Chapter 9

Ubiquitination and DNA Repair in Multiple Myeloma

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http://dx.doi.org/10.5772/intechopen.70800

Abstract

Multiple myeloma (MM) is a hematological neoplasm characterized by the clonal proliferation of malignant plasma cells in the bone marrow. MM cells are characterized by genomic abnormalities that arise during the pathogenesis of disease and accumulate during progression. DNA repair pathways are critical to repair the plethora of DNA lesions that occur in MM, and deregulation of these pathways is implicated in disease onset and survival. The ubiquitin proteasome system has emerged as a central player in the regulation of DNA damage response (DDR). In this chapter, we review defects within the ubiquitin proteasome system that are associated with abnormal DNA damage response in MM and discuss current and potential novel ways of targeting these aberrations in the clinic.

Keywords: multiple myeloma, ubiquitin proteasome system, DNA repair, proteasome inhibitors

1. Introduction

Multiple myeloma (MM) is a malignancy characterized by the abnormal proliferation of plasma cells in the bone marrow. Plasma cells are terminally differentiated B cells that provide protective immunity through the production and secretion of antibodies. During their maturation, plasma cells undergo physiological DNA rearrangements to generate a diverse range of antibodies. This process involves chromosomal breaks and subsequent DNA repair. In addition to the intrinsic genomic instability of plasma cells, clonal MM cells are exposed to enhanced exogenous stresses such as replication and proteotoxic stress. Defective DNA repair pathways are implicated in the pathogenesis and survival of MM cells. The importance of post-translational modification with ubiquitin in the regulation of DNA repair pathways is being increasingly recognized. The ubiquitin proteasome system is an important therapeutic target in MM. This chapter provides an overview of ubiquitin signaling, describes genomic

instability in MM cells and defects in ubiquitin-mediated regulation of DNA repair pathways in MM, and discusses the impact of current and potentially novel therapeutic approaches in targeting these aberrations.

2. Ubiquitination

Ubiquitin is a highly conserved, 76-amino acid protein that is expressed in the cytoplasm and nucleus of all cells. Post-translational modification with ubiquitin, a process known as ubiquitination or ubiquitylation, is involved in the regulation of a wide range of cellular processes. Ubiquitin modification is an ATP-dependent process involving the sequential action of three classes of enzymes. An E1 (ubiquitin activating enzyme) activates ubiquitin through the formation of a thioester bond and transfers it to an E2 (ubiquitin conjugating enzyme), and an E3 (ubiquitin ligase) then mediates the transfer of the activated ubiquitin to a lysine (K) residue on a target protein, thereby forming an isopeptide bond between ubiquitin and the protein. There is one main E1 enzyme in eukaryotic cells, over 30 E2s and more than 600 E3s [1]. Ubiquitin can be attached to a target protein as a monomer or a polymer, resulting in different fates (**Figure 1**), determined largely by the pairing of E2s and E3s [2, 3].

At the simplest level, a single ubiquitin moiety is added to the ε-amino of a lysine residue on a substrate protein, in a process termed mono-ubiquitination. Mono-ubiquitination is typically involved in protein localization, complex formation, or altering the activity of the modified protein [4]. A single ubiquitin molecule can be conjugated to multiple lysine residues on a target protein, termed multi-monoubiquitination, and this modification is important for receptor endocytosis [5]. Proteins may also be modified by attachment of a chain of ubiquitin molecules in a process known as polyubiquitination. Ubiquitin contains seven lysine residues, at positions 6, 11, 27, 29, 33, 48, and 63, and a methionine group at position 1 (M1), that provide attachment sites for further ubiquitin molecules, thereby allowing the formation of polyubiquitin chains [6]. K48-linked polyubiquitination is the most widely studied ubiquitin chain and labels a protein for degradation through the proteasome. K63-linked polyubiquitination functions in mediating protein-protein interactions or conformational changes and plays an established role in regulating the DNA damage response (DDR) [7]. The other lysine linkages are less abundant and therefore less well characterized; however, most have been implicated to some extent with proteasome-mediated degradation or DNA repair [8, 9]. M1-linked ubiquitin chains, also referred to as linear ubiquitin chains, play a central role in inflammatory signaling cascades by regulating the activation of the transcription factor NFKB [10]. Mixed and branched ubiquitin chains have also been described; however, the function of these has not been fully delineated.

