Am J Cancer Res 2023;13(7):2773-2789
www.ajcr.us /ISSN:2156-6976/ajcr0149630

Review Article

Zooming into the structure-function of RING finger proteins for anti-cancer therapeutic applications

Mary George[1], Priscilla Masamba[1], Bamidele Abiodun Iwalokun[2], Abidemi Paul Kappo[1]

[1]Molecular Biophysics and Structural Biology (MBSB) Group, Department of Biochemistry, Faculty of Science, University of Johannesburg, Auckland Park Kingsway Campus, Auckland Park, Johannesburg, South Africa; [2]Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria

Received February 12, 2023; Accepted May 22, 2023; Epub July 15, 2023; Published July 30, 2023

Abstract: Cancer is one of the most common and widely diagnosed diseases worldwide. With an increase in prevalence and incidence, many studies in cancer biology have been looking at the role pro-cancer proteins play. One of these proteins is the Really Interesting New Gene (RING), which has been studied extensively due to its structure and functions such as apoptosis, neddylation, and its role in ubiquitination. The RING domain is a cysteine-rich domain known to bind Cysteine and Histidine residues. It also binds two zinc ions that help stabilize the protein in various patterns, often with a 'cross-brace' topology. Different RING finger proteins have been studied and found to have suitable targets for developing anti-cancer therapeutics. These identified candidate proteins include Parkin, COP1, MDM2, BARD1, BRCA-1, PIRH2, c-CBL, SIAH1, RBX1 and RNF8. Inhibiting these candidate proteins provides opportunities for shutting down pathways associated with tumour development and metastasis.

Keywords: Anti-cancer therapeutics, neddylation, Parkin, RING finger proteins, ubiquitination

Introduction

Cancer is a non-communicable disease that has been increasing in incidence and prevalence globally. According to GLOBOCAN (2021) [1], the estimated number of new cases in 2020 stood at 19 million, which is predicted to increase to 28 million by 2040. Currently, the highest incidence rate globally in both sexes across all ages is cancer of the breast and lung [2]. Cancer is caused by uncontrollable cell growth that can metastasize from primary tumours [3, 4]. Several factors promote tumour growth and metastasis: sustaining proliferative signalling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, and resisting cell death; these are known as the hallmarks of cancer. There are also emerging hallmarks; avoiding immune destruction and tumour-promoting inflammation; and emerging characteristics; genome instability and mutation and deregulation of cellular energetics that are involved in the development and progression of cancer [5]. An understanding of these hallmarks and characteristics can help in the development of novel therapeutics.

Primary forms of cancer treatment include surgery, radiation therapy and chemotherapy [6]. Additional therapies include the use of newer drugs serving as Epidermal Growth Factor Receptor (EGFR) inhibitors, cyclin-dependent kinase inhibitors, inhibitors of Hepatocyte Growth Factor (HGF)/Mesenchymal-Epithelial Transition factor (c-MET) (HGF/c-Met), telomerase inhibitors, Vascular Endothelial Growth Factor (VEGF) signalling, proapoptotic inhibitors, Bcl-2 homology 3 (BH3) mimetics, immune-activating anti-Cytotoxic T Lymphocyte Antigen-4 monoclonal Antibody (anti-CTLA4 mAb), selective anti-inflammatory drugs, Poly (ADP-Ribose) Polymerase (PARP) inhibitors and aerobic glycolysis inhibitors [5]. Although these are effective, finding novel therapies targeting a combination of these factors must be developed as current therapies only target a single characteristic of the cancer hallmarks. Targeting a combination of these characteristics can help develop more lasting and effective therapies

[5]. Many challenges still need to be overcome with various treatments available to help treat cancer. These include combating drug-resistant cells, difficulty in diagnosis, side effects caused by multiple chemotherapeutic drugs, metastasis and more precise targeting of cancer cells- specificity [7, 8]. Several promising molecular targets have been identified in drug discovery and one of these are E3 ubiquitin ligases [9]. According to Sun (2003), E3 ubiquitin ligases are suitable cancer targets as they inhibit growth suppression, carcinogenesis, and apoptosis. They are also "druggable" as they can be screened easily for small molecular inhibitors and targeted by specific antibodies [9]. However, one of the main reasons that E3 ubiquitin ligases are a suitable target is that they are only expressed in low amounts and not in normal cells; hence their inhibition would result in little to no effect on the functioning of normal, healthy cells. Thus, E3 ubiquitin ligases are ideal targets for cancer. One of the E3 ubiquitin ligases is the Really Interesting New Gene (RING) finger protein, the most significant type in the E3 ubiquitin ligase family [9].

RING finger structure and function

Really Interesting New Gene (RING) finger proteins are found in various proteins. Its motif was first identified in the RING1 gene [10]. The RING finger is a 70-residue cysteine-rich domain [11], which coordinates eight conserved cysteine or histidine residues that can bind two zinc ions (Zn^{2+}) [11, 12]. The RING finger can have two types of motifs, a histidine (RING-H2) or a cysteine (RING-HC), which can be differentiated by their chelating metal residues [13, 14]. The motifs are arranged as Cys-X_2-Cys-X_{9-39}-Cys-X_{1-3}-His-X_{2-3}-Cys or His-X_2-Cys-X_{4-48}-Cys-X_2-Cys [13-15]. This arrangement shows the coordination of pairs 1 and 3 to the first Zn^{2+} ion and pairs 2 and 4 to the second Zn^{2+} ion [11, 16], with the formation of a cross-brace when cysteine and histidine residues bind [11, 13, 16]. The RING domain can be classified according to the various patterns that form, depending on how the Zn^{2+} ions bind; this includes C3HC4, C3HHC3, C2H2C4, C4C4 and C3HC4, which are the most common [11, 15]. The RING finger consists of an α-helix and three short-stranded β-sheets, all found close to each other and arranged next to the Zn^{2+} ions, where one helps stabilise the two large loops within the fold. A homodimer can also dimerize on the β-sheets, which are essential characteristics of the RING finger as it helps in its function [11]. The RING domain is involved in a variety of functions that include transcription and translation regulation, proteolysis [16], negative regulation of tumour suppressors such as p53 and Rb [17], neddylation [18], apoptosis and cell cycle regulation [15] with the essential function being ubiquitination by E3 ubiquitin ligases [11, 15-17, 19, 20].

Ubiquitination

Ubiquitination takes part in cell death, cell signalling and cell cycle regulation by removing toxic and damaged proteins and identifying the protein substrate that needs to undergo ubiquitination [21]. Ubiquitination begins with the ubiquitin-activating enzyme (E1), which activates ubiquitin with the help of adenosine triphosphate (ATP). The ubiquitin is then transferred to the ubiquitin-conjugating enzyme (E2) by forming a thioester bond between the active site on the cysteine residue on E1 and the C-terminal of the carboxyl group of ubiquitin. From E2, the ubiquitin gets transferred to ubiquitin ligase (E3). This transfer occurs through a catalytic reaction between E3 and ubiquitin. The ubiquitin is transferred to the lysine residue on the specific substrate that will be found bound to E3, thus forming an isopeptide bond between the lysine ε-amino group of the substrate and the carboxyl group on the C-terminal of ubiquitin. This process is repeated until a ubiquitin chain forms on the substrate. Once a ubiquitin chain is formed, a signal is sent, and the ubiquitin, along with the substrate, is sent for proteasomal degradation to the 26S proteasome [21-23] (**Figure 1**). The RING finger is known as an adapter-type ligase. This means that it allows a protein substrate and E2 enzyme to work in conjunction with it. This is done during the movement of ubiquitin from the E2 molecule during ubiquitination transfer without forming any thioester bonds. However, this is dependent on the location of the RING domain [13].

Apoptosis

Apoptosis is a form of programmed cell death that occurs during a cell's development to maintain homoeostasis in cell populations. It

