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Review

E3 ubiquitin ligases as drug targets and prognostic biomarkers in melanoma

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ABSTRACT

Melanomas are highly proliferative and invasive, and are most frequently metastatic. Despite many advances in cancer treatment over the last several decades, the prognosis for patients with advanced melanoma remains poor. New treatment methods and strategies are necessary.

The main hallmark of cancer is uncontrolled cellular proliferation with alterations in the expression of proteins. Ubiquitin and ubiquitin-related proteins posttranslationally modify proteins and thereby alter their functions. The ubiquitination process is involved in various physiological responses, including cell growth, cell death, and DNA damage repair. E3 ligases, the most specific enzymes of ubiquitination system, participate in the turnover of many key regulatory proteins and in the development of cancer.

E3 ligases are of interest as drug targets for their ability to regulate proteins stability and functions. Compared to the general proteasome inhibitor bortezomib, which blocks the entire protein degradation, drugs that target a particular E3 ligase are expected to have better selectivity with less associated toxicity. Components of different E3 ligases complexes (FBW7, MDM2, RBX1/ROC1, RBX2/ROC2, cullins and many others) are known as oncogenes or tumor suppressors in melanomagenesis. These proteins participate in regulation of different cellular pathways and such important proteins in cancer development as p53 and Notch. In this review we summarized published data on the role of known E3 ligases in the development of melanoma and discuss the inhibitors of E3 ligases as a novel approach for the treatment of malignant melanomas.

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1. Introduction

Ubiquitin and ubiquitin-related proteins posttranslationally modify proteins and so regulate functions of proteins. Ubiquitin is a 76-amino acid protein that is covalently conjugated to a lysine residue in proteins. Proteins can be modified by a single ubiquitin on a single lysine (monoubiquitination), by single ubiquitins on various lysines scattered over the substrate (multimonoubiquitination) or by ubiquitin chains on one or several lysines (polyubiquitination). Different ubiquitin chains (lysine 6, 11, 27, 29, 33, 48, 63 of ubiquitin) form various conformations [1] (Fig. 1).

Depending on modification type ubiquitinated substrates participate in different processes including proteins activation, inhibition, proteasomal or lysosomal degradation. Ubiquitination also regulates protein localization, sorting, and protein-protein interactions. Commonly chains are linked through lysine 48 of ubiquitin and target the substrates to proteasomal degradation. However, other linkages have been also detected in cells [2]. Ubiquitin is bonded to the protein by a series of enzymes: the ubiquitin activating enzyme (E1), the ubiquitin conjugating enzyme (E2), and the ubiquitin ligase (E3). Ubiquitin is linked to E1 and activated by using the energy of ATP. First ubiquitin is conjugated to a substrate by the formation of an isopeptide linkage between its last glycine residue and an internal lysine of the substrate (although alternative target amino acids have been described [3]). The activated ubiquitin is then transferred to E2 and in conjunction with E3, the ubiquitin is attached to the target proteins.

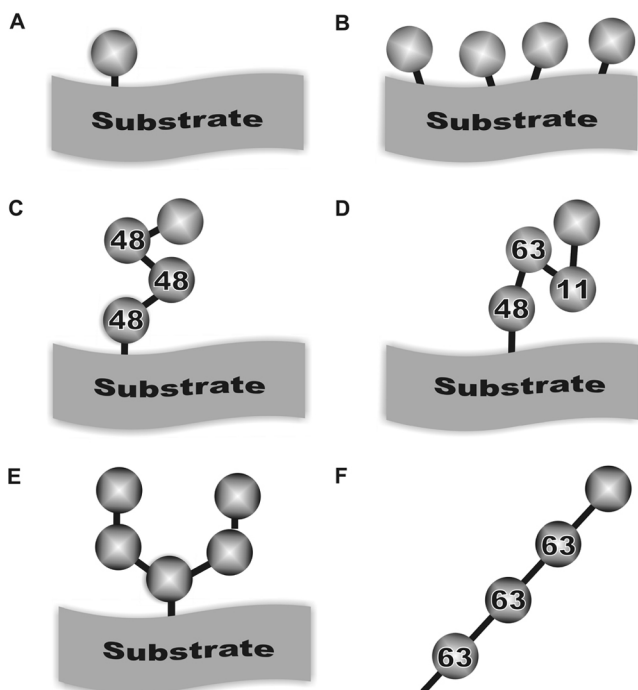


Fig. 1 – Type of protein ubiquitination: monoubiquitination (A), multimonoubiquitination (B), homogenous poliubiquitin chain (C), mixed poliubiquitin chain (D), branched poliubiquitin chain (E), and unanchored poliubiquitin chain (F).

Ubiquitin ligase E3 recognizes a substrate for ubiquitination, and is most important determining ubiquitination specificity [4]. Similar as phosphorylation, ubiquitination is reversible and linked with deubiquitination. Deubiquitinases (DUBs) belong to two main classes of proteases, cysteine proteases and metalloproteases [5]. There are about 100 DUBs in the mammalian genome and each DUB probably recognizes several substrates.

Group of proteins which are similar to ubiquitin are termed ubiquitin-like proteins (UBLs). UBLs include SUMO, NEDD8, ISG15, and FAT10. These proteins are conjugated to target proteins by an enzyme cascade analogous to ubiquitin E1, E2, and E3. UBL protein modifications are usually not associated with protein degradation, but participate in proteins localization, transportation, or protein-protein interactions. The ubiquitination process is involved in cell growth, cell death, DNA damage repair, and also in different types of cancers, including skin cancers [6,7].

E3 ubiquitin ligases recognize a substrate for ubiquitination and determine ubiquitination specificity. They are much more specific enzymes compared with ligase E2 and ligase E1, and also with DUBs and therefore they are potential therapeutic targets [4,8].

E3 ubiquitin ligases can be a single peptide, such as MDM2 or XIAP, or consist of multiple components such as APC/C (the anaphase-promoting complex/cyclosome) or SCF (SKP1-Cullin 1-F-box proteins). Most of E3 ligases contain additional linker proteins and may be divided in two main groups: the RING class and the HECT class. The RING E3s contain a subunit or domain with a RING motif, which coordinates a pair of zinc ions. RING E3s (and the structurally related but zinc-free U-box E3s) function at least in part as adaptors: they bind the ubiquitin-thioester-linked E2 and substrate protein simultaneously and position the substrate lysine nucleophile in close proximity to the reactive E2-ubiquitin thioester bond, facilitating transfer of the ubiquitin. Catalysis of ubiquitin-substrate modification by the HECT E3s follows a mechanism distinct from that of the RING E3s. In HECT E3s, the ubiquitin is first transferred from the E2 to an active-site cysteine in the conserved HECT domain of the E3. The thioester-linked ubiquitin is then transferred to substrate. Third group of E3 ligases – RING-between-RINGs (RBRs) or RING/HECT E3s – combine features of both RING- and HECT-type ligases. The ubiquitin-conjugated E2 binds to the RING1 domain and then ubiquitin is transferred from the E2 to the E3 RING2 domain from which it is transferred to the substrate [9,10] (Fig. 2).