Like all post-translation modifications, the process of ubiquitination is reversible and ubiquitin removal is mediated by a large and diverse family of proteins known as deubiquitinating enzymes (DUBs). DUBs function predominantly to generate free ubiquitin from ubiquitin precursors and to edit or disassemble ubiquitin chains. The balance between ubiquitination and deubiquitination plays a critical role in regulating protein turnover and function.

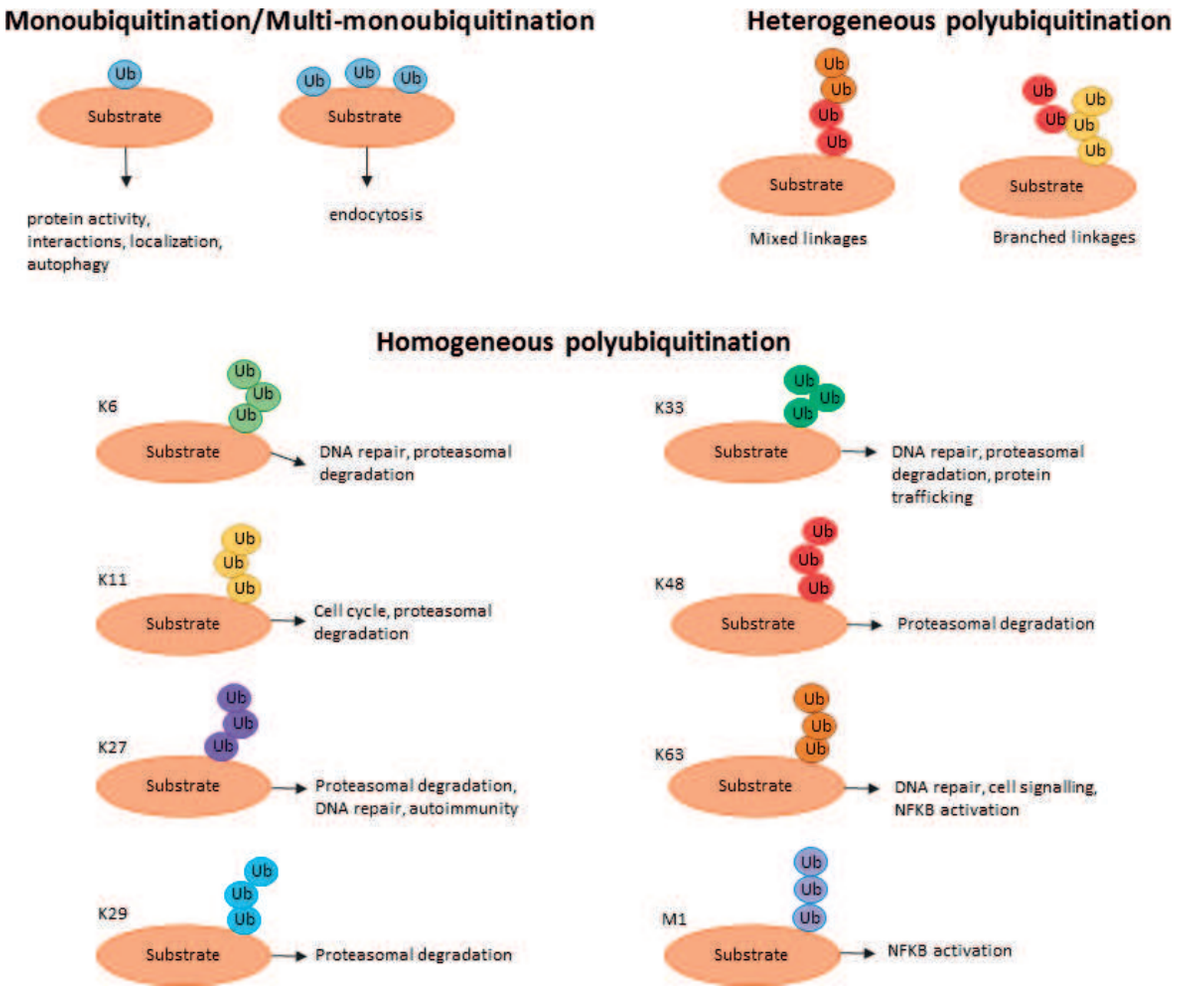

Figure 1. The ubiquitin code. An ubiquitin (Ub) molecule can be attached to a single site (monoubiquitination) or multiple sites (multi-monoubiquitination) on a substrate protein. In addition, ubiquitin contains seven lysine (K) residues and a methionine (M) residue that can support the assembly of homogenous or heterogeneous polyubiquitin chains.

2.1. The ubiquitin proteasome system

The ubiquitin proteasome system plays a central role in maintaining cellular protein homeostasis through the selective degradation of damaged, misfolded, and short-lived regulatory proteins that control essential cellular processes. The best-studied aspect of ubiquitination is the formation of a K48-linked polyubiquitin chain, which flags the target proteins for degradation through the 26S proteasome. The 26S or constitutive proteasome is a multicatalytic protease composed of two distinct subcomplexes: the 20S core particle and the 19S regulatory particle. The 19S regulatory particle is attached to one or both ends of the core particle and functions to recognize K48-linked polyubiquitin-tagged proteins, cleave and recycle ubiquitin, unfold the target protein, and feed it into the 20S proteolytic chamber for degradation. The 20S core particle is a barrel-shaped structure made up of 28 subunits arranged into four stacked rings. The two outer rings are composed of seven different α-subunits (α1–7), which serve as docking domains for the 19S regulatory caps.