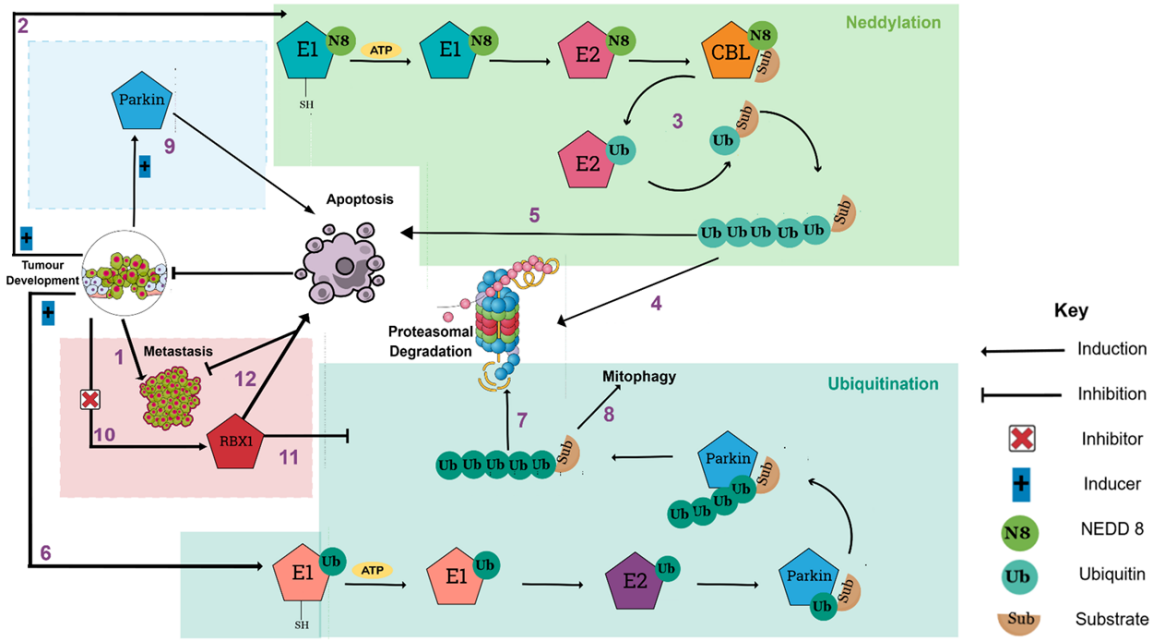


Figure 1. A model representing the various targets of RING finger proteins for use in cancer therapeutics. (1) Tumour development can lead directly to metastasis. Metastasis can occur due to overexpression of certain RING finger proteins such as RBX1; (2) Inducing of certain RING finger proteins such as CBL will lead to neddylation of the proteins that interact with them; (3) Neddylation causes a conformational change to occur and recruit ubiquitin; (4) And ultimately lead to proteasomal degradation; (5) Or can lead to apoptosis, which leads to the inhibition of tumour development; (6) Inducing of RING finger proteins such as Parkin; (7) This will lead to ubiquitination of the proteins that interact with them and ultimately lead to proteasomal degradation; (8) Or can also lead to mitophagy through a joint autophagic machinery; (9) The induction of Parkin can also lead to apoptosis; (10) Inhibition of RING finger proteins such as RBX1; (11) Will cause the inhibition of E3 activity such as ubiquitination; (12) But does lead to apoptosis and thus inhibiting metastasis.

also occurs when an immune reaction takes place for defence or when the cell has been damaged [24, 25]. Chemotherapeutic drugs, as well as irradiation, can cause cells to have DNA damage and thus lead to apoptosis using the p53-dependent pathway [25]. There are two main pathways in apoptosis, namely the extrinsic (death receptor) pathway and the intrinsic (mitochondrial) pathway [24, 25]. The extrinsic pathway begins when the death ligand binds to a death receptor with the help of adaptor proteins, which activates the caspase cascade; hence the activation of caspase 3 by caspase 8 leads to activating the execution pathway. The execution pathway begins with endonuclease activation, degradation of chromosomal DNA, protease activation, nuclear and cytoskeleton protein degradation leading to cytomorphological changes and finally, the formation of apoptotic bodies [24, 25]. The intrinsic pathway is activated by exo- and endogenous stimuli such as DNA damage, oxidative stress, radiation therapy, and chemotherapy. This leads to pro-apoptotic signalling where mitochondrial membrane perturbation allows for the release of cytochrome C, where the apoptosome forms and caspase 9 is activated, which leads to caspase 3 release and thus the execution pathway [24, 25].

Neddylation

Neddylation is a process like ubiquitination. This pathway has been found to play a role in apoptosis, senescence, angiogenesis, immune response [23], cell cycle regulation [18] as well as the regulation of protein stability and transcriptional activity [26]. It uses a ubiquitin-like molecule known as Neuronal precursor cell-expressed Developmentally Down-regulated protein 8 (NEDD8). It begins with the adenylation and activation of NEDD8-activating enzyme (NAE) E1. The NAE and a heterodimer known as Amyloid Precursor Protein-Binding Protein 1 (APPBP1) and Ubiquitin-like protein-Activating enzyme 3 (UBA3) form transferred to

Table 1. Expression pattern of RING finger proteins in common metastatic malignancies

Malignancy	RING finger Protein	Expression Pattern	References
Breast Cancer	RNF126	High	[28-30]
	RNF6	Normal	[31]
	RNF31	High	[32]
	RING1	Low	[33]
	COP1	Normal	[34]
	BRCA1	High	[35]
	PIRH2	High	[36]
	RBX1	Normal	[37-39]
	SIAH1	Low	[40]
	RNF8	Normal	[41-43]
Prostate Cancer	RNF126	High	[29, 30]
	RNF19A	High	[44, 45]
	RNF6	Normal	[31, 46, 47]
	RBX1	High	[48]
	MDM2	Normal	[49]
	PIRH2	High	[36, 50, 51]
	RNF8	Low	[41]
Lung Cancer	RBX2	High	[48]
	RNF19A	Normal	[45]
	RNF180	Low	[52, 53]
	RNF38	High	[54]
	MDM2	Normal	[49]
	PIRH2	Normal	[36, 50, 51]
	RBX1	Normal	[37-39]
	RNF8	High	[41]
Kidney Cancer	RING1	Low	[33]
	RNF121	Low	[55]
	Parkin	Low	[56]
	RNF8	Low	[41]
Thyroid Cancer	RING1	Low	[33]
	RNF150	Low	[57]
	RNF8	Low	[41]
Colon Cancer	RNF220	High	[58]
	RING1	Low	[33]
	RNF2	High	[59]
	RNF128	High	[60]
	MDM2	Normal	[49]
	BARD1	High	[61-63]
	RBX1	Normal	[39]
	SIAH1	Low	[64]
	Parkin	Low	[65]
	RNF8	High	[41]
Pancreatic Cancer	RNF223	High	[66]
	RNF13	Normal	[67, 68]
	MDM2	Normal	[49]
	Parkin	Low	[56]
Bone Cancer	RNF6	High	[69]
	PIRH2	High	[36]

either Ubiquitin Conjugating Enzyme E2M (UBE2M) also known as Ubiquitin Conjugating enzyme 12 (UBC12) or Ubiquitin Conjugating Enzyme E2F (UBE2F), NEDD8-conjugating enzymes (E2) by a trans-thiolation reaction. The NEDD8 is then transferred from the E2 of a lysine residue on substrate-specific E3 ligase (RING Box protein 1/2 (RBX1/2) or Defective in Cullin Neddylation protein 1 (DCN1)) through covalent bonding to the target protein [18, 26, 27] (**Figure 1**).

Many RING proteins and/or RING-containing proteins have been found to play various roles and could be used in anti-cancer therapeutics. Several RING finger proteins are known to be differentially expressed in various common metastatic malignancies; the expression pattern of these RING finger proteins (specifically those reviewed in this manuscript) is shown in **Table 1**. These RING proteins include COP1, MDM2, BARD1, BRCA-1, PIRH2, RBX1, CBL, SIAH1, Parkin and RNF8.

RING finger proteins and anti-cancer drug design

COP1

Constitutive Photomorphogenic 1 (COP1) is a protein that contains a RING domain. It is also known as the RING finger and WD repeat domain 2 (RFWD2), and it is a conserved E3 ubiquitin ligase [70, 71]. COP1 is found in many organisms and on chromosome 1q24.1 of the Cop1 gene in humans [70]. It consists of three conserved domains; the RING domain on the N-terminal, the coiled-coil domain and seven WD40 repeats on the C-terminal [72-74]. COP1 may also contain a glycine-serine-rich 70 amino acid extension at the N-terminal in the human COP1 [73, 74]. The RING domain of COP1 is a cysteine-rich domain that binds two Zn^{2+} ions in a cross-brace formation like most RING proteins. It follows the RING-H2 motif and participates in protein-protein interactions [75]. The RING domain interacts with other proteins, allowing COP1 to play a role in ubiquitination and other functions such as nuclear import [74], DNA repair,

cell cycle arrest and apoptosis [70, 72, 76]. The RING domain has interacted with tumour suppressor genes such as p53 and p27, thus mediating its degradation [72, 77]. The Polyomavirus Enhancer Activator 3 (PEA3) group also interacts with RING. It plays a role in ubiquitination and proteasomal degradation of transcriptional factors, one of which is Jun Proto-Oncogene (c-Jun), known to play a role in regulating tumorigenesis [70, 73, 78] (**Table 2**). According to Song et al. (2020), COP1 can be used as a target for cancer therapy due to its structure and function in carcinogenesis. Increasing COP1 expression levels elicits its function as a tumour suppressor in certain cancers.

MDM2

The Mouse Double Minute 2 (MDM2), also known as the Human Double Minute 2 (HDM2), is another RING-containing protein. It is a 491 amino acid residue protein on the 12q13-14 chromosome [49, 79, 80]. The protein contains four conserved regions: the p53 binding domain, central acidic part, and zinc finger motif with the RING finger domain at the C-terminal [79-81]. The RING domain of the protein is encoded by residues 429-491, approximately 60 residues long [80-82], and folds into a C2H2C4 pattern. It follows the cross-brace motif but with a symmetrical dimer. Its fold is similar to that of other RING finger domains, following a ββαβ fold that binds two zinc ions and contains a hydrophobic core [80]. The RING domain is involved in protein-protein and protein-nucleic acid interactions [79]. In MDM2, the RING domain plays a role in autoubiquitination of MDM2 and the ubiquitination of p53 [83]. The RING domain, an E3 ubiquitin ligase, is well studied primarily on its interaction with p53, where it is found to play a role as a negative regulator of p53 [79, 80, 84]. Expression levels of MDM2 and p53 are low during normal cell cycle but increase during stress conditions [79, 80]. When MDM2 binds with p53, its conformation and interaction with other molecules change. This interaction activates E3 ligase activity on both MDM2 as well as on p53, thus leading to proteasomal degradation of p53 [49, 85]. Through this interaction, many possible targets have been or are being developed for cancer treatment. The first set of inhibitors was designed to inhibit the interaction of MDM2 and p53, but recently, small molecules have been designed to inhibit E3 activity [49, 80]; these include HDM2 Ligase Inhibitor 98 class (HLI98) and Serdemetan (JNJ-26854165: a Tryptamine derivative) [49, 85] (**Table 2**). Other small molecule inhibitors include Partheolide and Sempervirine; these are natural-origin molecules which have not been clinically tested yet [49] (**Table 2**). MDM2 is a thoroughly studied protein and is one of the main targets of drug design in cancer therapy.