This review summarizes published data about structure and functions of SCF E3 ligases and their role in the development of cancer. Well-known E3 ligases and proteins associated with them in melanoma will be discussed in the most detail.

2. SCF E3 ligases in melanoma

The largest family of E3 ubiquitin ligases is the RING type SCF E3 ligases. They are conserved among eukaryotic cells and consist of four structural and functional components: a substrate-recognizing F-box protein (WD40 domain containing FBXWs, leucine-rich repeats-containing FBXLs and other

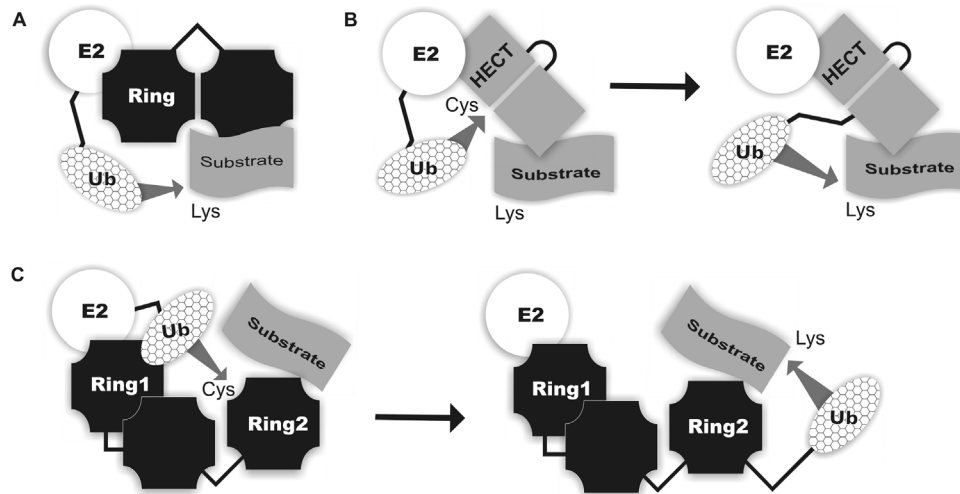


Fig. 2 – Ubiquitination of proteins. (A) RING E3 ligase is bound to an ubiquitin-conjugated E2, from which the ubiquitin is transferred to a lysine on the substrate. (B) HECT E3 ligase is bound to an ubiquitin-conjugated E2, from which ubiquitin is first transferred to the active-site cysteine of the E3, and is then transferred to a lysine on the substrate bound to the E3. (C) RING-between-RINGS (RBRs) or RING/HECT E3 ligases. The ubiquitin-conjugated E2 binds to the RING1 domain and then ubiquitin is transferred from the E2 to the E3 RING2 domain from which it is transferred to the substrate.

diverse domains-containing FBXOs), an adaptor protein SKP1, scaffold protein cullin (CUL-1, -2, -3, -4A, -4B, -5, and -7) and two RING proteins, RBX1/ROC1 and RBX2/ROC2, also known as SAG (sensitive to apoptosis gene). All SCF E3 ligases share a similar structure in which CUL binds to SKP1 and an F-box protein at the N-terminus and a RING protein RBX1 or RBX2 at the C-terminus [11]. Substrate specificity of SCF E3 ligases is determined largely by the F-box protein, which usually recognizes phosphorylated target protein, whereas CUL-RBX1 or CUL-RBX2 constitutes the core ligase activity, catalyzing the transfer of the ubiquitin from an E2 to the substrate [11,12] (Fig. 3).

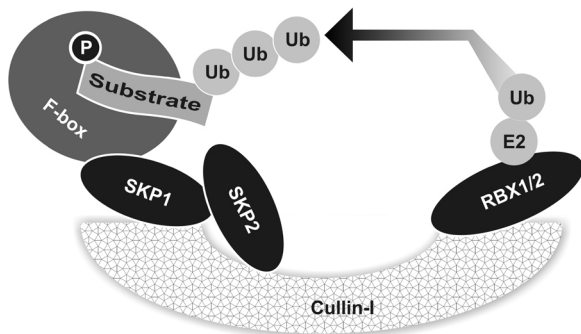


Fig. 3 – Components of the SCF E3 ubiquitin ligase complex. A scaffold protein Cullin is associated with an adaptor protein SKP1(SKP2) and a RING finger protein RBX1 or RBX2. F-box protein determines the specificity of the target proteins. CUL-RBX1 or CUL-RBX2 catalyzes the transfer of the ubiquitin from an E2 to the substrate.

3. F-box proteins

The F-box proteins are the substrate recognizing subunits of SCF E3 ligases, thus determining their substrate specificity [12]. The human genome encodes 69 F-box proteins [11], but only some of them are well-characterized.

SCFb-TrCP (b-transducin repeats-containing proteins) E3 ligase contains at the N-terminus an F-box domain which facilitates its binding to SKP1 and CUL1 and at the C-terminus a WD40 repeat domain for substrate recognition [12,13]. SCFb-TrCP E3 ligase promotes the degradation of many key regulatory proteins, including I κ B, b-catenin, cyclin D1, p53, MCL1, Pro-caspase-3, WEE1, and CDC25. In most cases, b-TrCPs function as oncoproteins, whereas in a few others, they have displayed tumor suppressive functions. Neither mutations nor complete loss of expression of b-TrCP were detected in malignant melanomas.

It was indicated that increased expression of oncogenic BRAF (V660E) in melanocytes enhances b-TrCP expression with subsequent I κ Ba degradation. Activating mutations of the protooncogene BRAF have been observed in approximately 70% of malignant melanomas [14]. Blockage of BRAF signaling by a small molecular inhibitor or by BRAF siRNA knockdown in melanoma cells reduces b-TrCP expression with subsequent I κ Ba stabilization and NF- κ B inactivation, leading to apoptosis sensitization [15]. On the other hand, a recent study showed that silymarin, a plant flavonoid derived from *Silybum marianum*, actively suppresses migration and invasion of human melanoma cells in part through b-TrCP-mediated degradation of b-catenin, suggesting that b-TrCP may act as a negative regulator of migration and invasion of melanoma cells [16].