Alpha subunits function as a gate controlling entry of substrates to the central chamber where proteolysis occurs. The two inner rings are composed of seven different β-subunits (β1–7), at least three of which contain catalytic sites, which are classified based upon preference to cleave after a particular amino acid residue. Chymotrypsin-like, trypsin-like, and caspase-like catalytic activities are associated with the β5, β2, and β1 subunits, respectively, due to their preference to cleave after hydrophobic, basic or acidic amino acid residues [11, 12]. An additional proteasome isoform known as the immunoproteasome also exists and is composed of an alternative set of catalytic beta subunits and regulatory cap. Constitutive subunits β5, β2, and β1 are replaced with subunits β5i (LMP7), β2i (MECL1), and β1i (LMP2), and the 19S regulatory cap is replaced with an 11S regulatory structure. These modifications allow the immunoproteasome to generate peptides for antigen presentation by major histocompatibility complex (MHC) class 1 molecules [13]. Immunoproteasomes are predominantly expressed in lymphoid tissues and hematopoietic cells, but can be formed in other cell types in response to stimuli such as interferon-γ and tumor necrosis factor-α.

3. Multiple myeloma

Multiple myeloma is a malignancy of plasma cells. It is almost always preceded by an asymptomatic premalignant stage called monoclonal gammopathy of undetermined significance (MGUS). An intermediate asymptomatic stage between MGUS and MM is referred to as smoldering MM (SMM). MM has historically been defined by four key features: hyper**c**alcemia, **r**enal failure, **a**nemia, and the presence of osteolytic **b**one lesions. These are commonly referred to as CRAB features. The International Myeloma Working Group revised these diagnostic criteria in 2014 to allow the inclusion of specific biomarkers and the addition of modern imaging tools to define MM bone disease [14]. The revised criteria made it possible to distinguish patients with SMM with high risk of progression to symptomatic MM. This facilitates the possibility of therapeutic intervention before end organ damage occurs and increases the possibility of success. Following diagnosis, patients can be further stratified into distinct prognostic subgroups using the revised international staging system (ISS) [15]. This algorithm builds on the original ISS (β2 microglobulin and serum albumin levels) and further includes cytogenetic abnormalities (outlined below) and serum lactate dehydrogenase.