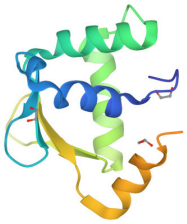
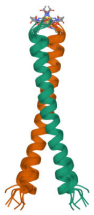
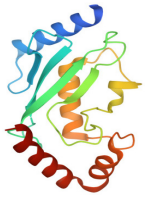
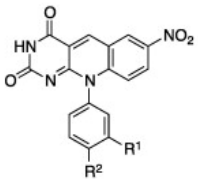
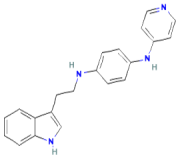
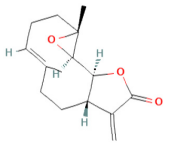
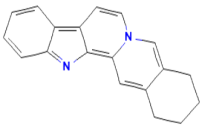
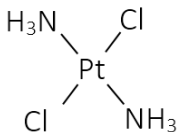
BARD1

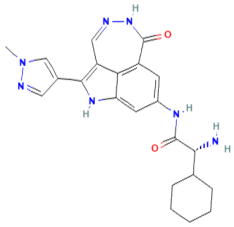
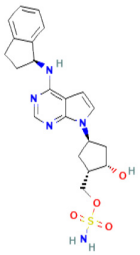
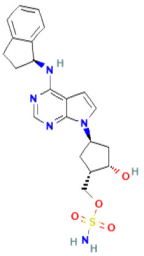
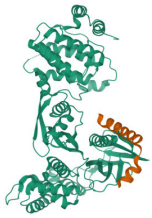
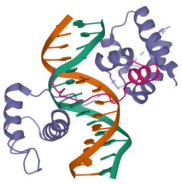
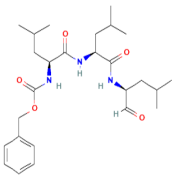
The BRCA1-Associated RING Domain protein 1 (BARD1) is associated with breast and ovarian cancer. It is a 777 amino acid residue protein on the 2q34-q35 chromosome [86]. It contains an N-terminal RING domain and three Ankyrin repeats (ANK) domains, and on the C-terminal, there are two tandem BRCA1 C-terminal repeat (BRCT) domains [86-89]. The RING finger domain of the BARD1 consists of approximately 60 residues that form two α-helices and two β-strands secondary structural el ements, arranged as αββα. Like most RING finger domains, it contains the conserved eight residues of cysteine and histidine that bind two Zn^{2+} ions [90, 91]. BARD1 does not have E3 ligase activity until it undergoes heterodimerisation with BRCA1 [92]. Even though the BARD1 RING domain does not have E3 activity, it is known to play a role in DNA repair [92, 93]. BARD1 is a binding partner for BRCA1, thus allowing for a BRCA1-BARD1 heterodimer complex to form through binding at their RING domains [90, 91, 94]. Inhibition of this binding causes tumorigenesis and this is why the heterodimerisation of BARD1 alongside BRCA1 is crucial for its structure-function characteristics [94]. Therefore, developing drugs for cancer using BARD1 as a target should involve the induction of the BRCA1-BARD1 heterodimer complex facilitated by the RING finger domain.

BRCA-1

Breast Cancer-1 (BRCA1) is a protein involved in breast and ovarian cancer [90]. It is an 1863 amino acid residue protein that consists of an N-terminal RING domain and two tandem BRCA1 C-terminal repeat (BRCT) domains [90, 95, 96]. The RING domain of BRCA1 follows the C3HC4 pattern and contains two anti-parallel α-helices [97]. The RING of BRCA1 has E3 activity and is also involved in DNA repair [92]. The

Table 2. Small molecule inhibitors and protein-protein interactions target RING proteins in anti-cancer therapeutics

Target protein	Small Molecule Inhibitor/Protein-Protein Partner	Structure of Small Molecule Inhibitors/ Protein	Mechanism of Action	Clinical Trial Stage	References
COP1	PEA3		COP1 interacts with PEA3 causing its degradation, leading cancer cell proliferation.	No clinical trials	[70]
	c-JUN		COP1 interacts with c-JUN causing its degradation, leading to inhibition of cancer development and progression.	No clinical trials	[70]
	p28		Binds to p53 and inhibits COP1-mediated ubiquitination causing apoptosis of cancer cells as well as cell cycle arrest.	Phase I	[70]
MDM2	HLI98		Inhibits MDM2 ubiquitin ligase activity.	No clinical trials	[85, 137]
	JNJ-26854165		Inhibits MDM2 ubiquitin ligase activity and has p53 independent activity.	Phase I	[49]
	Parthenolide		Induces MDM2 ubiquitination.	No clinical trials for cancer	[49]
	Sempervirine		Inhibits MDM2 ubiquitin ligase activity, inhibits MDM2-mediated p53 ubiquitination and MDM2 autoubiquitination.	Preclinical	[49]
BRCA-1	Transplatin		Inhibits BRCA-1 E3 ligase activity.	Clinically ineffective	[96]

RBX1	CHK1 inhibitors		Silences RBX1 and thus inducing G_2 phase arrest in the cell cycle.	No clinical trials	[39, 105]
	MLN4924		Inhibits the E3 ligase through deneddylation. leading to apoptosis.	Phase Ib/II	[38, 105]
CBL	MLN4924		Inhibits neddylation, thus inducing apoptosis.	Phase Ib/II	[26]
SIAH1	N-CoR		Binding with SIAH 1 promotes ubiquitination.	No clinical trials	[121]
	DCC		Binding with SIAH1 promotes proteolysis through the ubiquitination and proteasomal degradation pathways.	No clinical trials	[119, 121]
	OBF-1		Binding with SIAH1 promotes proteasomal degradation.	No clinical trials	[121]
	YBX-1		Binds to SIAH1 and leading to the ubiquitination of YBX1, thus stabilizing mRNAs and overcoming chemoresistance.	No clinical trials	[117]
	MG132		Inhibition of proteasomal activity, thus inhibiting ubiquitination of SIAH2.	Unknown/ Not Reported	[119, 138]

Parkin	B5G1	Not Available	Induces mitochondrial apoptosis and mitophagy when interacts with PINK1-Parkin pathway.	Unknown/ Not Reported	[130, 131]

RING domain allows BRCA1 to cause a double-strand break [96] and is involved in tumour suppression [98, 99]. As aforementioned, BRCA1 binds with BARD1 through its RING domains forming a heterodimer complex [90, 91, 94]. This heterodimerisation creates a four-helix bundle, allowing ubiquitin-binding activity through E2 binding. The E2 enzyme only interacts with the BRCA1 RING domain and not that of BARD1. However, BARD1 stabilises BRCA1, so E2 binding takes place [95, 98]. BRCA1 E3 ligase activity is an essential function performed by the RING domain. It targets estrogen receptor-alpha, CtBP-Interacting Protein (CtIP), histone protein H2A and the progesterone receptor leading to changes in gene activation, DNA condensation and DNA repair. BRCA1 has also been found to participate in autoubiquitination [98]. The BRCA1 RING domain has been used in cancer therapy by targeting the DNA repair pathway. As E3 ligases play an essential role in many cell processes, it has become a suitable drug target and prognostic biomarker in cancer therapy. There have been possible uses of the BRCA1 RING as a potential molecular target in disrupting E3 ligase activity. Use of platinum-based agents (*trans*-platinum) or other metal-based drugs that target the RING domain of BRCA1 to inhibit E3 activity can potentially improve the efficacy of anticancer drugs [96] (**Table 2**).

PIRH2

The p53-Induced RING-H2 protein (PIRH2), also known as the RING-finger and CHY zinc-finger domain-containing protein 1 (RCHY1) is an E3 ubiquitin ligase [50, 100-102]. It is found on chromosome 4p21.1 in humans [51]. It was first seen interacting with the androgen receptor as an N-terminal interacting protein [50, 101] and is a 261-residue protein containing three domains: the Amino Terminal Domain (NTD), RING, and Carboxy Terminal Domain (CTD) domain. These domains are found on residues 1-137, 138-189 and 190-261, respectively [102]. The RING domain of PIRH2 is a cysteine-rich central domain in the form of a RING-H2 motif, thus following a C3H2C3 pattern [51, 102-104]. The RING domain folds like most RING fingers into a globular structure that binds two zinc ions in a cross-brace formation. The RING structure for PIRH2 is similar to RING structures found in RBX1 and MDM2 [102, 103]. Like MDM2, it has been found to participate in the ubiquitylation of p53 through its interaction with p53 [50, 100, 102, 103]; this interaction can suppress transcription activation, thus degrading p53 using a negative feedback loop [100]. The RING domain of PIRH2 has been found to interact with E2, which binds to the RING domain of PIRH2 through a hydrophobic and shallow groove on the surface of the RING domain; this is essential for E3 ligase activity [102, 103]. Sheng and colleagues looked at developing inhibitors with new targets for p53-dependent cancer therapy [102]. Developing inhibitors targeting E3 ligase activity is also being examined to create a new target in cancer with PIRH2 [51].

