the secretion of transforming growth factor beta (TGFβ) or accumulation of Treg cells, both of which severely dampen the activation, expansion, and tumor homing of CD8+ T cells [79]. Another study reveals that silencing cbl-b reduces TGFβ sensitivity *in vitro* and enhances anti-tumor effects *in vivo*. Adoptive transfer of Cbl-b-silenced CD8+ T lymphocytes augments tumor vaccine to suppress tumor growth and prolong the survival in a B16F10 melanoma model [80].

FBXO38 belongs to the SCF family of E3 ubiquitin ligase of PD-1, and mediates Lys48-linked poly-ubiquitination and substrate proteasome degradation [81]. Previous research investigates that FBXO38 mediates PD-1 ubiquitination and maintains the anti-tumour activity of T cells in melanoma cells [82]. It offers an alternative method to block the PD-1 and highlights the clinical potential of the regulation of anti-tumour immunity through ubiquitination of FBXO38.

SIAH2, potent E3 RING finger ubiquitin ligases, mediates the cell cycle, apoptosis, and DNA repair regulation through targeting subsequent related proteins [83]. Previous study finds that hypoxia activates Siah2 E3 ligase, and then enhances the Warburg effect and pro-tumor immune response via degrading nuclear respiratory factor 1 (NRF1) through ubiquitination on lysine 230 [84]. A recent study reveals the effect and mechanism of Siah2 on the T cells and immune therapy. As is shown in this article, in the one way, Siah2-deficient mice suppress melanoma growth, increase the infiltration of T effector cells, and decrease number of FOXP3+ Treg cells. Inhibition of Siah2−/− melanoma cell proliferation is p27 dependent. Moreover, Siah2−/− BM-transplanted mice inhibit the melanoma growth, which may be a clinical potential of new adoptive cell therapy. On the other hand, loss of Siah2 exhibits synergy with anti-PD1 therapy in melanoma.

In addition to Ub, lots of Ub-related proteins display an immune regulation function in melanoma. A family of Toll-like receptors (TLRs) involves in the recognition of microbial components and regulates innate immune responses [85, 86]. TNFAIP3 (TNF-α induced protein 3), an ubiquitin-editing enzyme, can negatively regulate the TLRs via function as an ubiquitin-editing molecule [87]. E3 ligase NEDD4 mediates the function of immune regulation. Silence of NEDD4 inhibits FOXP3+ Treg cells through mediating GITR degradation, and then contributes to melanoma progression [88]. A previous study finds that USP15 was highly expressed in immune cells through analysis of the BioGPS database. In naïve CD4+ T cells, loss of USP15 stimulates the TCR + CD28 to produce cytokines, such as interleukin 2 (IL-2) and interferon-γ (IFN-γ). Moreover, USP15 inhibits the naïve CD4+ T cell activation and suppresses TH1 differentiation. MDM2, which is recognized as substrate protein of USP15, targets a T cell transcription factor, NFATc2, and negatively regulates T cell activation, which was independent of p53. Later, the author testifies the function of USP15 in B16F10 melanoma models. This study investigated that USP15−/− mice increase IFN-γ + CD4+ T cell infiltration to the tumors, and deficiency of USP15 reduces melanoma tumors size and tumor-induced lethality [89].

4. Ubiquitination of key transcription factors in melanoma

4.1 Ubiquitination of p53

The tumor suppressor protein p53 is a transcription factor that can affect cell proliferation by regulating the expression of its target protein [90]. P53 interacts with E3 ligase MDM2 in the nucleus, and is transferred from the nucleus to the cytoplasm

following ubiquitin, resulting in proteasome degrading [91]. In 2003, Leonard Girnita et al. discovered that inhibition of p53 leads to ubiquitination and down-regulation of the IGF-1R in human malignant melanoma cells. This impact was independent of the p53 status (wild type or mutated) but can be rescued by coinhibition of MDM2. Mdm2 serves as a ligase in ubiquitination of the IGF-1R [92]. Unlike other solid tumors, malignant melanomas retain the expression of wild-type p53 and typically lack p53 mutations [93, 94]. Adil Anwar et al. reveal that the wild-type p53 is the target for the ubiquitin-proteasomal pathway (UPP) degradation. The residues serine 15 and serine 20 are also essential for the binding of MDM2, which control p53 destruction via UPP pathway. In this article, p53 stabilization mediated by UPP inhibitors is independent of phosphorylation at residues serine 15 and serine 20 of p53 in melanoma cells [95]. MKRN2 is recognized as a novel ubiquitin E3 ligase targeting the p65 subunit of NF-κB to negatively regulate inflammatory responses [96]. A recent study indicates that the MKRN2 expression increases in the human melanoma cell lines, and silence of this gene leads to the suppression of melanoma proliferation by upregulation of p53. To investigate the mechanism of this effect, authors take co-immunoprecipitation and glutathione S-transferase pulldown assays to confirm the interaction of MKRN2 with p53 and take *in vitro* ubiquitination assays to study the ubiquitination of p53 by MKRN2. The result shows that MKRN2 interacts with p53, and ubiquitylates p53, leading to the influence of melanoma cell proliferation [97].