Substantial progress has been made in the treatment of MM over the past decade. The approval of new types of biological agents, such as proteasome inhibitors and immunomodulatory drugs, has improved treatment options and led to improved overall survival [16]. The first-in-class proteasome inhibitor, bortezomib, was approved by the FDA for relapsed and refractory MM in 2003, and bortezomib-based combinations now form the backbone of many treatment regimens, across all stages of disease [17]. In addition, two second generation proteasome inhibitors, carfilzomib and ixazomib, have recently been approved highlighting the importance of the UPS as a therapeutic target for MM [18, 19].

4. Genomic instability in multiple myeloma

Genomic instability is a hallmark of MM and is associated with the evolution and progression of the disease [20]. Almost all patients display cytogenetic abnormalities including ploidy changes, deletions, amplifications, and chromosomal translocations. Based on initiating events, MM can be broadly categorized into hyperdiploid or nonhyperdiploid depending on the number of chromosomes present [21]. Hyperdiploid tumors, characterized by the presence of 48–75 chromosomes, make up approximately half of the MM cases and often have multiple trisomies of odd numbered chromosomes (3, 5, 7, 11, 15, 19, 21). Primary translocations involving the IGH locus on 14q32 with five recurrent partners are found in the majority (≥ 70%) of nonhyperdiploid MM cases and are associated with poorer overall survival. MM cells originate from postgerminal center B cells, which have undergone physiological DNA rearrangement during their maturation, including immunoglobulin (Ig) variable region rearrangement, somatic hypermutation, and Ig class switch recombination. Errors in these physiological processes can result in translocations that juxtapose one of five oncogenes [CCND1 (11q13), MMSET/FGFR3 (4p16), MAF (16q23), CCND3 (6p21), MAFB (20q11)] under the control of an immunoglobulin heavy (IGH) locus enhancer [22]. These initiating events are present in the majority of MGUS patients, and secondary genetic alterations, along with intraclonal heterogeneity, occur with increased incidence in disease progression from MGUS to MM [23]. These secondary events include further translocations, deletions, and chromosome gains, involving genes such as MYC, KRAS, NRAS, TP53, and NFKB-related genes (NIK, BIRC2/3, TRAF3), all of which are involved in DNA damage response and repair pathways. There is accumulating evidence eluding to deregulation of DNA repair pathways in MM as a mediator of the onset and progression of the disease, as well as survival. Changes in the expression of DNA repair genes have been suggested to play an important role in the pathogenesis of MM by leading to genomic instability and accumulation of genetic mutations [24]. In addition, ongoing DNA damage has been shown to intensify across the disease spectrum from MGUS to MM, thus providing a mechanism by which chromosomal abnormalities and tumor heterogeneity may be acquired in malignant plasma cells [25]. Furthermore, a number of studies report that alterations in DNA damage repair pathways are associated with poor prognosis in MM [26, 27].