SCF-SKP2 (S-phase kinase associated protein 2) is the specific substrate recognizing subunit of SCF type protein

ligases. The E3 ligase SCF-SKP2 conjugated with SKP1 and its accessory protein Cdc kinase subunit 1 (Cks1) promote proliferation largely by inducing the degradation of the cyclin-dependent kinase (CDK) inhibitors p27, p21, and p57 [8,17]. SCF-SKP2 recognized p27 degron by a complex consisting of SCF-SKP2 and Cks1, after phosphorylation on Thr-187 by cyclin E-CDK2 [18]. Overexpression of SKP2 mRNA and protein levels was observed in many aggressive cancers and was commonly associated with downregulation of p27 levels and loss of tumor differentiation. p27 is a negative regulator of the cell cycle that plays an important role in tumor suppression. Loss of p27 results in uncontrolled proliferation and promotes tumor progression. During human melanomagenesis starting from melanocytic proliferation to melanocytic nevi, then to melanoma in situ and primary melanoma, and finally to metastatic melanoma, a progressive increase of SKP2 and loss of p27 were observed [19]. It is not completely clear nuclear or cytoplasmic SKP2 is responsible for tumor progression. Thus, cytoplasmic expression of SKP2 correlates with worse 10-year overall survival in patients with primary melanoma [19,20]. However, neither SKP2 nor p27 nuclear expression has significant impact on patient prognosis [20,21]. Other authors indicated that nuclear SKP2 expression is inversely correlated with p27 levels and clinically associated with increasing malignancy and poorer patient survival [15,21]. Thus, the exact role of cytoplasmic and nuclear SKP2 and p27 in melanomagenesis remains to be determined.

Furthermore, it was indicated that in melanoma cells, mutation in BRAF activates BRAF signaling, which regulates Cks1/SKP2-mediated p27 degradation and controls G1 cell cycle event. Silencing of SKP2 by siRNA inhibited the melanoma cell growth, suggesting a potential tool of cancer gene therapy in malignant melanoma. It was indicated, that the combined suppression of mutated BRAF and SKP2 inhibited cell growth and attenuated the invasive potential of melanoma cell lines in vitro. Thus, combination therapy targeting BRAF and SKP2 may be perspective for melanoma treatment [22].

No mutations were identified in SKP2. Copy number gain is a major contributing mechanism of SKP2 overexpression in metastatic melanoma [21]. In addition to p27, SKP2 was found to target the degradation of ING3 (inhibitor of growth family member 3), a putative tumor suppressor whose expression is found to be reduced remarkably in melanomas, which correlated with poorer patient survival. Taken together, these findings indicate that the SKP2-mediated reduction, as a result of enhanced degradation of tumor suppressors p27, p21, p57 and ING3, contributes to the development of melanoma [17].

Small heterocyclic molecules that stabilize p27 were identified. These compounds either inhibited 26S proteasome activity, prevented SKP2 from incorporating into the SCF complex, or downregulated SKP2 mRNA [23]. Inhibitors that inactivate SCF E3 ligases via disrupting the protein-protein interaction (e.g., binding of F-box proteins and their substrates; binding of E2 and E3, or binding among individual components) were discovered. Recently, four new selective inhibitors of SKP2 ligase activity acting on SKP2-p27 interaction were identified [18]. These inhibitors increased both p27 protein level and half-life in metastatic melanoma cell lines. Inhibitor treatments in various cancer cells also shifted the population of cells into G1, or G2/M phase, and this phenotype was both

p27 and cell type dependent. These compounds show high selectivity for SCF-SKP2 ligase, they do not exhibit activity against SKP1, CyclinE/Cdk2 kinase activity, and a MDM2 [18]. Other inhibitor hinokitiol (b-thujaplicin), a tropolone-related compound, increased the stability of the p27 protein by inhibiting p27 phosphorylation at Thr187 and by downregulating SKP2 expression. Hinokitiol inhibited cell growth and DNA synthesis in human melanoma cells [15]. Recently it was indicated that accumulation of SKP2 and c-Myc proteins was significantly higher in metastatic melanoma as compared with that in primary melanoma and normal skin or nevi. TGF- β 1 (transforming growth factor β 1) signals to SKP2 via Akt1 and c-Myc during EMT (epithelial mesenchymal transition), and induced metastasis in human melanoma [24].

SCF-FBW7 (F-box and WD repeat domain-containing 7) (also known as Sel-10, hCdc4, hAgo, or FBW7), is the substrate recognition and binding component in SCF (SKP1-CULL-F-box protein-RBX1) ubiquitin ligase complex. FBW7 could either suppress or promote carcinogenesis by targeted degradation of c-Jun, c-Myc, Notch or other known substrates [25-28]. Human FBW7 is located on chromosome 4 and encodes three transcripts (isoforms α , β and γ) derived from the same gene locus by alternative splicing. All three isoforms differ at the N-terminal region but contain conserved domains in the C-terminus (F-box and WD40 repeats). The C-terminal region participates in substrate recognition and ubiquitination. F-box domain interacts with SKP1; D-domain participates in dimerization; eight WD40 repeats form a β -propeller structure termed phosphodegron binding pockets, which recognize and bind to substrates after they have been phosphorylated by various kinases in different cells [25]. The N-terminal end is responsible for localization. FBW7 α localizes to the nucleus, FBW7 β to cytoplasm and FBW7 γ to nucleolus. FBW7 can function as monomer or dimer. FBW7 dimerization depends on the degron strength and determines the specificity of substrate degradation [27,29-31].

Numerous cancer-associated mutations in FBW7 and its substrates have been identified, and loss of FBW7 function causes chromosomal instability and tumorigenesis [29,30,32,33]. FBW7 mutation rates in cholangiocarcinoma, T-cell acute lymphocytic leukemia, endometrial carcinoma, and colorectal cancer were reported as 35%, 31%, 9%, and 9%, respectively [29,30,34].

The mechanism of FBW7 reduction in tumors is not well understood. Recent studies indicated that several regulators such as p53, and C/EBP-d (CCAAT/enhancer-binding protein-d), as well as miRNAs (microRNAs) including miR-27a and miR-223 can directly modulate the FBW7 expression that lead to inactivation of FBW7 in human malignancies [29,35]. Activity levels of FBW7 substrate cyclin E could be upregulated by the overexpression of miR-223 through reducing FBW7 mRNA level, and increased genomic instability was observed as a consequence. It was indicated that miR-27a inhibits FBW7-mediated ubiquitination and degradation of the cyclin E [35]. These data indicate that the degradation of FBW7 substrate could be affected by the upstream regulators that transcriptionally regulate FBW7 expression.