4.2 Ubiquitination of c-Myc

The transcription factor c-Myc plays an important role in cell proliferation and differentiation, cell cycle, metabolism, and apoptosis [98]. C-Myc is a protein that is very unstable and vulnerable to degradation in a proteasome-dependent manner. Research has identified the E3 ligase of c-Myc in melanoma. Also, c-Myc can be specifically bound by the E3 ligase SKP2 [99].

5. Melanoma therapeutic strategy via targeting the ubiquitin-proteasome system (UPS)

In protein degradation and melanoma pathogenesis, the UPS plays a crucial role, as shown above. The pathogenesis of malignant melanoma leads to genetic changes, irregular expression, or dysfunction [100]. Hence, targeting the UPS may be a potential therapeutic strategy for melanoma. Currently, many small molecule inhibitors targeting different components of the UPS, including the proteasome, E3 ligases, E1 enzymes, E2 enzymes, ubiquitin-like proteins, and DUBs, have been developed [101].

Bortezomib is the first proteasome inhibitor approved by FDA, which was originally used for multiple myeloma treatment [102]. However, due to the clinical safety, the study in other cancer researches, including melanoma, has been discontinued. Compared to the proteasome inhibitor bortezomib, drugs targeting a particular E3 ubiquitin ligase are expected to have better selectivity with less associated toxicity relative to the proteasome inhibitor bortezomib [103]. MDM2 is an E3 ubiquitin ligase with the ability to regulate tumor suppressor p53 and potentiate Notch signaling by degrading Numb [104, 105]. Nutlin-3a, an imidazoline compound, has been generally known as a MDM2 inhibitor. Nutlin-3a can suppress melanoma and other cancers, including retinoblastoma, leukemia, and neuroblastoma [106]. Meanwhile, WIP1 inhibitor (WIP1i), GSK2830371, can enhance p53-mediated tumor suppression by MDM2–p53 inhibitors, nutlin-3, RG7388, and HDM201 in cutaneous melanoma [107]. Therefore, more findings from the phase I clinical trials are needed to evaluate whether there exist any significant side effects.

Besides, Siah2 is known as a RING finger E3 ubiquitin ligase. Inhibition of Siah2 activity using a peptide is reported to be able to weaken its effect on hypoxia, effectively leading to melanoma metastasis inhibition, while suppression of Siah2 activities prevents the tumorigenicity of melanoma by disrupting Ras/MAPK signaling pathways [108]. Menadione (MEN), also known as vitamin K3, is a quinone used for cancer chemotherapeutic agents. A recent research identifies MEN as a novel Siah2 inhibitor, which attenuates hypoxia and MAPK signaling, and blocks melanoma tumorigenesis [109]. This study revealed that targeting Siah2 by MEN may be a new therapeutic strategy in melanoma treatment.

Cullin-RING ligases (CRLs) are a subgroup of the E3 ligases, and play an important role in the degradation of oncology relative proteins. It is activated by the neddylation pathway, such as NEDD8 conjugation [110]. The NEDD8-activating enzyme (NAE) is a critical regulator of the neddylation pathway [111]. Pevonedistat (TAK-924/MLN4924) is reported as a first-in-class, small-molecule inhibitor of NAE. Previous preclinical studies reveal that Pevonedistat is associated with tumor growth inhibition of a range of cell lines and primary human cancer cells derived from solid tumors, including malignant melanoma [112, 113]. A phase I study of Pevonedistat about patients with advanced solid tumors was undertaken. The results find that, in nine melanoma patients, one achieved a partial response (PR) while another 8 patients achieved stable disease (SD) lasting 6 months [114]. In addition, another phase I study (NCT01011530) was conducted to assess the safety, pharmacokinetics (PK), pharmacodynamic (PD), and antitumor activity of Pevonedistat in metastatic melanoma patients. The maximum tolerated dose (MTD) is reported as 209 mg/m^2. Most patients have a well toleration to Pevonedistat, only 16% patients experience drug-related serious adverse event (SAE), such as drug-related grade 4 acute renal failure, grade 3 myocarditis, and grade 3 small intestinal obstruction. At the end of the research, the research results show that one patient achieves a partial response, and stable disease is reported in 15 patients with lasting for 6.5 months or more in 4 patients [115].