RBX1

The RING Box protein 1 (RBX1), also known as Regulator of Cullins1 (ROC1), is one of the RING components of the Skp1-cullin-F-box proteins (SCF), also known as Cullin-based RING ligase (CRL) protein, is a large family of E3 ligases [37-39, 105]. RBX1 is expressed in various cancer types and the expression levels are related to the survival rates of cancer patients as it causes cell proliferation of cancer cells [37, 105]. RBX1 is a 108 amino acid residue protein with a C-terminal RING domain that follows the RING-H2 motif with a C3H2C3 pattern [38, 39, 105]. Like most RING fingers, the RING domain of RBX1 binds two zinc ions in a cross-brace formation [38] and is needed for E3 ubiquitin ligase activity, whereby E2 binds to the RING domain [38, 106]. RBX1 is a potential target for anti-cancer therapy due to its role in cell cycle regulation, cancer cell proliferation and survival [37, 39, 105]. When silenced, RBX1 reduces cell proliferation and causes no apoptosis in normal cells but increases the number of cell death in cancerous cells. This shows that RBX1

can be used to develop siRNA-based therapy, which silences RBX1 or the use of small-molecule inhibitors that inhibits the activity of E3 ubiquitin ligases such as CHK1 (Checkpoint Kinase 1) inhibitors and MLN4924 (Pevonedistat) [38, 39, 105] (**Figure 1** and **Table 2**).

C-CBL

Casitas B-lineage Lymphoma (c-CBL) is a RING finger protein containing an E3 ubiquitin ligase [107-110]. CBL is a family of proteins that contain a Tyrosine Kinase-Binding domain (TKB), a linker region in the N-terminal, a RING domain, and a proline-rich domain in the C-terminal. The TKB domain contains 4-helix bundles, a calcium-binding EF-hand and an SH2 domain [109-112]. The RING domain of CBL follows the C3H2C3 pattern [109, 112] consisting of three β-sheet strands, an α-helix, and two big loops stabilized like most RING structures by two zinc ions [113]. The RING domain in this protein is known to perform many functions, majorly the binding of E2 and mediation of ubiquitination [109, 111-113]. When the TKB domain is phosphorylated, it allows the RING domain to have increased binding affinity to E2 [109]. Other functions relate to the degradation of Protein Tyrosine Kinases (PTK) [112] as it promotes the epidermal growth factor receptor (EGFR) activation, which is important as it causes auto-phosphorylation, thereby leading to multiple mono-ubiquitination. Therefore, EGFR is downregulated during ubiquitination [26, 108, 111, 112]. Added to this, the RING domain also participates in lysosome degradation [24, 77]; it stabilises Transforming Growth Factor-Beta Receptor II (TGF-βRII) through ubiquitination and degradation. The CBL RING domain has also been found to play a role in the NEDD8 pathway, thus promoting protein neddylation [26] (**Figure 1**). Due to the relationship between CBL and EGFR, overexpression of EGFR in certain cancers can be used as a target for anti-cancer therapies [26]. Other treatments have looked at developing small molecules that mimic phosphorylated tyrosine, which binds to the linker region, thus allowing ubiquitination and inhibiting tumour cell growth. Thus, CBL proteins can be used to help develop anti-cancer therapies with these proteins as targets [109] (**Table 2**).

SIAH1

The Seven In Absentia Homolog 1 (SIAH1) is an E3 ubiquitin ligase, RING-containing protein [114-116]. It is a mammalian ortholog of Seven-In Absentia (SIAN) that is highly conserved [115, 117, 118]. SIAH1 is a 282 amino acid residue protein [119] that has conserved domains, namely the RING domain on the N-terminal that is followed by the Substrate Binding Domain (SBD) at the C-terminal, which consists of the SIAN-type Zinc Fingers (SZF), Substrate-Binding Site (SBS) and the Dimerization (DIMER) domain [114, 116-118, 120]. The SBS plays a role in substrate recognition, interaction, targeting, and degradation with the DIMER specifically found to allow for the formation of homo- and heterodimers between SIAN/SIAH proteins [120]. The RING domain of SIAH1 is found to follow the C3HC4 motif [119]. It is an E3 ubiquitin ligase involved in the interaction, modification and targeting of different substrates and regulators, allowing them to perform ubiquitin-mediated proteolysis [119, 120]. It also plays a role in complex protein assembly, protein stability and protein subcellular localisation [114, 120]. The RING domain of SIAH1 has been found to interact with different proteins, leading to proteolysis, which can help develop anti-cancer therapies. Some of these proteins include the N-CoR (Nuclear receptor Corepressor), DCC (Deleted in Colorectal Cancer) [119, 121], OBF-1 (Oct-Binding Factor 1) [121] and YBX-1 (Y-Box Binding protein 1) [117] (**Table 2**). There have been many obstacles in developing anti-cancer therapies with SIAH1, as it promotes breast cancer metastasis and invasion; hence inhibition of SIAH1 could be used in anti-cancer therapy [122]. Gao and colleagues (2022) discovered that SIAH1 helps regulate EOC cell drug sensitivity through the ubiquitin-proteasome pathway by degradation of YBX-1. In addition, the SIAH1-YBX-1-E2F5/YY1/RCC2 (Seven In Absentia Homolog 1-Y-Box Binding Protein 1-E2F Transcription Factor 5/Yin Yang 1/Regulator of Chromosome Condensation 2) axis could be a potential target. Due to SIAH interaction with adaptor proteins, inhibition of this protein-protein interaction showed potential, even though no possible ligands have been discovered to be used as a target [115]. In this light, Stebbins and colleagues (2013) showed that covalent peptide-based inhibitors were more successful and could be used to develop selective and irreversible peptide inhibitors for anti-cancer therapeutics (**Table 2**). As SIAH is involved in signalling pathways during tumour progression, it is essential to understand these processes and

their interaction with other proteins when developing inhibitors for anti-cancer therapy [118].

Parkin

Parkin is a 465 amino acid residue multi-domain protein consisting of six domains [123-125]; the Ubiquitin-Like domain (UBL), RING0, RING1, In-Between-Ring domain (IBR), Repressor Element of Parkin (REP) and RING2. The RING1-IBR-RING2 is also known as the RBR domain collectively [123, 124, 126, 127]. The RING domains of Parkin follow the C3HC4 motif but have different topologies. The RING0 has a hairpin topology, RING1 follows the usual cross-brace formation, while IBR and RING2 pursue a sequential topology as seen in LIM domains [127]. The RING domains of Parkin are involved in protein-protein interactions via the E2 enzymes that mediate ubiquitination, with UbcH7 (Ubiquitin Conjugating enzyme H7) and UbcH8 (Ubiquitin Conjugating enzyme H8) being the two main E2 interacting enzymes [123, 125]. Parkin also mediates dopaminergic neuron functioning through the ubiquitination of substrates such as Parkin-associated endothelin-like receptor (Pael-R), α-synaptotagmin XI, Septin-5 (Sept5), transcription factor SIM2 (Single-minded homolog 2) [123, 125], O-glycosylated α-synuclein, synphilin-1 [123-125], cyclin E and p53 synthase [124, 128]. The ubiquitination of these proteins is primarily due to their interaction with the C-terminal RING domains and RBR [125] (**Figure 1**). It is a protein often associated with Parkinson's disease but has also been found to play a role in cancer. The Parkin gene has been mutated in many cancers, and there is a loss of heterozygosity and copy number in breast, ovarian, lung and colorectal cancer [56, 124, 129]. The overexpression of the protein inhibits cancer cell proliferation, whereas the inactivation causes cancer cells' proliferation, thus showing Parkin's role as a tumour suppressor [124]. Furthermore, Parkin promotes cancer cell apoptosis by promoting mitochondrial depolarization. Added to this, it promotes ubiquitination and degradation of Mcl-1 (Myeloid leukaemia 1) and Bcl-2 (B-cell lymphoma protein 2) and opens the Bax/Bak (Bcl2 associated X protein/Bcl2 Antagonist/Killer protein) channel, thus allowing the cells to undergo apoptosis [56, 124]. Parkin also plays a role in mitochondrial autophagy, known as mitophagy, which occurs via the Parkin/PTEN-induced kinase 1 (PINK1) pathway. The mitochondria to be removed cause the attachment of PINK1, which then becomes activated and phosphorylates substrates like Parkin and ubiquitin. The recruitment of E2s is then promoted, activating Parkin, thus catalysing ubiquitination of the mitochondrial proteins through the RING domain. The ubiquitinated mitochondrial proteosomes are then joined to the autophagic machinery, where autophagy is initiated [124, 126, 130, 131] (**Figure 1**). Understanding these pathways and mechanisms has been used to help develop anticancer therapies. One such is a derivative of Betulinic acid (BA), B5G1, which has potent anticancer activity in multi-drug resistant cells by triggering mitophagy dependent on PINK1 and Parkin [130, 131] (**Table 2**). Thus, the mitochondria have been identified as a target for anticancer therapy, where inducers and inhibitors of mitophagy can be used for treatment [130].