5. DNA repair pathways

Cells are continuously challenged by DNA damage, induced through endogenous and exogenous sources. The ability to repair this damage is essential for the maintenance of genome integrity. Multiple proteins function together to detect and repair DNA damage, a process collectively termed the DNA damage response (DDR). There are six major DNA repair pathways involved in the DDR in mammalian cells. Base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) pathways repair nucleotide lesions on single strands. The BER pathway repairs small lesions induced by UV radiation, ionizing radiation, oxidative stressors, and alkylating agents [28]. The NER pathway removes bulky lesions

induced by UV light, environmental mutagens, and some chemotherapeutic agents [29]. The MMR pathway corrects replication errors such as base mismatches and insertion/deletion loops that occur during replication [30]. DNA double-strand breaks (DSBs), which are a more serious form of DNA damage, are generated by exogenous agents such as ionizing radiation or chemicals or endogenously by reactive oxygen species, replication of single-strand breaks, replication stress, or class switch recombination. DSBs can be repaired by two main pathways, homologous recombination (HR) and nonhomologous end joining (NHEJ). HR takes place in late S- and G2-phases of the cell cycle and corrects DSBs in an error-free manner using an undamaged sister chromatid as a homologous DNA template [31]. NHEJ can take place in all phases of the cell cycle and repairs DSBs in an error-prone manner by direct ligation of the two broken ends [32]. Interstrand crosslinks (ICL), which are covalent links between two opposite strands of DNA, are induced by endogenous metabolites and exogenous chemicals such as alkylating agents [33]. The Fanconi anemia (FA) pathway detects ICL and repairs ICL lesions in co-operation with NER and HR pathways [34].

6. Ubiquitin-mediated DDR signaling in MM

All DDR pathways are tightly controlled by reversible post-translational modifications, including ubiquitination and de-ubiquitination, which act to regulate protein stability, localization, and activity. Deregulation of these DNA repair pathways can lead to genomic instability and promote tumorigenesis. Aberrant ubiquitin-mediated signaling of DNA damage repair in MM is summarized below.

6.1. DSB repair

Nonproteolytic ubiquitination of histones at the chromatin surrounding DSBs is a key step in DDR activation. Two E3 ubiquitin ligases, RING finger 8 (RNF8) and RNF168, are critical mediators of DSB repair. DSBs trigger activation of the ataxia-telengiectasia mutated (ATM) kinase, which phosphorylates histone H2AX (referred to as γ-H2AX) and mediator of DNA damage checkpoint protein 1 (MDC1). Phosphorylated MDC1 recruits RNF8, which promotes K63-linked polyubiquitination of H1 linker histones at DSB sites. This in turn recruits RNF168, leading to mono-ubiquitination of H2A-type histones at K13 and K15. These modifications provide a platform for binding of two essential effectors of the DDR to the DSB site, p53 binding protein 1 (53BP1), and breast cancer 1 (BRCA1). 53BP1 and BRCA1 have key roles in DSB repair pathway choice: BRCA1 promotes the HR pathway, whereas 53BP1 facilitates repair through the NHEJ pathway. A number of studies have reported deregulation of DSB repair in MM. Walters and colleagues demonstrated that H2AX is constitutively phosphorylated in MM, leading to constitutive activation of DSB repair pathways [25]. Consistent with this, elevated activity of both HR and NHEJ repair pathways has been observed in MM [35]. Furthermore, upregulation of NHEJ pathway-related gene expression is significantly associated with poor overall survival in MM [36]. This upregulation of DSB repair pathways likely contributes to the inherent genomic instability of malignant plasma cells with consequences for disease progression and acquisition of drug resistance.

Increased NHEJ, in particular, is associated with frequent chromosome aberrations and translocations that may contribute to tumor heterogeneity. RNF168 plays a crucial role in the recruitment of 53BP1 to sites of DNA damage by both recruiting 53BP1 and removing competing proteins from sites of DNA damage. Overexpression of RNF168 in tumor cells has been found to alter the DSB DNA repair response by shifting the balance from HR to NHEJ [37]. Proteotoxic stress, arising due to aneuploidy, copy-number variations, and transcriptional alterations, is an emerging hallmark of cancer cells. Previous studies have reported aberrant ubiquitin-mediated signaling of DNA damage under proteotoxic stress, whereby there is a depletion of free ubiquitin available for ubiquitin-dependent aspects of the DSB response, as a consequence of accumulating ubiquitinated substrates. However, a recent study identified a subset of cancer cell lines overexpressing RNF168 that could preferentially exploit the residual free ubiquitin to recruit 53BP1 and activate the NHEJ pathway [37]. Proteotoxic stress is particularly prominent in immunoglobulin-producing myeloma cells, and therefore, using MM cell lines as a model, this altered DSB response was found to be even more pronounced in MM cells, correlating with higher expression of RNF168. Furthermore, upregulation of RNF168 was found to influence the response of tumor cells to cancer therapies. RNF168-high tumors exhibit increased resistance to ionizing radiation under conditions of enhanced proteotoxic stress. However, this phenotype and associated alterations in DSB repair pathways render cells sensitive to topoisomerase and poly-ADP-ribose-polymerase (PARP) inhibitors.