As mentioned, ubiquitination removes the process of Ub and plays an important role in genomic instability regulation and tumorigenesis processes. Thus, several DUB inhibitors have been developed and identified as potential anticancer agents [116]. G9 is described as small molecule Usp9x inhibitor suppressing Usp9x activity [117]. G9 can inhibit NRAS mutant melanoma growth by decreasing Ets-1 protein content and NRAS expression. G9 also has a synergistic effect with PD0325901, a MEK inhibitor [60]. For specific Usp9x inhibitors such as G9 targeting two other DUBs, namely Usp24 and Usp5, more drug testing is needed [117, 118]. In addition, Spautin-1 is recognized a potent USP10/13 deubiquitinating activity antagonist. A recent study revealed that Spautin-1 plays an anti-tumor role in melanoma suppression via DNA damage by increasing ROS levels and has a synergistic effect with Cisplatin [119]. Targeting USP10/13 by Spautin-1 may be a new therapeutic strategy in melanoma patient treatment.

6. Conclusions

Melanoma has a low 5-year survival rate due to being susceptible to invasion and metastasis. Recently, growing evidence identified the critical role of ubiquitination and de-ubiquitination in malignant melanoma progression, which may be the novel targets for cancer therapy. In this article, we make a brief conclusion that the misregulated expressions of the E2 ubiquitin conjugating-enzymes, E3 ubiquitin ligases, and DUBs lead to aberrant oncogenic signaling in malignant melanoma (**Figure 1**). The ubiquitination plays a vital role in melanoma not only through ubiquitination of key transcription factors or key cell signaling but also immunological

microenvironment modulation. We also make a conclusion of the target UPS components, the corresponding therapeutic drugs or potential therapeutic targets, and the molecular mechanism (**Table 1**). Understanding of ubiquitination and

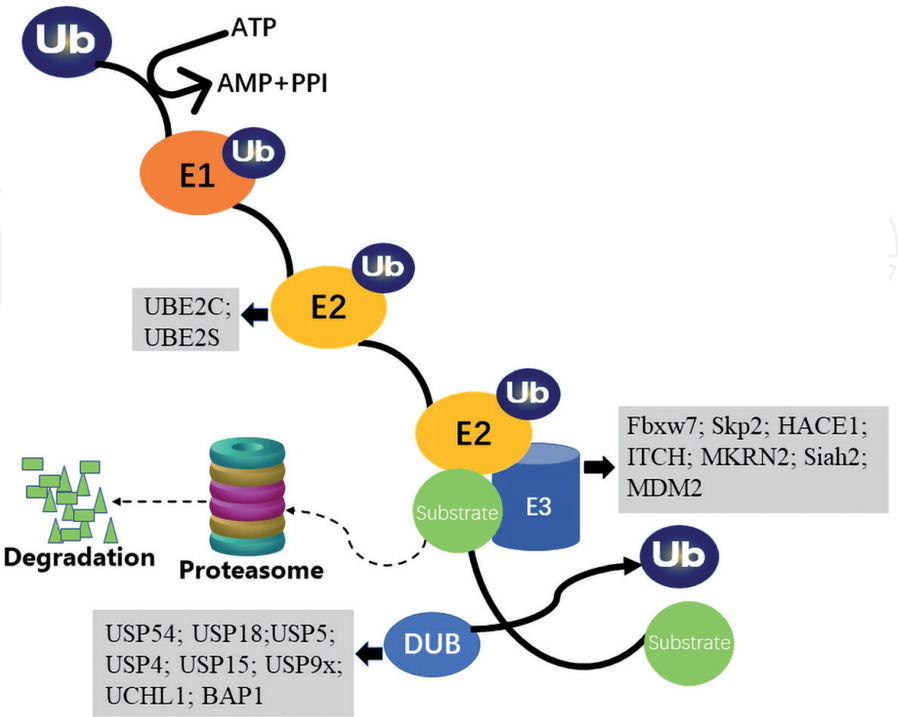

Figure 1.
Important UPS components and therapeutic targets toward melanoma pathogenesis.