Notch, a well characterized FBW7 substrate, is an oncogenic protein in various tissues but may serve as a tumor suppressor in others. Regulation of Notch intracellular domain (NICD) by

ubiquitination and proteasomal degradation is critical for an appropriate Notch signaling, since maintaining Notch signaling over a long period leads to severe pathologies. Moreover, the intracellular domain of Jagged-1 was also found to interact with Notch1 intracellular domain and promote its degradation through FBW7-dependent proteasomal pathway. Interestingly, presenilin, one of the components of the γ -secretase complex was found to regulate the ubiquitin ligase FBW7 to modulate EGFR signaling and cell transformation [36]. FBW7 mutations in leukemic cells mediate Notch pathway activation and resistance to γ -secretase inhibitors [33]. FBW7 mutants cannot bind to the NICD. Although the mutant forms of FBW7 were still able to bind to Myc, they do not target it for degradation [29,33]. In melanoma Notch expression is increased. It is possible that FBW7 mutation or inactivation is responsible for this increase. The expression of FBW7 is significantly reduced in primary melanoma and metastatic melanoma as compared with dysplastic nevi. Nuclear FBW7 α is the predominant form of FBW7 protein expressed in melanoma. Reduced level of FBW7 correlated with melanoma progression. FBW7 regulates the proliferation and differentiation of keratinocytes by mediating the degradation of c-Myc and Notch proteins. FBW7-deficient keratinocytes showed an increased proliferative capacity that was dependent on the accumulation of c-Myc but not on that of Notch [29].

Recent studies suggest that FBW7 may have functions in cell migration and metastasis [36]. *Fbw7* knockdown cells showed a remarkable increase of cell migration and stress fiber formation. It was indicated that MEK (MAPK/ERK kinase) inhibitor abrogated FBW7 α knockdown-induced melanoma cell migration. FBW7 has an important role in melanoma progression, and it inhibits melanoma cell migration through the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway [36,37]. As the majority of FBW7 substrates are oncoproteins, it stands to reason that enhancing FBW7-mediated degradation would be important goal of chemotherapeutics. However, the problem is how to find specific compounds to restore only FBW7 activity, but not affecting other signaling pathways without causing unwanted side effects. Recently, natural compound oridonin has been reported to activate FBW7 E3 ubiquitin ligase, leading to inhibition of c-Myc pathway [12,38].

However, further in depth investigation is required to define the exact molecular mechanism underlying the anti-tumor activity of FBW7 as well as the mechanism by which the expression of FBW7 is regulated, and how the loss of FBW7 contributes to the development of human cancers [7,27].

4. MDM2 (HDM2) E3 ubiquitin ligase

MDM2 is E3 ubiquitin ligase with strong clinical relevance by virtue of its ability to regulate the abundance of the tumor suppressor p53. The N-terminal hydrophobic pocket of MDM2 binds to p53 and thereby inhibits the transcription of p53 target genes. Additionally, the C-terminus of MDM2 contains a RING domain with intrinsic ubiquitin E3 ligase activity [39]. By recruiting E2 ubiquitin conjugating enzyme(s), MDM2 acts as an E3 ubiquitin ligase that facilitates the export of p53 from the nucleus to the cytoplasm and targets p53 for

ubiquitin-dependent proteasome degradation. In addition, MDM2 inhibits p53 function by direct binding to the transcriptional binding site of p53, thereby preventing its interaction with the transcription machinery. p53 and MDM2 interact to form an auto-regulatory loop, where increased level of p53 transcriptionally activates MDM2 and the latter in turn decreases the level of p53 [40-42]. Recently, it was established that NEDD4-1 is an E3 ligase for MDM2 that contributes to the regulation of MDM2 protein stability in cells. Also, NEDD4-1 is a novel component of the p53/MDM2 regulatory feedback loop that controls p53 activity during stress responses [43]. MDM2 is also a major target of the Ras-Raf-MAP kinase pathway. Ras, a well-known oncogene, may contribute to suppression of p53 activity and the cancer phenotype partially through induction of MDM2 [44]. Recently, it was shown that the melanoma growth suppression mediated by MAPK/ERK kinase inhibition was potentiated by MDM2 antagonism [45]. MDM2 known as a main regulator of p53 tumor suppressor protein also plays a role in Notch signaling: first by upregulating the ubiquitination of Numb leading to Numb degradation, and thus indirectly to an increase of Notch signaling; second by directly targeting Notch1, resulting in stabilization and activation of NICD [46,47]. However, another study proposes Notch4 to be a substrate for MDM2-mediated ubiquitination and degradation, in a manner inversely proportional to the quantity of p53 protein in the cell [48]. It is thus possible that p53-MDM2-Numb complexes coordinate the regulation of both the p53 and the Notch pathways [47,49]. MDM2 oncoprotein is overexpressed in many human tumors that retain the wild type p53 allele [50,51]. High MDM2 levels are associated with poor prognosis of several human cancers [52]. MDM2 is overexpressed in malignant melanomas. Recently, Rajabi et al. have shown evident relationship between MDM2 expression and tumor thickness and invasion in primary cutaneous malignant melanoma [53]. Overexpression of Notch1 and Notch4 in malignant melanoma is correlated with tumor development and progression [54]. As p53 is often structurally preserved, but functionally crippled, by CDKN2A/ARF loss in melanoma, restore of p53 function represents an attractive point of vulnerability in melanoma. Thus, MDM2 can be attractive target for p53 and Notch signaling pathways in malignant melanoma. Due to the lack of MDM2 E3 ligase specific inhibitors, the most common approaches used for target validation have been aimed at disrupting MDM2-p53 interactions, or blocking MDM2 expression rather than directly targeting E3 ubiquitin ligase activity. Nutlin class of imidazoline compounds has been successfully developed that inactivate SCF E3 ligases via disrupting the protein-protein interaction including p53-MDM2 [55]. Nutlin-3a is the most promising candidate [56-58] and is under investigation in preclinical models for a variety of malignancies, including neuroblastoma [59], retinoblastoma [60], leukemia [61], and melanoma [45].

5. MDM2-MDMX E3 ubiquitin ligase

MDMX (also known as a MDM4 or HDMX), an MDM2 homolog, sharing substantial structural homology with MDM2, also has an important role in regulating p53. In addition to inhibiting the transcriptional activity of p53, MDMX forms a hetero-complex with MDM2 that potentiates the ubiquitination and