RNF8

The Really Interesting New Gene (RING) finger protein 8 (RNF8) is a RING protein that was first described in 1998. RNF8 is found in different human tissues except for the spleen on chromosome 6p21.3 [42]. It consists of two conserved domains, namely the Forkhead-Associated (FHA) on the N-terminal; which specifically binds the phospho-peptide motifs in target proteins, thus mediating protein-protein interactions; and the RING finger domain on the C-terminal, which is found to possess E3 ubiquitin ligase activity [42, 132]. Like most RING finger domains, RNF8 follows the C3HC4 motif that binds two zinc ions [132, 133] and consists of an α-helix followed by a two-stranded antiparallel β-sheet. It contains an extended dimeric coiled-coil at the N-terminal and a C-terminal helix [133]. The RNF8 RING plays a role in ubiquitination where it interacts with H2A and H2B histones, allowing them to undergo mono-ubiquitination. This is essential in DNA damage and mediating the DNA damage signal, which in turn allows mediating DNA damage repair and activation of cell cycle checkpoints, thus maintaining genomic stability [42, 134-136]. RNF8 RING also interacts with class III E2 ubiquitin ligases such as UBE2E2 (Ubiquitin Conjugation enzyme E2 E2), UbcH6 (Ubiquitin Conjugation enzyme H6),

UBE2E3 (Ubiquitin Conjugation enzyme E2 E3) and UBC13 (Ubiquitin Conjugation enzyme 13), thus catalysing K48-linked poly-ubiquitination [42, 132, 133, 135, 136]. RNF8 also recruits other E3 ligases such as RNF168 to help facilitate ubiquitination [43, 136]. Lee and colleagues (2016) found that through the K63-linked ubiquitination, DSB-dissociated RNF8 activates Twist, a chromosome-independent substrate, and thus promotes epithelial-mesenchymal transition (EMT) of cancer cells, development of cancer stem cells, cancer progression and chemoresistance. In colorectal cancer, RNF8 promotes tumorigenesis but also has tumour suppressor activities in other cancer types [42]. There have been several therapies and strategies developed using RNF8. Targeting RNF8 has been proposed for breast cancer as RNF8, when overexpressed, is found to promote tumour metastasis. Thus, targeting RNF8 could cause suppression of cancer cells, thereby increasing the sensitivity of cancer cells to anti-cancer therapies [132]. Another proposed treatment targets E3 ligases for the K63-linked ubiquitination [43]. Thus, it is essential to study these pathways and functions to develop strategies for the appropriate cancer therapy for different cancer types.

Conclusion

Various studies have looked at multiple targets and proteins for anti-cancer therapy. With the increasing incidence and prevalence of the disease, the search for specific cancer therapeutics to combat drug resistance and minimise the side effects caused by chemotherapeutic drugs has been increasingly studied. E3 ubiquitin ligases such as the RING finger proteins have been of interest as a suitable target in anti-cancer therapeutics due to their various functions, such as apoptosis and ubiquitination. Proteins such as COP1, MDM2, BARD1, BRCA-1, PIRH2, RBX1, CBL, SIAH1, Parkin and RNF8 have been recognised as suitable targets in anti-cancer therapy due to the roles played by their RING finger domains. More so, some studies have suggested appropriate targets and drugs for anti-cancer therapeutics targeting ubiquitin pathways. By studying these pathways and functions, strategies must still be developed to discover suitable cancer therapies. Presently, various new anticancer treatments have been in the development pipeline, but many cancer challenges remain. Although some treatments have been used for years and have greatly helped in cancer treatment, the search for more effective and specific novel therapies still needs to be developed. The RING finger domain is now seen as an attractive druggable target in cancer therapy. Various RING finger proteins have now been identified and are being proposed to treat different cancer types, usually as inhibitors to tumour development and metastasis. Applying these RING finger proteins as a target in anti-cancer therapeutics can ultimately help in the search for effective anticancer treatment.

Acknowledgements

Mary George is profoundly thankful to the National Research Foundation (NRF) of South Africa for a Postgraduate Research Bursary, while Priscilla Masamba is grateful to Department of Science and Innovation (DSI) and the National Research Foundation (NRF) for a Postdoctoral Research Fellowship. Abidemi Paul Kappo is grateful for support provided by the University of Johannesburg Research Committee.

Disclosure of conflict of interest

None.

Address correspondence to: Abidemi Paul Kappo, Molecular Biophysics and Structural Biology (MBSB) Group, Department of Biochemistry, C2 Lab Room 426, Faculty of Science, University of Johannesburg, Auckland Park Kingsway Campus, Auckland Park 2006, Johannesburg, South Africa. Tel: +27-11-559-3182; Fax: +27-11-559-2605; E-mail: akappo@uj.ac.za

References

[1] GLOBOCAN. GLOBOCAN 2020: New Global Cancer Data. 2021; Available: https://www.uicc.org/news/globocan-2020-new-global-cancer-data (Accessed 17 January 2022).

[2] International Agency for Research on Cancer. All Cancers. 2020; Available: https://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf (Accessed 17 January 2022).

[3] Ikwegbue PC, Masamba P, Mbatha LS, Oyinloye BE and Kappo AP. Interplay between heat shock proteins, inflammation and cancer: a potential cancer therapeutic target. Am J Cancer Res 2019; 9: 242-249.

[4] Adekiya TA, Aruleba RT, Khanyile S, Masamba P, Oyinloye BE and Kappo AP. Structural analysis and epitope prediction of MHC class-1-chain related protein-a for cancer vaccine development. Vaccines (Basel) 2017; 6: 1.
[5] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-674.
[6] Morales-Cruz M, Delgado Y, Castillo B, Figueroa CM, Molina AM, Torres A, Milián M and Griebenow K. Smart targeting to improve cancer therapeutics. Drug Des Devel Ther 2019; 13: 3753-3772.
[7] Zugazagoitia J, Guedes C, Ponce S, Ferrer I, Molina-Pinelo S and Paz-Ares L. Current challenges in cancer treatment. Clin Ther 2016; 38: 1551-1566.
[8] Chakraborty S and Rahman T. The difficulties in cancer treatment. Ecancermedicalscience 2012; 6: ed16.
[9] Sun Y. Targeting E3 ubiquitin ligases for cancer therapy. Cancer Biol Ther 2003; 2: 623-629.
[10] Freemont PS, Hanson IM and Trowsdale J. A novel cysteine-rich sequence motif. Cell 1991; 64: 483-484.
[11] Kappo MA, Ab E, Hassem F, Atkinson RA, Faro A, Muleya V, Mulaudzi T, Poole JO, McKenzie JM, Chibi M, Moolman-Smook JC, Rees DJ and Pugh DJ. Solution structure of RING finger-like domain of retinoblastoma-binding protein-6 (RBBP6) suggests it functions as a U-box. J Biol Chem 2012; 287: 7146-7158.
[12] Alam I, Yang YQ, Wang Y, Zhu ML, Wang HB, Chalhoub B and Lu YH. Genome-wide identification, evolution and expression analysis of RING finger protein genes in Brassica rapa OPEN. Sci Rep 2017; 7: 1-11.
[13] Kandias NG, Chasapis CT, Bentrop D, Episkopou V and Spyroulias GA. High yield expression and NMR characterization of Arkadia E3 ubiquitin ligase RING-H2 finger domain. Biochem Biophys Res Commun 2009; 378: 498-502.
[14] Yang Y, Lorick KL, Jensen JP and Weissman AM. Expression and evaluation of RING finger proteins. Methods Enzymol 2005; 398: 103-112.
[15] Pugh DJ, Ab E, Faro A, Lutya PT, Hoffmann E and Rees DJ. DWNN, a novel ubiquitin-like domain, implicates RBBP6 in mRNA processing and ubiquitin-like pathways. BMC Struct Biol 2006; 6: 1.
[16] Kosarev P, Mayer KF and Hardtke CS. Evaluation and classification of RING-finger domains encoded by the Arabidopsis genome. Genome Biol 2002; 3: RESEARCH0016.
[17] Xiao C, Wu G, Zhou Z, Zhang X, Wang Y, Song G, Ding E, Sun X, Zhong L, Li S, Weng J, Zhu Z, Chen J and Wang X. RBBP6, a RING finger-domain E3 ubiquitin ligase, induces epithelial-mesenchymal transition and promotes metastasis of colorectal cancer. Cell Death Dis 2019; 10: 833.
[18] Soucy TA, Dick LR, Smith PG, Milhollen MA and Brownell JE. The NEDD8 conjugation pathway and its relevance in cancer biology and therapy. Genes Cancer 2010; 1: 708-716.
[19] Mbita Z, Meyer M, Skepu A, Hosie M, Rees J and Dlamini Z. De-regulation of the RBBP6 isoform 3/DWNN in human cancers. Mol Cell Biochem 2012; 362: 249-262.
[20] Chibi M, Meyer M, Skepu A, G Rees DJ, Moolman-Smook JC and Pugh DJ. RBBP6 interacts with multifunctional protein YB-1 through its RING finger domain, leading to ubiquitination and proteosomal degradation of YB-1. J Mol Biol 2008; 384: 908-916.
[21] Oh CK, Choi YK, Hwang IY, Ko YU, Chung IK, Yun N and Oh YJ. RING-finger protein 166 plays a novel pro-apoptotic role in neurotoxin-induced neurodegeneration via ubiquitination of XIAP. Cell Death Dis 2020; 11: 939.
[22] Deng L, Meng T, Chen L, Wei W and Wang P. The role of ubiquitination in tumorigenesis and targeted drug discovery. Signal Transduct Target Ther 2020; 5: 11.
[23] Joazeiro CA and Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. Cell 2000; 102: 549-552.
[24] Jan R and Chaudhry GE. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. Adv Pharm Bull 2019; 9: 205-218.
[25] Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007; 35: 495-516.
[26] Zhao Y, Morgan MA and Sun Y. Targeting neddylation pathways to inactivate Cullin-RING ligases for anticancer therapy. Antioxid Redox Signal 2014; 21: 2383-400.
[27] Gai W, Peng Z, Liu CH, Zhang L and Jiang H. Advances in cancer treatment by targeting the neddylation pathway. Front Cell Dev Biol 2021; 9: 653882.
[28] Pan Y, Yang Y, Huang R, Yang H, Huang Q, Ji Y, Dai J, Qiao K, Tang W, Xie L, Yin M, Ouyang J, Ning S and Su D. Ring finger protein 126 promotes breast cancer metastasis and serves as a potential target to improve the therapeutic sensitivity of ATR inhibitors. Breast Cancer Res 2022; 24: 92.
[29] Xu H, Ju L, Xiong Y, Yu M, Zhou F, Qian K, Wang G, Xiao Y and Wang X. E3 ubiquitin ligase RNF126 affects bladder cancer progression through regulation of PTEN stability. Cell Death Dis 2021; 12: 239.
[30] Zhi X, Zhao D, Wang Z, Zhou Z, Wang C, Chen W, Liu R and Chen C. E3 ubiquitin ligase RNF126 promotes cancer cell proliferation by targeting the tumor suppressor p21 for ubiqui-