Reference	UPS component	Potential therapeutic targets or drugs	Experimental model	Molecular mechanism	Clinical trial
[10]	Fbxw7	Targeting NOTCH signaling	Human and cells	Inhibiting NOTCH activation, unleashing HSF1, and activating the MAPK/ERK signaling	None
[19]	SKP2	None	Human and cells	Stabilizing the MTH1 expression via K63-linked polyubiquitination, and mediating degradation of cyclin A	None
[22, 24]	HACE1	Targeting HACE1	Human and cells	Inhibiting YAY1 signaling, and activating RAC-Family GTPases, and regulating K27 ubiquitination of FN	None
[26]	ITCH	None	Cells	Mediating BRAF polyubiquitination	None
[30, 31]	UBE2C	None	Cells	Suppressing both the level and the activity of MPF	None

Reference	UPS component	Potential therapeutic targets or drugs	Experimental model	Molecular mechanism	Clinical trial
[32]	UBE2S	Targeting UBE2S	Human, animal and cells	Catalyzing Lys11-linkage ubiquitination, and inhibiting EMT	None
[35]	MKRN2	Targeting MKRN2	Cells	Interacting with ubiquitylated P53	None
[50]	USP54	Targeting USP54	Animal and cells	Unknown	None
[52]	USP18	Targeting USP18	Animal and cells	Bing stimulated by IFN-γ, and regulating CTL CD8+ immune-cell function	None
[53]	USP5	Targeting USP5	Cells	Blocking p53 and FAS induction, and then suppressing cell cycle checkpoint and apoptosis	None
[54]	USP4	Targeting USP4	Cells	Promoting EMT	None
[55]	USP15	Targeting USP15	Animal and cells	Downregulating MDM2 expression, and increasing frequency of CD8+ effector T cell tumor-infiltrating	None
[62]	UCHL1	Targeting UCHL1	Cells	Regulating stability of MITF in human melanocytes	None
[67, 68]	BAP1	Targeting BAP1	Human, animal and cells	Unknown	None
[105, 106]	MDM2	Nutlin-3a	Human, animal and cells	Inhibiting MDM2 and cyclin B1/CDK1-phosphorylated nuclear iASPP	None
[107, 108]	Siah2	Menadione	Cells	Attenuating hypoxia and MAPK signaling	None
[59, 116]	Usp9x	G9	Cells	Decreasing Ets-1 protein content and NRAS expression, and having a synergistic effect with PD0325901	None
[111–113]	NEDD8	Pevonedistat	Human, animal and cells	Inhibiting the activity of cullin E3 ligases and then stabilizing cullin substrates	NCT01011530
[118]	USP10/13	Spautin-1	Animal and cells	Inducing DNA damage by increasing ROS levels, and having synergistic effect with Cisplatin	None

Table 1.
Summarization of the target UPS components.

de-ubiquitination mechanisms and their regulation in melanoma will help us to better understand the pathogenesis of this cancer, and develop effective therapeutic approaches, which lets us see a promising future for the application of these advancements owing to the prosperity and success of drugs targeting ubiquitination and de-ubiquitination in melanoma.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

JLZ developed the ideas and revised the manuscript. JG wrote the main manuscript.

Acronyms and abbreviations

ACT	Adoptive cell therapy
BAP1	BRCA1-associated protein-1
CRC	Colorectal cancer
DUB	Deubiquitinating enzymes
E1	The ubiquitin-activating enzyme
E2	The ubiquitin-conjugating enzyme
E3	The ubiquitin ligase
EMT	Epithelial-mesenchymal transition
Fbxw7	F-box/WD repeat-containing protein 7
FN	Fibronectin
HACE1	HECT domain and ankyrin repeat-containing E3 ubiquitin protein ligase 1
HSF1	Heat-shock factor 1
ISG15	Interferon-stimulated gene 15
MAGE-A11	Melanoma antigen-A11
MEN	Menadione
miRNAs	MicroRNAs
MITF	Microphthalmia-associated transcription factor
MKRN2	Makorin ring finger protein 2
MPF	M-phase-promoting factor
NEDD8	Neural precursor cell expressed, developmentally down-regulated8
NRF1	Nuclear Respiratory Factor 1
Skp2	S-phase kinase-associated protein 2
SUMO	Small ubiquitin-like modifiers
TLRs	Toll-like receptors
TME	Tumor microenvironment
UBE2S	Ubiquitin-conjugating enzyme E2S
UBLs	Ubiquitin-like proteins

UCHL1	Ubiquitin C-terminal hydrolase 1
UPP	Ubiquitin proteasomal pathway
UPS	Ubuiquitin-proteasome system
Usp9x	Ubiquitin specific peptidase 9, X-linked
WIP1i	WIP1 inhibitor

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