degradation of p53. Unlike MDM2, MDMX is not a transcriptional target of p53. MDMX, also has a RING domain and hetero-oligomerizes with MDM2 to stimulate its E3 ligase activity. Recent studies have shown that C-terminal residues adjacent to the RING domain of both MDM2 and MDMX contribute to MDM2 E3 ligase activity. However, the molecular mechanisms mediating this process remain unclear, and the biological consequences of inhibiting MDM2/MDMX cooperation or blocking MDM2 ligase function are relatively unexplored [41,62]. There are currently two distinct models proposed to explain why both MDM2 and MDMX are required in p53 control: (1) these two proteins function independently, (2) MDM2 and MDMX work together in p53 regulation. Abundant evidence indicates an intricate interplay between MDM2 and MDMX in p53 regulation [63,64]. Similarly to MDM2, MDMX is overexpressed in human tumors generally distinct from those containing p53 mutations and exert its oncogenic activity predominantly by inhibiting the p53 tumor suppressor [41,65]. MDMX oncoprotein may inhibit tumorigenesis by regulating the apoptotic mediator p53. Analysis of the prognostic parameters indicated that MDMX expression was positively correlated with an increased likelihood for survival. Compared with the poor prognosis patients, mitochondria from good prognosis glioma patients contained higher levels of both MDMX and the proapoptotic protein p53Ser46P [66]. However, the MDM4 proteins modulate and respond to many other signaling networks in which they are embedded. Recent mechanistic studies and animal models have demonstrated how functional interactions in these networks are crucial for maintaining normal tissue homeostasis, and for determining responses to oncogenic and therapeutic factors [41]. It was indicated, that MDMX impaired early DNA damage-response signaling, such as phosphorylation of the serine/threonine-glutamine motif, mediated by the ATM kinase [67]. Moreover, MDMX associated with Nbs1 of the Mre11-Rad50-Nbs1 (MRN) DNA repair complex, and this association increased upon DNA damage and was detected at chromatin. Elevated MDMX levels also increased cellular transformation in a p53-independent manner. Unexpectedly, all MDMX-mediated phenotypes also occurred in cells lacking MDM2 and were independent of the MDM2-binding domain (RING) of MDMX. Therefore, MDMX-mediated inhibition of the DNA damage response resulted in delayed DNA repair and increased genome instability and transformation independent of p53 and MDM2. Certain human cancer cells overexpressed MDM2 and MDMX suppress p53, prompting a pharmacological quest to target these negative regulators for cancer therapy [68]. Amplification of MDMX is seen in many tumors, including melanoma, breast, head and neck, hepatocellular, and retinoblastoma, and, interestingly, amplification of MDMX appears to correlate with both p53WT status and an absence of MDM2 amplification [69]. One major limitation of MDM2-specific therapy with Nutlins is that tumor cells in which MDM4 expression is high but MDM2 is low respond poorly to MDM2 inhibition [69,70]. Bernal et al. by using chemical strategy termed "hydrocarbon stapling" generated stabilized Alpha-Helix of p53 (SAH-p53) peptides, which inhibits MDMX [68]. MDMX, a negative regulator of p53, is upregulated in a substantial proportion (~65%) of stage I-IV human melanomas and melanocyte-specific MDMX overexpression enhanced tumorigenesis in a mouse model of

melanoma induced by the oncogene NRAS. MDMX promotes the survival of human metastatic melanoma by antagonizing p53 proapoptotic function. Inhibition of the p53-MDMX interaction restored p53 function in melanoma cells, resulting in increased sensitivity to cytotoxic chemotherapy and to inhibitors of the BRAF (V600E) oncogene. MDMX as a key determinant of impaired p53 function in human melanoma and designate MDMX as a promising target for antimelanoma combination therapy [70]. Targeting the p53-MDMX interaction with stapled α -helical peptide SAH-p53-8 was sufficient to suppress the growth of metastatic melanoma cells with wild-type p53 [70]. Stapled α -helical peptides have emerged as a promising new modality for a wide range of therapeutic targets. Now, new stapled α -helical peptides are under development. Recently a potent and selective dual inhibitor of MDM2 and MDMX, ATSP-7041, was discovered, which effectively activates the p53 pathway in tumors in vitro and in vivo [69].

6. RING component of SCF

In mammals, the RING component of SCF has two family members, namely RBX1 (RING box protein 1), also known as ROC1 (regulator of Cullins-1), and the RBX2 or ROC2 also known as SAG (sensitive to apoptosis gene). The function of RBX is to bring the ubiquitin-loaded E2 into close proximity to the targeted substrate [71].

RBX1/ROC1, an essential component required for full activity of SCF E3 ligases RBX1 is overexpressed in various cancers, including carcinomas derived from liver, kidney, lung, and breast [72,73]. Very little is currently known about the potential pathological involvement of RBX1/ROC1 in melanoma. It was indicated that expression of ROC1 is higher in nevi than in melanomas, which was just opposite for cyclin D1 expression [74]. ROC1 may play an antiproliferative role in part by targeting cyclin D1 for degradation in nevi, which is attenuated during melanomagenesis [74].

SAG/RBX2/ROC2 regulates cell proliferation, apoptosis, vasculogenesis, and tumorigenesis by targeting the degradation of many critical cellular regulators including c-Jun, I κ B α , HIF-1 α , NF1 [12,71,72]. RBX2 regulates skin tumorigenesis in a stage-dependent manner. RBX2 is overexpressed in multiple human tumor tissues, and patients with RBX2 overexpression have a poor prognosis. RBX2 siRNA silencing selectively inhibited cancer cell proliferation via apoptosis induction, suppressed in vivo tumor growth, and sensitized cancer cells to chemotherapeutic drugs and radiation, suggesting its potential as an anticancer target [75].

Cullins are scaffold proteins which assembly with other components of SCF into four functionally distinct E3 ubiquitin ligases [75,76]. So far, seven cullin family members (cullin 1, 2, 3, 4A, 4B, 5, and 7) have been identified. Cullin family proteins are involved in a diverse array of functions, including cell-cycle control, DNA replication and development [77]. Aberrant expression of cullins was found in a number of human cancers which is closely associated with poor patient prognosis [75,78,79]. The cullin family proteins seem to be widely expressed and located both to the nucleus and cytoplasm, but there are no compelling data suggesting that cullin activity is

controlled by subcellular localization or by differential expression in a tissue-specific manner [76]. CUL1 is overexpressed in 40% of lung cancers, with active neddylation forms specifically expressed in high-grade neuroendocrine lung tumor tissues [75]. It was indicated that both cytoplasmic and nuclear CUL1 levels are increased at the early stages of melanoma development (between dysplastic nevi and primary melanoma), but no significant difference was observed between primary and metastatic melanoma. CUL1 expression was also characterized as one of the significant biomarkers which may be used to discriminate melanoma from dysplastic nevi [80]. Recently it was found that CUL1 expression is increased in early stages of melanoma. Also, the in vitro cell culture work showed that knockdown of CUL1 inhibits melanoma cell growth by arresting cells at the G1 phase, likely through p27 accumulation, resulting at least in part from blockage of the functional SKP2-SCF E3 ligase (substrate receptor of CUL1) [81]. Little is known about role of other cullins (CUL2, CUL3, CUL4, CUL5, CUL6, and CUL7) in melanomagenesis. CUL2 frameshift mutations were detected in two out of 41 colon cancers. CUL3 is downregulated in breast and kidney cancer, suggesting a tumor suppressive role [80]. CUL5 is a putative tumor suppressor that blocks Src activity and inhibits breast cancer cell growth upon overexpression [79]. CUL7 mRNA is significantly overexpressed in non-small cell lung carcinoma and is associated with poor patient prognosis. CUL7 is a putative tumor-promoting protein, as it binds directly to the p53 tetramerization domain and antagonizes p53 function [78]. CUL4A is overexpressed in a number of human cancers, including breast cancers, hepatocellular carcinomas, and mesotheliomas. According to recent findings it is supposed that CUL4A amplification and overexpression plays an oncogenic role in carcinogenesis, and that CUL4A could be an attractive target for anticancer therapies [12,75,82].