tin-mediated degradation. Cancer Res 2013; 73: 385-394.
[31] Zeng Y, Xu X, Wang S, Zhang Z, Liu Y, Han K, Cao B and Mao X. Ring finger protein 6 promotes breast cancer cell proliferation by stabilizing estrogen receptor alpha. Oncotarget 2017; 8: 20103-20112.
[32] Zhu J, Zhao C, Zhuang T, Jonsson P, Sinha I, Williams C, Strömblad S and Dahlman-Wright K. RING finger protein 31 promotes p53 degradation in breast cancer cells. Oncogene 2016; 35: 1955-1964.
[33] Gao S, Wang SY, Zhang XD, Wu H and Pang D. Low expression of the polycomb protein RING1 predicts poor prognosis in human breast cancer. Front Oncol 2021; 10: 618768.
[34] Li YF, Wang DD, Zhao BW, Wang W, Huang CY, Chen YM, Zheng Y, Keshari RP, Xia JC and Zhou ZW. High level of COP1 expression is associated with poor prognosis in primary gastric cancer. Int J Biol Sci 2012; 8: 1168-77.
[35] Chang HJ, Yang UC, Lai MY, Chen CH and Fann YC. High BRCA1 gene expression increases the risk of early distant metastasis in ER+ breast cancers. Sci Rep 2022; 12: 77.
[36] Daks A, Fedorova O, Parfenyev S, Nevzorov I, Shuvalov O and Barlev NA. The role of E3 ligase Pirh2 in disease. Cells 2022; 11: 1515.
[37] Kunishige T, Migita K, Matsumoto S, Wakatsuki K, Nakade H, Miyao S, Kuniyasu H and Sho M. Ring box protein-1 is associated with a poor prognosis and tumor progression in esophageal cancer. Oncol Lett 2020; 20: 2919-2927.
[38] Wei D and Sun Y. Small RING finger proteins RBX1 and RBX2 of SCF E3 ubiquitin ligases: the role in cancer and as cancer targets. Genes Cancer 2010; 1: 700-707.
[39] Jia L and Sun Y. RBX1/ROC1-SCF E3 ubiquitin ligase is required for mouse embryogenesis and cancer cell survival. Cell Div 2009; 4: 16.
[40] Zhang H, Wang J, Ge Y, Ye M and Jin X. Siah1 in cancer and nervous system diseases (review). Oncol Rep 2022; 47: 35.
[41] Liu C, Kuang J, Wang Y, Duan T, Min L, Lu C, Zhang T, Chen R, Wu Y and Zhu L. A functional reference map of the RNF8 interactome in cancer. Biol Direct 2022; 17: 17.
[42] Zhou T, Yi F, Wang Z, Guo Q, Liu J, Bai N, Li X, Dong X, Ren L, Cao L and Song X. The functions of DNA damage factor RNF8 in the pathogenesis and progression of cancer. Int J Biol Sci 2019; 15: 909-918.
[43] Lee HJ, Li CF, Ruan D, Powers S, Thompson PA, Frohman MA and Chan CH. The DNA damage transducer RNF8 facilitates cancer chemoresistance and progression through twist activation. Mol Cell 2016; 63: 1021-1033.
[44] Zhang N, Huang D, Ruan X, Ng AT, Tsu JH, Jiang G, Huang J, Zhan Y and Na R. CRISPR screening reveals gleason score and castration resistance related oncodriver ring finger protein 19 A (RNF19A) in prostate cancer. Drug Resist Updat 2023; 67: 100912.
[45] Cheng Y, Hu Y, Wang H, Zhao Z, Jiang X, Zhang Y, Zhang J, Tong Y and Qiu X. Ring finger protein 19A is overexpressed in non-small cell lung cancer and mediates p53 ubiquitin-degradation to promote cancer growth. J Cell Mol Med 2021; 25: 7796-7808.
[46] Xu H, Wong CC, Li W, Zhou Y, Li Y, Wang L, Liu L and Yu J. RING-finger protein 6 promotes colorectal tumorigenesis by transcriptionally activating SF3B2. Oncogene 2021; 40: 6513-6526.
[47] Xu K, Qiu Y, Shimelis H and Yang X. RING-finger protein 6 (Rnf6) is upregulated during prostate cancer progression and promotes prostate cancer growth via inducing atypical ubiquitylation of androgen receptor. Cancer Res 2008; 68: 1219.
[48] Huang T, Li J, Liu X, Shi B, Li S and An HX. An integrative pan-cancer analysis revealing the difference in small ring finger family of SCF E3 ubiquitin ligases. Front Immunol 2022; 13: 968777.
[49] Nag S, Zhang X, Srivenugopal KS, Wang MH, Wang W and Zhang R. Targeting MDM2-p53 interaction for cancer therapy: are we there yet? Curr Med Chem 2014; 21: 553-574.
[50] Daks A, Petukhov A, Fedorova O, Shuvalov O, Kizenko A, Tananykina E, Vasileva E, Semenov O, Bottrill A and Barlev N. The RNA-binding protein HuR is a novel target of Pirh2 E3 ubiquitin ligase. Cell Death Dis 2021; 12: 581.
[51] Jung YS, Qian Y and Chen X. Pirh2 RING-finger E3 ubiquitin ligase: its role in tumorigenesis and cancer therapy. FEBS Lett 2012; 586: 1397-1402.
[52] Ding Y, Lu Y, Xie X, Cao L and Zheng S. Ring finger protein 180 suppresses cell proliferation and energy metabolism of non-small cell lung cancer through downregulating C-myc. World J Surg Oncol 2022; 20: 162.
[53] Liu H, Yang P, Li X and Jia Y. Ring finger protein 180 is associated with biological behavior and prognosis in patients with non-small cell lung cancer. Oncol Lett 2020; 20: 35.
[54] Xiong D, Zhu SQ, Wu YB, Jin C, Jiang JH, Liao YF, Long X, Wu HB, Xu JJ, Li JJ and Ding JY. Ring finger protein 38 promote non-small cell lung cancer progression by endowing cell EMT phenotype. J Cancer 2018; 9: 841-850.
[55] Xiang P, Sun Y, Liu Y, Shu Q and Zhu Y. Really interesting new gene finger protein 121 is a tumor suppressor of renal cell carcinoma. Gene 2018; 676: 322-328.
[56] Liu J, Zhang C, Hu W and Feng Z. Parkinson's disease-associated protein Parkin: an unusual