6.2. The Fanconi anemia pathway

The FA pathway is a DNA damage activated pathway required for the repair of ICLs. ICLs are covalent bonds between two strands of DNA leading to a block in DNA replication and translation. Ubiquitination plays a pivotal role in the regulation of ICL repair by the FA pathway. In response to ICLs, a complex of eight FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) along with two associated proteins, FANCA-associated polypeptide 24 (FAAP24) and FAAP100, forms a multisubunit E3 ligase complex required for the monoubiquitination of FANCD2 on K561 and FANCI on K523. Monoubiquitinated FANCD2-FANCI is recruited to sites of DNA damage, where as a complex, they co-localize with BRCA1 and RAD51 to co-ordinate downstream reactions including nucleolytic incision, translesion synthesis (TLS), and DSB repair. Nucleolytic incisions unhook the ICL, creating a DSB and translesion synthesis that allows the bypass of unhooked crosslinked oligonucleotides. The DSB is then repaired by HR and remaining adducts are excised by the NER repair pathway [38, 39].

The FA pathway plays a key role in the cellular response to alkylating agents. The DNA alkylating agent, melphalan, is a commonly used chemotherapeutic agent in MM therapy. The majority of MM patients are initially sensitive to alkylating agents such as melphalan but inevitably acquire resistance, leading to disease progression. Studies into the mechanisms of resistance to melphalan in MM identified an increase in expression of genes coding for FA and HR pathways along with enhanced ICL repair and decreased DNA damage [40, 41] in melphalan-resistant cells. Further studies revealed a role of the NFKB pathway as a regulator of the FA pathway in response to melphalan-induced DNA damage [42]. The NFKB pathway is frequently dysregulated in MM and plays a central role in survival, proliferation, and resistance of MM cells to anticancer therapies. NFKB subunits RelB/p50 are transcriptional

activators of the FA pathway through binding to the FANCD2 promoter. Chronic exposure of MM cells to melphalan resulted in an increased NFKB activity and associated FA pathway activity in MM cell lines and patient cells. Inhibition of the NFKB pathway, both using a proteasome inhibitor or a selective NFKB inhibitor, results in decreased expression of FA pathway genes in melphalan-sensitive and melphalan-resistant cell lines [42].

7. Targeting ubiquitin-mediated DDR signaling in MM

The UPS is recognized as an important therapeutic target in MM. Proteasome inhibitors are a principal component of current anti-MM therapy, and there is an increasing interest in targeting other parts of the UPS. An overview of current and novel UPS drugs that act directly on DNA repair pathways is given below and in **Figure 2**.