It was indicated, that for activity of SCF E3 ubiquitin ligases cullins neddylation is required [75]. Neddylation, a process of addition of ubiquitin-like protein NEDD8 to target proteins has been studied in certain cancer cells. Results have revealed that in highly proliferative cell lines the levels of conjugated NEDD8 expression were high [75]. According to recent data, an increase in neddylation was identified in melanoma cell lines and tissues [82,83]. It was concluded that the neddylation pathway may be involved in the development of melanoma. Inhibition of the neddylation pathway affects cell cycle regulators and apoptosis promoters, leading to the depression of melanoma growth. Thus, it was suggested that neddylation-related regulatory enzymes are potential targets for melanoma therapy [82]. A small molecule inhibitor (MLN4924) suppressing the NEDD8 activating pathway is currently in clinical trials, having demonstrated success in tumor suppression in animal model studies, and two hydrophobic synthetic inhibitors have been identified recently to inhibit SCF activities by different mechanisms [84].

7. Concluding remarks

The main goal of studying E3-ligases is to develop a drug that specifically targets these enzymes for cancer therapy. Compared to the general proteasome inhibitor bortezomib, which

blocks the proteasome mediated protein degradation, inhibitors that target a particular E3 ligase are expected to have better selectivity with less associated toxicity. Indeed, SCF complex E3 ligase inhibitor MLN4924, which blocks ~20% of all cellular proteins subjected to proteasomal degradation, is well-tolerated in mice and has entered phase I clinical trials for hematological and solid tumor malignancies. Although compared with bortezomib MLN4924 appears to be a better candidate drug as it targets particular superfamily of E3 ligases, it would be judicious to wait for the results of phase I clinical trials to see whether it exhibits any serious side effects.

However, E3 ligases do not contain a canonical active site and to develop highly selective and specific inhibitors against these proteins is expected to be difficult. Their mode of action involves protein-protein interactions. For example, a small compound named Nutlins was shown to inhibit MDM2 (the ligase for p53) by binding to its p53-binding pocket and resulting in p53 accumulation. As a result, Nutlin has shown promising anti-cancer efficacy in cancer cell line xenograft assays. Nutlin-3 and its pharmacologically optimized form, RG7112 are currently undergoing phase I clinical trials for the treatment of retinoblastoma, liposarcomas, and hematological malignancies.

The results of recent studies are very important and promising, whereas the overexpression of E3 ligases (MDM2, RBX1/ROC1, and others) were found in melanomas and their inhibition is a novel approach for the treatment of malignant melanomas. This calls for extensive research in this emerging area involving functional delineation of E3 ubiquitin ligases and their substrates and study of deregulated pathways in human malignant melanoma.

Conflict of interest

The authors state no conflict of interest.

REFERENCES

- [1] Komander D, Michael R. The ubiquitin code. *Annu Rev Biochem* 2012;81:203-29.
- [2] Xu P, Duong DM, Seyfried NT, Cheng D, Xie Y, Robert J, et al. Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell* 2009;137:133-45.
- [3] Shimizu Y, Okuda-Shimizu Y, Hendershot LM. Ubiquitylation of an ERAD substrate occurs on multiple types of amino acids. *Mol Cell* 2010;40:917-26.
- [4] Moretti J, Brou Ch. Ubiquitinations in the Notch signaling pathway. *Int J Mol Sci* 2013;14:6359-81.
- [5] Fraile JM, Quesada V, Rodriguez D, Freije JMP, Lopez-Otin L. Deubiquitinases in cancer: new functions and therapeutic options. *Oncogene* 2012;31:2373-88.
- [6] Nakayama K. Growth and progression of melanoma and non-melanoma skin cancers regulated by ubiquitination. *Pigment Cell Melanoma Res* 2010;23:338-51.
- [7] Lipkowitz S, Weissman AM. RINGS of good and evil: RING finger ubiquitin ligases at the crossroads of tumour suppression and oncogenesis. *Nat Rev Cancer* 2011;11:629-44.
- [8] Sun Y, Li H. Functional characterization of SAG/RBX2/ROC2/RNF7, an antioxidant protein and an E3 ubiquitin ligase. *Protein Cell* 2013;4(2):103-16.