player in cancer. Cancer Commun (Lond) 2018; 38: 40.
[57] Deng W, Wu J, Zheng W, Wang Q, Li D and Kuang H. RNF150 suppresses papillary thyroid carcinoma with ASK1 ubiquitination presenting a direct target via inactivating p38 signaling axis. Cell Biol Int 2023; [Epub ahead of print].
[58] Yan J, Tan M, Yu L, Jin X and Li Y. Ring finger 220 promotes the stemness and progression of colon cancer cells via Ubiquitin specific peptidase 22-BMI1 axis. Bioengineered 2021; 12: 12060-12069.
[59] Wei F, Jing H, Wei M, Liu L, Wu J, Wang M, Han D, Yang F, Yang B, Jiao D, Zheng G, Zhang L, Xi W, Guo Z, Yang AG, Qin W, Zhou Y and Wen W. Ring finger protein 2 promotes colorectal cancer progression by suppressing early growth response 1. Aging (Albany NY) 2020; 12: 26199-26220.
[60] Ning S, Chen Y, Wang G, Liu Y, Yang Y and Zhang Z. Ring finger protein 128 promotes, rather than inhibits, colorectal cancer progression by regulating the Hippo signaling pathway. Front Oncol 2022; 12: 1031160.
[61] Hawsawi YM, Shams A, Theyab A, Abdali WA, Hussien NA, Alatwi HE, Alzahrani OR, Oyouni AAA, Babalghith AO and Alreshidi M. BARD1 mystery: tumor suppressors are cancer susceptibility genes. BMC Cancer 2022; 22: 599.
[62] Zhang YQ, Pilyugin M, Kuester D, Leoni VP, Li L, Casula G, Zorcolo L, Schneider-Stock R, Atzori L and Irminger-Finger I. Expression of oncogenic BARD1 isoforms affects colon cancer progression and correlates with clinical outcome. Br J Cancer 2012; 107: 675-683.
[63] Sporn JC, Hothorn T and Jung B. BARD1 expression predicts outcome in colon cancer. Clin Cancer Res 2011; 17: 5451-62.
[64] Xiao Z, Wei Z, Deng D, Zheng Z, Zhao Y, Jiang S, Zhang D, Zhang LJ, Fan M, Chen S, Wang S, Ding Y, Ye Y and Jiao H. Downregulation of Siah1 promotes colorectal cancer cell proliferation and migration by regulating AKT and YAP ubiquitylation and proteasome degradation. Cancer Cell Int 2020; 20: 50.
[65] Da Silva-Camargo CCV, Baldin RK, Polli NL, Agostinho AP, Olandosk M, De Noronha L and Sotomaior VS. Parkin protein expression and its impact on survival of patients with advanced colorectal cancer. Cancer Biol Med 2018; 15: 61-69.
[66] Feng L, Wang J, Zhang J, Diao J, He L, Fu C, Liao H, Xu X, Gao Y and Zhou C. Comprehensive analysis of E3 ubiquitin ligases reveals ring finger protein 223 as a novel oncogene activated by KLF4 in pancreatic cancer. Front Cell Dev Biol 2021; 9: 738709.
[67] Jin X, Cheng H, Chen J and Zhu D. RNF13: an emerging RING finger ubiquitin ligase important in cell proliferation. FEBS J 2011; 278: 78-84.
[68] Zhang Q, Meng Y, Zhang L, Chen J and Zhu D. RNF13: a novel RING-type ubiquitin ligase over-expressed in pancreatic cancer. Cell Res 2009; 19: 348-357.
[69] Ren Y, Xu X, Mao CY, Han KK, Xu YJ, Cao BY, Zhang ZB, Sethi G, Tang XW and Mao XL. RNF6 promotes myeloma cell proliferation and survival by inducing glucocorticoid receptor polyubiquitination. Acta Pharmacol Sin 2020; 41: 394-403.
[70] Song Y, Liu Y, Pan S, Xie S, Wang ZW and Zhu X. Role of the COP1 protein in cancer development and therapy. Semin Cancer Biol 2020; 67: 43-52.
[71] Li DQ, Ohshiro K, Reddy SD, Pakala SB, Lee MH, Zhang Y, Rayala SK and Kumar R. E3 ubiquitin ligase COP1 regulates the stability and functions of MTA1. Proc Natl Acad Sci U S A 2009; 106: 17493-17498.
[72] Zhou K, Wang L, Sun Z, Liu Y, Zhu Y, Liu Z, Zhang B and Shi H. COP1 acts as a ubiquitin ligase for PCDH9 ubiquitination and degradation in human glioma. Mol Neurobiol 2022; 59: 2378-2388.
[73] Yi C and Deng XW. COP1 - from plant photomorphogenesis to mammalian tumorigenesis. Trends Cell Biol 2005; 15: 618-625.
[74] Yi C, Wang H, Wei N and Deng XW. An initial biochemical and cell biological characterization of the mammalian homologue of a central plant developmental switch, COP1. BMC Cell Biol 2002; 3: 30.
[75] Torii KU, Stoop-Myer CD, Okamoto H, Coleman JE, Matsui M and Deng XW. The RING finger motif of photomorphogenic repressor COP1 specifically interacts with the RING-H2 motif of a novel Arabidopsis protein. J Biol Chem 1999; 274: 27674-27681.
[76] Guo M, Ding P, Zhu Z, Fan L, Zhou Y, Yang S, Yang Y and Gu C. Targeting RFWD2 as an effective strategy to inhibit cellular proliferation and overcome drug resistance to proteasome inhibitor in multiple myeloma. Front Cell Dev Biol 2021; 9: 675939.
[77] Ponnu J and Hoecker U. Illuminating the COP1/SPA ubiquitin ligase: fresh insights into its structure and functions during plant photomorphogenesis. Front Plant Sci 2021; 12: 662793.
[78] Baert JL, Monte D, Verreman K, Degerny C, Coutte L and de Launoit Y. The E3 ubiquitin ligase complex component COP1 regulates PEA3 group member stability and transcriptional activity. Oncogene 2010; 29: 1810-1820.

[79] Hou H, Sun D and Zhang X. The role of MDM2 amplification and overexpression in therapeutic resistance of malignant tumors. Cancer Cell Int 2019; 19: 216.
[80] Kostic M, Matt T, Martinez-Yamout MA, Dyson HJ and Wright PE. Solution structure of the Hdm2 C2H2C4 RING, a domain critical for ubiquitination of p53. J Mol Biol 2006; 363: 433-450.
[81] Linke K, Mace PD, Smith CA, Vaux DL, Silke J and Day CL. Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans. Cell Death Differ 2008; 15: 841-848.
[82] Leslie PL, Ke H and Zhang Y. The MDM2 RING domain and central acidic domain play distinct roles in MDM2 protein homodimerization and MDM2-MDMX protein heterodimerization. J Biol Chem 2015; 290: 12941-12950.
[83] Zheng J, Lang Y, Zhang Q, Cui D, Sun H, Jiang L, Chen Z, Zhang R, Gao Y, Tian W, Wu W, Tang J and Chen Z. Structure of human MDM2 complexed with RPL11 reveals the molecular basis of p53 activation. Genes Dev 2015; 29: 1524-1534.
[84] Iwakuma T and Lozano G. MDM2, an introduction. Mol Cancer Res 2003; 1: 993-1000.
[85] Wade M, Li YC and Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer 2013; 13: 83-96.
[86] Sniadecki M, Brzezinski M, Darecka K, Klasa-Mazurkiewicz D, Poniewierza P, Krzeszowiec M, Kmiec N and Wydra D. BARD1 and breast cancer: the possibility of creating screening tests and new preventive and therapeutic pathways for predisposed women. Genes (Basel) 2020; 11: 1251.
[87] Alenezi WM, Fierheller CT, Recio N and Tonin PN. Literature review of BARD1 as a cancer predisposing gene with a focus on breast and ovarian cancers. Genes (Basel) 2020; 11: 856.
[88] Fox D 3rd, Le Trong I, Rajagopal P, Brzovic PS, Stenkamp RE and Klevit RE. Crystal structure of the BARD1 ankyrin repeat domain and its functional consequences. J Biol Chem 2008; 283: 21179-21186.
[89] Edwards RA, Lee MS, Tsutakawa SE, Williams RS, Tainer JA and Glover JN. Literature review of BARD1 as a cancer predisposing gene with a focus on breast and ovarian cancers. Biochemistry 2008; 47: 11446-11456.
[90] Stewart MD, Duncan ED, Coronado E, DaRosa PA, Pruneda JN, Brzovic PS and Klevit RE. Tuning BRCA1 and BARD1 activity to investigate RING ubiquitin ligase mechanisms. Protein Sci 2017; 26: 475-483.
[91] Brzovic PS, Rajagopal P, Hoyt DW, King MC and Klevit RE. Structure of a BRCA1 - BARD1 heterodimeric RING - RING complex. Nat Struct Biol 2001; 8: 833-837.
[92] Lipkowitz S and Weissman AM. RINGs of good and evil: RING finger ubiquitin ligases at the crossroads of tumour suppression and oncogenesis. Nat Rev Cancer 2011; 11: 629-643.
[93] Adamovich AI, Banerjee T, Wingo M, Duncan K, Ning J, Martins Rodrigues F, Huang KL, Lee C, Chen F, Ding L and Parvin JD. Functional analysis of BARD1 missense variants in homology-directed repair and damage sensitivity. PLoS Genet 2019; 15: e1008049.
[94] Wu J, Aini A and Ma B. Mutations in exon region of BRCA1-related RING domain 1 gene and risk of breast cancer. Mol Genet Genomic Med 2022; 10: e1847.
[95] Elia AE and Elledge SJ. BRCA1 as tumor suppressor: lord without its RING? Breast Cancer Res 2012; 14: 306.
[96] Atipairin A, Canyuk B and Ratanaphan A. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by the platinum-based anticancer drugs. Breast Cancer Res Treat 2011; 126: 203-209.
[97] Masso M, Bansal A, Bansal A and Henderson A. Structure-based functional analysis of BRCA1 RING domain variants: concordance of computational mutagenesis, experimental assay, and clinical data. Biophys Chem 2020; 266: 106442.
[98] Clark SL, Rodriguez AM, Snyder RR, Hankins GD and Boehning D. Structure-function of the tumor suppressor BRCA1. Comput Struct Biotechnol J 2012; 1: e201204005.
[99] Drost R, Bouwman P, Rottenberg S, Boon U, Schut E, Klarenbeek S, Klijn C, van der Heijden I, van der Gulden H, Wientjens E, Pieterse M, Catteau A, Green P, Solomon E, Morris JR and Jonkers J. BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. Cancer Cell 2011; 20: 797-809.
[100] Bang S, Kaur S and Kurokawa M. Regulation of the p53 family proteins by the ubiquitin proteasomal pathway. Int J Mol Sci 2020; 21: 261.
[101] Halaby MJ, Hakem R and Hakem A. Pirh2: an E3 ligase with central roles in the regulation of cell cycle, DNA damage response, and differentiation. Cell Cycle 2013; 12: 2733-2737.
[102] Sheng Y, Laister RC, Lemak A, Wu B, Tai E, Duan S, Lukin J, Sunnerhagen M, Srisailam S, Karra M, Benchimol S and Arrowsmith CH. Molecular basis of Pirh2-mediated p53 ubiquitylation. Nat Struct Mol Biol 2008; 15: 1334-1342.
[103] Shloush J, Vlassov JE, Engson I, Duan S, Saridakis V, Dhe-paganon S, Raught B, Sheng Y and Arrowsmith CH. Structural and functional comparison of the RING domains of two p53