7.1. Proteasome inhibitors

Proteasome inhibition has emerged as a powerful strategy to treat MM. Since its introduction into the clinic in 2003, mechanisms underlying the therapeutic effect of the first-in-class proteasome inhibitor, bortezomib, have been widely investigated. One of the first mechanisms of action attributed to bortezomib was the inhibition of the inflammation-associated transcription factor nuclear factor kappa B (NFKB), a key signaling molecule in MM, through stabilization of its inhibitor IKB [43]. Initial studies also suggested that the unique sensitivity of MM cells to bortezomib was largely related to their high proteasome load, with inhibition of the proteasome resulting in an increased endoplasmic reticulum (ER) stress and a prolonged activation of the unfolded protein response (UPR) [44]. In addition, bortezomib was found to promote apoptosis through upregulation of proapoptotic proteins and induce cell cycle arrest through stabilization of cyclin-dependent kinase inhibitors [45]. Furthermore, bortezomib was found to exert an effect on the protective bone marrow microenvironment through inhibition of the angiogenic factor vascular endothelial growth factor (VEGF) and decreased binding of MM cells to bone marrow stromal cells [46]. Later studies found that proteasome inhibitors impair the DDR in MM in a number of ways [42, 47–50].

As mentioned previously, proteotoxic stress can impact ubiquitin-mediated DDR pathways through depletion of available nuclear ubiquitin. Inhibition of proteasome activity exacerbates endogenous proteotoxic stress in MM by preventing degradation of polyubiquitinated proteins, leading to a reduction in the amount of free ubiquitin in the cell [47, 48]. This results in a loss of ubiquitination at sites of DSBs and consequently impairs DSB repair. Bortezomib has been shown to alter HR by abrogating K63 polyubiquitination of H1 histones and subsequently impairing recruitment of BRCA1 and RAD51 [49]. Proteasome inhibition also leads to a reduction in FANCD2/FANCI monoubiquitination, thereby blocking a critical step in the FA pathway [42]. Along with altered ubiquitin signaling, proteasome nuclear activity is important for DSB repair. Proteasomes are recruited to sites of DSBs to degrade key regulatory proteins. Inhibition of proteasome activity blocks degradation of MDC1 at DSB sites, thereby inhibiting recruitment of BRCA1 in an RNF8 independent manner. Furthermore, proteasome inhibition blocks NFKB-mediated activation of the FA pathway [50].