- [9] Wenzel DM, Klevit RE. Following Ariadne's thread: a new perspective on RBR ubiquitin ligases. *BMC Biol* 2012;10:24.
- [10] Lorenz S, Cantor AJ, Rape M, Kuriyan J. Macromolecular juggling by ubiquitylation enzymes. *BMC Biol* 2013;11:65.
- [11] Jianping J, Cardozo T, Lovering RC, Elledge SJ, Pagano M, Harper JW. Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev* 2004;18:2573-80.
- [12] Xie C, Wei W, Sun Y. Role of SKP1-CUL1-F-box-protein (SCF) E3 ubiquitin ligases in skin cancer. *J Genet Genomics* 2013;40:97e106.
- [13] Fuchs SY, Spiegelman VS, Kumar KG. The many faces of beta-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer. *Oncogene* 2004;23:2028-36.
- [14] Bucheit AD, Syklawer E, Jakob JA, Bassett RL, Curry JL, Gershenwald JE, et al. Clinical characteristics and outcomes with specific BRAF and NRAS mutations in patients with metastatic melanoma. *Cancer* 2013;119(21):3821-9.
- [15] Liu S, Yamauchi H. p27-Associated G1 arrest induced by hinokitiol in human malignant melanoma cells is mediated via down-regulation of pRb, Skp2 ubiquitin ligase, and impairment of Cdk2 function. *Cancer Lett* 2009;286:240-9.
- [16] Vaid M, Prasad R, Sun Q, Katiyar SK. Silymarin targets beta catenin signaling in blocking migration/invasion of human melanoma cells. *PLoS ONE* 2011;6:e23000.
- [17] Hershko DD. Oncogenic properties and prognostic implications of the ubiquitin ligase Skp2 in cancer. *Cancer* 2008;112:1415-24.
- [18] Wu L, Grigoryan A, Li Y, Hao B, Pagano M, Cardozo T. Specific small molecule inhibitors of Skp2-mediated p27. *Chem Biol* 2012;19(12):1515-24.
- [19] Chen G, Cheng Y, Martinka M, Li G. Cytoplasmic Skp2 expression is increased in human melanoma and correlated with patient survival. *PLoS ONE* 2011;6(2):e17578. PMID: 21386910.
- [20] Li Q, Murphy M, Ross J, Sheehan C, Carlson JA. Skp2 and p27 kip1 expression in melanocytic nevi and melanoma: an inverse relationship. *J Cutan Pathol* 2004;31:633-42.
- [21] Rose AE, Wang G, Hanniford D, Monni S, Tu T, Shapiro R, et al. Clinical relevance of SKP2 alterations in metastatic melanoma. *Pigment Cell Melanoma Res* 2011;24(1):197-206.
- [22] Sumimoto H, Hirata K, Yamagata S, Miyoshi H, Miyagishi M, Taira K, et al. Effective inhibition of cell growth and invasion of melanoma by combined suppression of BRAF (V599E) and Skp2 with lentiviral RNAi. *Int J Cancer* 2006;118:472-6.
- [23] Rico-Bautista E, Yang CC, Lu L, Roth GP, Wolf DA. Chemical genetics approach to restoring p27Kip1 reveals novel compounds with antiproliferative activity in prostate cancer cells. *BMC Biol* 2010;8:153.
- [24] Qu X, Shen L, Zheng Y, Cui Y, Feng Z, Liuand F, et al. A signal transduction pathway from TGF- β 1 to SKP2 via Akt1 and c-Myc and its correlation with progression in human melanoma. *J Invest Dermatol* 2014;134:159-67.
- [25] Cheng Y, Li G. Role of the ubiquitin ligase Fbw7 in cancer progression. *Cancer Metastasis Rev* 2012;31:75-87.
- [26] Busino L, Millman SE, Scotti L, Kyratsous CA, Basrur V, O'Connor O, et al. Fbxw7a- and GSK3-mediated degradation of p100 is a pro-survival mechanism in multiple myeloma. *Nat Cell Biol* 2012;14(4):375-85.
- [27] Wang Z, Inuzuka H, Zhong J, Wan L, Fukushima H, Sarkar F, et al. Tumor suppressor functions of FBW7 in cancer development and progression. *FEBS Lett* 2012;586:1409-18.
- [28] Zhou W, Wei W, Sun Y. Genetically engineered mouse models for functional studies. *Cell Res* 2013;23:599-619.
- [29] Welcker M, Clurman B. FBW7 ubiquitin ligase a tumour suppressor at the crossroads of cell division, growth and differentiation. *Nat Rev Cancer* 2008;8:83-93.
- [30] Bhaskaran N, van Drogen F, Ng HF, Kumar R, Ekholm-Reed S, Peter M, et al. Fbw7 α and Fbw7 γ collaborate to shuttle cyclin E1 into the nucleolus for multiubiquitylation. *Mol Cell Biol* 2013;33(1):85-97.
- [31] Welcker M, Larimore EA, Swanger J, Bengoechea-Alonso MT, Grim JE, Ericsson J, et al. Fbw7 dimerization determines the specificity and robustness of substrate degradation. *Genes Dev* 2013;27:2531-6.
- [32] Tan Y, Sangfelt O, Spruck C. The Fbxw7/hCdc4 tumor suppressor in human cancer. *Cancer Lett* 2008;271:1-12.
- [33] Ishikawa Y, Hosogane M, Okuyama R, Aoyama S, Onoyama I, Nakayama KI, et al. Opposing functions of Fbxw7 in keratinocyte growth, differentiation and skin tumorigenesis mediated through negative regulation of c-Myc and Notch. *Oncogene* 2013;32(15):1921-32.
- [34] O'Neil J, Grim J, Strack P, Rao S, Tibbitts D, Winter Ch, et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to γ -secretase inhibitors. *J Exp Med* 2007;204(8):1813-24.
- [35] Lerner M, Lundgren J, Akhoondi S, Jahn A, Ng HF, Moqadam FA, et al. MiRNA-27a controls FBW7/hCDC4-dependent cyclin E degradation and cell cycle progression. *Cell Cycle* 2011;10:2172-83.
- [36] Cheng Y, Chen G, Martinka M, Ho V, Li G. Prognostic significance of Fbw7 in human melanoma and its role in cell migration. *J Invest Dermatol* 2013;133(7):1794-802.
- [37] Li J, Cheng Y, Tai D, Martinka M, Welch DR, Li G. Prognostic significance of BRMS1 expression in human melanoma and its role in tumor angiogenesis. *Oncogene* 2011;30:896-906.
- [38] Huang HL, Weng HY, Wang LQ, Yu CH, Huang QJ, Zhao PP, et al. Triggering Fbw7-mediated proteasomal degradation of c-Myc by oridonin induces cell growth inhibition and apoptosis. *Mol Cancer Ther* 2012;11(5):1155-65.
- [39] Wade M, Li Y, Matani A, Braun S, Milanesi F, Rodewald L, et al. Functional analysis and consequences of Mdm2 E3 ligase. *Oncogene* 2012;31(45):4789-97.
- [40] Vassilev LT. MDM2 inhibitors for cancer therapy. *Trends Mol Med* 2006;13(1):23-30.
- [41] Wade M, Li Y-Ch, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer* 2013;13:83-96.
- [42] Nag S, Zhang X, Srivenugopal KS, Wang MH, Wang W, Zhang R. Targeting MDM2-p53 interaction for cancer therapy: are we there yet? *Curr Med Chem* 2014;21(5):553-74.
- [43] Xu C, Fan CD, Wang X. Regulation of Mdm2 protein stability and the p53 response by NEDD4-1 E3 ligase. *Oncogene* 2015;34(3):281-9.
- [44] Sun Y. Targeting E3 ubiquitin ligases for cancer therapy. *Cancer Biol Ther* 2003;2(6):623-9.
- [45] Ji Z, Njauw Ch.N., Taylor M, Neel V, Flaherty KT, Tsao H. p53 rescue through HDM2 antagonism suppresses melanoma growth and potentiates MEK inhibition. *J Invest Dermatol* 2012;132:356-64.
- [46] Pettersson S, Sczaniecka M, McLaren L, Russel F, Gladstone K, Hupp T, et al. Non-degradative ubiquitination of the Notch1 receptor by the E3 ligase MDM2 activates the Notch signaling pathway. *Biochem J* 2013;450(3):523-36.
- [47] Colaluca IN, Tosoni D, Nuciforo P, Senic-Matuglia F, Galimberti V, Viale G, et al. NUMB controls p53 tumour suppressor activity. *Nature* 2008;451:76-80.
- [48] Sun Y, Klauzinska M, Lake RJ, Lee JM, Santopietro S, Raaf A, et al. Trp53 regulates Notch4 signaling through Mdm2. *J Cell Sci* 2010;124:1067-76.
- [49] Sczaniecka M, Gladstone K, Pettersson S, McLaren L, Huart AS, Wallace M. MDM2 protein-mediated ubiquitination of NUMB protein identification of a second physiological substrate of MDM2 that employs a dual-site docking mechanism. *Biol Chem* 2012;287(17):14052-68.
- [50] Li Q, Lozano G. Molecular pathways: targeting Mdm2 and Mdm4 in cancer therapy. *Clin Cancer Res* 2012;19(1):1-8.