E3 ligases, Mdm2 and Pirh2. J Biol Chem 2011; 286: 4796-4808.
[104] Chen M, Cortay JC, Logan IR, Sapountzi V, Robson CN and Gerlier D. Inhibition of ubiquitination and stabilization of human ubiquitin E3 ligase PIRH2 by measles virus phosphoprotein. J Virol 2005; 79: 11824-11836.
[105] Jia L, Bickel JS, Wu J, Morgan MA, Li H, Yang J, Yu X, Chan RC and Sun Y. RBX1 (RING box protein 1) E3 ubiquitin ligase is required for genomic integrity by modulating DNA replication licensing proteins. J Biol Chem 2011; 286: 3379-3386.
[106] Cardote TAF, Gadd MS and Ciulli A. Crystal structure of the Cul2-Rbx1-EloBC-VHL ubiquitin ligase complex data resources 5N4W. Structure 2017; 25: 901-911, e3.
[107] Lyle C, Richards S, Yasuda K, Napoleon MA, Walker J, Arinze N, Belghasem M, Vellard I, Yin W, Ravid JD, Zavaro E, Amraei R, Francis J, Phatak U, Rifkin IR, Rahimi N and Chitalia VC. c-Cbl targets PD-1 in immune cells for proteasomal degradation and modulates colorectal tumor growth. Sci Re 2019; 9: 20257.
[108] Wei TT, Lin YC, Lin PH, Shih JY, Chou CW, Huang WJ, Yang YC, Hsiao PW and Chen CC. Induction of c-Cbl contributes to anti-cancer effects of HDAC inhibitor in lung cancer. Oncotarget 2015; 6: 12481-12492.
[109] Kales SC, Ryan PE and Lipkowitz S. Cbl exposes its RING finger. Nat Struct Mol Biol 2012; 19: 131-133.
[110] Meng W, Sawasdikosol S, Burakoff SJ and Eck MJ. Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. Nature 1999; 398: 84-90.
[111] Dou H, Buetow L, Hock A, Sibbet GJ, Vousden KH and Huang DT. Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. Nat Struct Mol Biol 2012; 19: 184-192.
[112] Swaminathan G and Tsygankov AY. The Cbl family proteins: ring leaders in regulation of cell signaling. J Cell Physiol 2006; 209: 21-43.
[113] Zheng N, Wang P, Jeffrey PD and Pavletich NP. Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases. Cell 2000; 102: 533-539.
[114] Siswanto FM, Jawi IM and Kartiko BH. The role of E3 ubiquitin ligase seven in absentia homolog in the innate immune system: an overview. Vet World 2018; 11: 1551-1557.
[115] Stebbins JL, Santelli E, Feng Y, De SK, Purves A, Motamedchaboki K, Wu B, Ronai ZA, Liddington RC and Pellecchia M. Structure-based design of covalent siah inhibitors. Chem Biol 2013; 20: 973-982.
[116] House CM, Hancock NC, Möller A, Cromer BA, Fedorov V, Bowtell DD, Parker MW and Polekhina G. Elucidation of the substrate binding site of siah ubiquitin ligase. Structure 2006; 14: 695-701.
[117] Gao W, Chen L, Lin L, Yang M, Li T, Wei H, Sha C, Xing J, Zhang M, Zhao S, Chen Q, Xu W, Li Y and Zhu X. SIAH1 reverses chemoresistance in epithelial ovarian cancer via ubiquitination of YBX-1. Oncogenesis 2022; 11: 13.
[118] Baba K and Miyazaki T. Critical function of Siah2 in tumorigenesis. AIMS Mol Sci 2017; 4: 415-423.
[119] Hu G and Fearon ER. Siah-1 N-terminal RING domain is required for proteolysis function, and C-terminal sequences regulate oligomerization and binding to target proteins. Mol Cell Biol 1999; 19: 724-732.
[120] Pepper IJ, Van Sciver RE and Tang AH. Phylogenetic analysis of the SINA/SIAH ubiquitin E3 ligase family in Metazoa. BMC Evol Biol 2017; 17: 182.
[121] Tiedt R, Bartholdy BA, Matthias G, Newell JW and Matthias P. The RING finger protein Siah-1 regulates the level of the transcriptional coactivator OBF-1. EMBO J 2001; 20: 4143-4152.
[122] Adam MG, Matt S, Christian S, Hess-Stumpp H, Haegebarth A, Hofmann TG and Algire C. SIAH ubiquitin ligases regulate breast cancer cell migration and invasion independent of the oxygen status. Cell Cycle 2015; 14: 3734-3747.
[123] Biswas S and Bagchi A. Mutational impact on "in-Between-Ring" (IBR) domain of PARKIN on protein stability and function. Appl Biochem Biotechnol 2021; 193: 1603-1616.
[124] Ding D, Ao X, Liu Y, Wang YY, Fa HG, Wang MY, He YQ and Wang JX. Post-translational modification of Parkin and its research progress in cancer. Cancer Commun (Lond) 2019; 39: 77.
[125] Beasley SA, Hristova VA and Shaw GS. Structure of the Parkin in-between-ring domain provides insights for dysfunction in autosomal recessive Parkinson's disease. Proc Natl Acad Sci U S A 2007; 104: 3095-3100.
[126] Seirafi M, Kozlov G and Gehring K. Parkin structure and function. FEBS J 2015; 282: 2076-2088.
[127] Trempe JF, Sauvé V, Grenier K, Seirafi M, Tang MY, Meńade M, Al-Abdul-Wahid S, Krett J, Wong K, Kozlov G, Nagar B, Fon EA and Gehring K. Structure of parkin reveals mechanisms for ubiquitin ligase activation. Science 2013; 340: 1451-1455.
[128] Zhang X, Lin C, Song J, Chen H, Chen X, Ren L, Zhou Z, Pan J, Yang Z, Bao W, Ke X, Yang J, Liang Y, Huang H, Tang D, Jiang L and Liu J. Parkin facilitates proteasome inhibitor-induced apoptosis via suppression of NF-κB activity in hepatocellular carcinoma. Cell Death Dis 2019; 10: 719.

[129] Rouland L, Duplan E, Ramos Dos Santos L, Bernardin A, Katula KS, Manfioletti G, Idbaih A, Checler F and da Costa CA. Therapeutic potential of parkin as a tumor suppressor via transcriptional control of cyclins in glioblastoma cell and animal models. Theranostics 2021; 11: 10047-10063.
[130] Guan Y, Wang Y, Li B, Shen K, Li Q, Ni Y and Huang L. Mitophagy in carcinogenesis, drug resistance and anticancer therapeutics. Cancer Cell Int 2021; 21: 350.
[131] Yao N, Wang C, Hu N, Li Y, Liu M, Lei Y, Chen M, Chen L, Chen C, Lan P, Chen W, Chen Z, Fu D, Ye W and Zhang D. Inhibition of PINK1/Parkin-dependent mitophagy sensitizes multidrug-resistant cancer cells to B5G1, a new betulinic acid analog. Cell Death Dis 2019; 10: 232.
[132] Kuang J, Li L, Guo L, Su Y, Wang Y, Xu Y, Wang X, Meng S, Lei L, Xu L and Shao G. RNF8 promotes epithelial-mesenchymal transition of breast cancer cells. J Exp Clin Cancer Res 2016; 35: 88.
[133] Campbell SJ, Edwards RA, Leung CC, Neculai D, Hodge CD, Dhe-Paganon S and Glover JN. Molecular insights into the function of RING finger (RNF)-containing proteins hRNF8 and hRNF168 in Ubc13/Mms2-dependent ubiquitylation. J Biol Chem 2012; 287: 23900-23910.
[134] Bartocci C and Denchi EL. Put a RING on it: regulation and inhibition of RNF8 and RNF168 RING finger E3 ligases at DNA damage sites. Front Genet 2013; 4: 128.
[135] Zhang X, Chen J, Wu M, Wu H, Arokiaraj AW, Wang C, Zhang W, Tao Y, Huen MS and Zang J. Structural basis for role of ring finger protein RNF168 RING domain. Cell Cycle 2013; 12: 312-321.
[136] Lu CS, Truong LN, Aslanian A, Shi LZ, Li Y, Hwang PY, Koh KH, Hunter T, Yates JR 3rd, Berns MW and Wu X. The RING finger protein RNF8 ubiquitinates Nbs1 to promote DNA double-strand break repair by homologous recombination. J Biol Chem 2012; 287: 43984-43994.
[137] Dickens MP, Roxburgh P, Hock A, Mezna M, Kellam B, Vousden KH and Fischer PM. 5-Deazaflavin derivatives as inhibitors of p53 ubiquitination by HDM2. Bioorg Med Chem 2013; 21: 6868-6877.
[138] Zhu W, Zhan D, Wang L, Ma D, Cheng M, Wang H, Zhao J, Cai Y and Cheng Z. Proteasome inhibitor MG132 potentiates TRAIL-induced apoptosis in gallbladder carcinoma GBC-SD cells via DR5-dependent pathway. Oncol Rep 2016; 36: 845-852.