- [51] Panayi ND, Mendoza EE, Breshears ES, Burd R. In: Lester D, editor. Aberrant death pathways in melanoma, recent advances in the biology, therapy and management of melanoma. 2013;978-953-51-0976-1.
- [52] Sun Y. E3 ubiquitin ligases as cancer targets and biomarkers. *Neoplasia* 2006;8(8):645-54.
- [53] Rajabi P, Karimian P, Heidarpour M. The relationship between MDM2 expression and tumor thickness and invasion in primary cutaneous malignant melanoma. *J Res Med Sci* 2012;7(5):452-5.
- [54] Müller CS. Notch signaling and malignant melanoma. *Adv Exp Med Biol* 2012;727:258-64.
- [55] Zhao Y, Sun Y. Cullin-RING ligases as attractive anti-cancer targets. *Curr Pharm Des* 2013;19(18):3215-25.
- [56] Tovar Ch, Rosinski J, Filipovic Z, Higgins B, Kolinsky K, Hilton H, et al. Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. *PNAS* 2006;103(6):1888-93.
- [57] Yang Y, Ludwig RL, Jensen JP, Pierre SA, Medaglia MV, Davydov IV, et al. Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells. *Cancer Cell* 2005;7:547-59.
- [58] Peirce SK, Findley HW. Targeting the MDM2-p53 interaction as a therapeutic strategy for the treatment of cancer. *Cell Health Cytoskeleton* 2010;2:49-58.
- [59] Maerken TV, Rihani A, Gothem AV, De Paepe A, Speleman F, Vandesompele J. Selective inhibition of the p53-MDM2 interaction by nutlin drugs: a new therapeutic perspective for neuroblastoma. *Belg R Acad Med* 2013;2:198-207.
- [60] Brennan RC, Federico S, Bradley C, Zhang J, Flores-Otero J, Wilson M, et al. Targeting the p53 pathway in retinoblastoma with subconjunctival Nutlin-3a. *Cancer Res* 2011;71(12):4205-13.
- [61] Secchiero P, Melloni E, di Iasio MG, Tiribelli M, Rimondi E, Corallini F, et al. Nutlin-3 up-regulates the expression of Notch1 in both myeloid and lymphoid leukemic cells, as part of a negative feedback antiapoptotic mechanism. *Blood* 2009;113:4300-8.
- [62] Metzger MB, Hristova VA, Weissman AM. HECT and RING finger families of E3 ubiquitin ligases at a glance. *J Cell Sci* 2012;125:531-7.
- [63] Huang L, Yan Z, Liao X, Li Y, Yang J, Wang Z-G, et al. The p53 inhibitors MDM2/MDMX complex is required for control of p53 activity in vivo. *PNAS* 2011;108(29):12001-6.
- [64] Marine JC. MDM2 and MDMX in cancer and development. *Curr Top Dev Biol* 2011;94:45-75.
- [65] Herman AG, Hayano M, Poyurovsky MV, Shimada K, Skouta R, Prives C, et al. Discovery of Mdm2-MdmX E3 ligase inhibitors using a cell-based ubiquitination assay. *Cancer Discov* 2011;1(4):312-25.
- [66] Wang Ch-L, Wang JY, Liu ZY, Ma XM, Wang XW, Jin H, et al. Ubiquitin-specific protease 2a stabilizes MDM4 and facilitates the p53-mediated intrinsic apoptotic pathway in glioblastoma. *Carcinogenesis* 2014;35(7):1500-9.
- [67] Carrillo AM, Bouska A, Arrate MP, Eischen CM. Mdmx promotes genomic instability independent of p53 and Mdm2. *Oncogene* 2014;(March). <http://dx.doi.org/10.1038/onc.2014.27>
- [68] Bernal F, Wade M, Godes M, Davis TN, Whitehead DG, Kung AL, et al. A stapled p53 helix overcomes HDMX-mediated suppression of p53. *Cancer Cell* 2010;18(5):411-22.
- [69] Chang YS, Graves B, Guerlavais V, Tovar Ch, Packman K, To K-H, et al. Stapled α -helical peptide drug development: a potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. *PNAS* 2011;108(29):12001-6.
- [70] Gembarska A, Luciani F, Fedele C, Russell EA, Dewaele M, Villar S, et al. MDM4 is a key therapeutic target in cutaneous melanoma. *Nat Med* 2012;18(8):1239-47.
- [71] Tan M, Zhao Y, Kim SJ, Liu M, Jia L, Saunders TL, et al. SAG/RBX2/ROC2 E3 ubiquitin ligase is essential for vascular and neural development by targeting NF1 for degradation. *Dev Cell* 2011;21:1062-76.
- [72] Gu Q, Bowden TG, Normolle D, Sun Y. SAG/ROC2 E3 ligase regulates skin carcinogenesis by stage dependent targeting of c-Jun/AP1 and I κ B/NF- κ B. *J Cell Biol* 2007;178:1009-23.
- [73] Jia L, Yang J, Hao X, Zheng M, He H, Xiong X, et al. Validation of SAG/RBX2/ROC2 E3 ubiquitin ligase as an anticancer and radiosensitizing target. *Clin Cancer Res* 2010;16:814-24.
- [74] Nai G, Marques M. Role of ROC1 protein in the control of cyclin D1 protein expression in skin melanomas. *Pathol Res Pract* 2011;207:174e181.
- [75] Jia L, Sun Y. SCF E3 ubiquitin ligases as anticancer targets. *Curr Cancer Drug Targets* 2011;11(3):347-56.
- [76] Sarikas A, Hartmann T, Pan Z-Q. The cullin protein family. *Genome Biol* 2011;12:220.
- [77] Marin I. Diversification of the cullin family. *BMC Evol Biol* 2009;9:267.
- [78] Lee J, Zhou P. Cullins and cancer. *Genes Cancer* 2010;1(7):690-9.
- [79] Laszlo GS, Cooper JA. Restriction of Src activity by Cullin-5. *Curr Biol* 2009;19(2):157-62.
- [80] Zhang G, Li G. Novel multiple markers to distinguish melanoma from dysplastic nevi. *PLoS ONE* 2012;7(9):e45037.
- [81] Chen G, Li G. Increased Cul1 expression promotes melanoma cell proliferation through regulating p27 expression. *Int J Oncol* 2010;37:1339-44.
- [82] Cheng F, He R, Zhang L, Li H, Zhang W, Ji X, et al. Expression of neddylation-related proteins in melanoma cell lines and the effect of neddylation on melanoma proliferation. *Oncol Lett* 2014;7:1645-50.
- [83] Jackson S, Xiong Y. CRL4s: the CUL4-RING E3 ubiquitin ligases. *Trends Biochem Sci* 2009;34(11):562-70.
- [84] Du W, Mei Q. Ubiquitin-proteasome system, a new anti-tumor target. *Acta Pharmacol Sin* 2013;34:187-8.