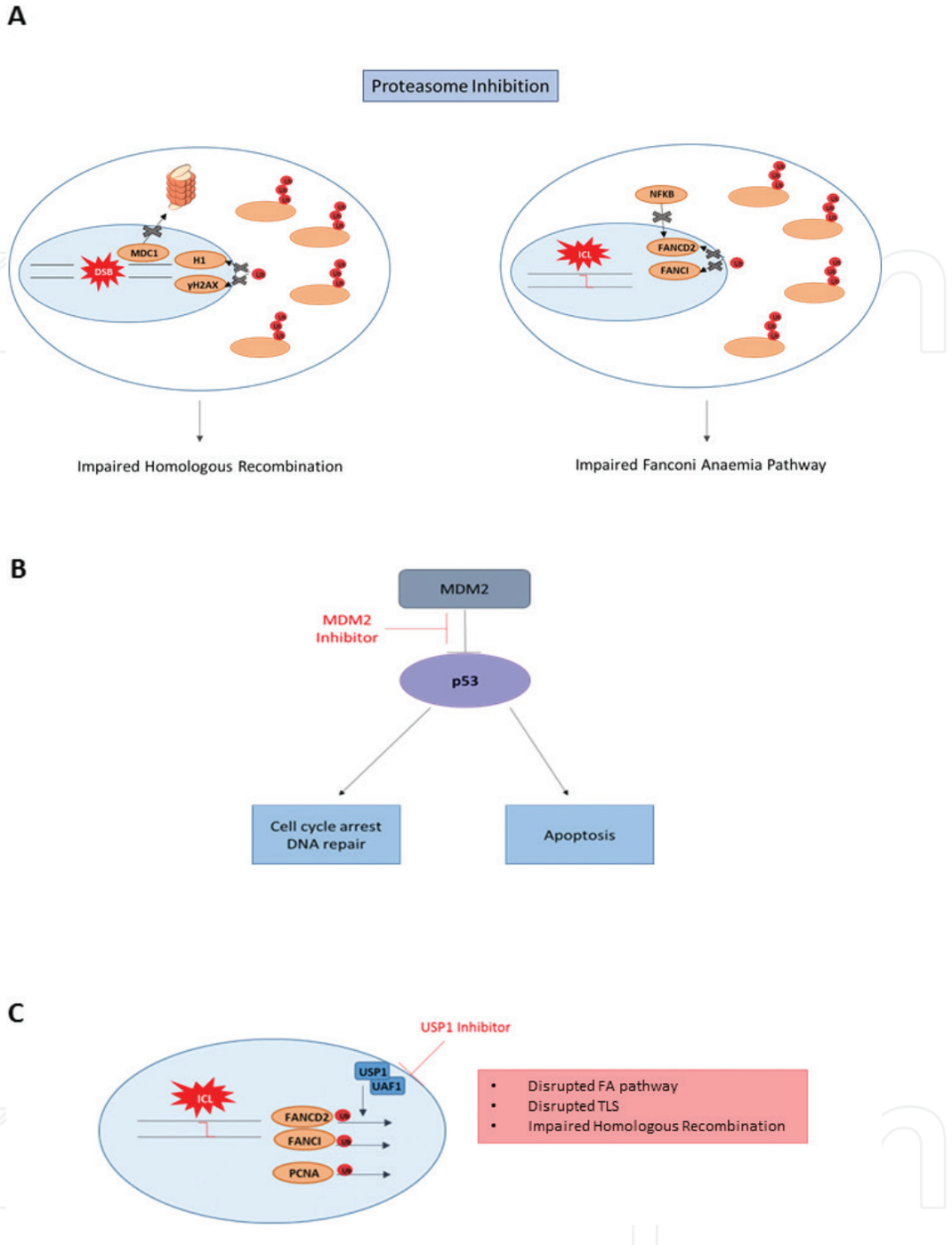

Figure 2. Targeting ubiquitin-mediated DNA damage repair signaling in MM. (A) Proteasome inhibitors prevent degradation of polyubiquitinated proteins leading to a depletion of available nuclear ubiquitin. Proteasome inhibition leads to reduced ubiquitination of H1 histones and γH2AX and reduced degradation of mediator of DNA damage checkpoint protein 1 (MDC1), resulting in impaired repair of double-strand breaks (DSBs) through homologous recombination. Proteasome inhibition disrupts repair of interstrand crosslinks (ICLs) by the Fanconi anemia (FA) pathway through reduced ubiquitination of FANCD2 and FANCI and a block in nuclear factor kappa B (NFKB)-mediated activation of the FA pathway. (B) In response to DNA damage, p53 is activated to induce cell cycle arrest to allow DNA repair or to induce apoptosis of a damaged cell. Mouse double minute 2 (MDM2) inhibitors block the interaction of p53 and its endogenous inhibitor MDM2 to stabilize p53 levels and increase its activity. (C) Ubiquitin-specific protease 1 (USP1) along with its binding partner USP1-associated factor (UAF1) regulate the FA pathway and translesion synthesis (TLS) through the de-ubiquitination of FAND2, FANCI, and PCNA. Inhibition of USP1 leads to a disruption of the FA pathway, TLS, and impaired homologous recombination.

Bortezomib was initially approved as a single agent; however, its predominant use is in combination therapies with steroids and/or standard chemotherapy drugs. Given its effect on DSB repair, it is not surprising that bortezomib is reported to sensitize MM cells to DNA damage inducing chemotherapeutics. In preclinical studies, bortezomib was found to both sensitize tumor cells to conventional DNA damaging agents, doxorubicin and melphalan, and to overcome resistance to these therapies [51]. Furthermore, bortezomib demonstrates clinical efficacy in combination with doxorubicin, melphalan, and cyclophosphamide [52]. Finally, bortezomib has been shown to act in synergy with PARP inhibitors by blocking HR, resulting in marked cell death [49].

7.2. p53 as a therapeutic target

The tumor suppressor p53 plays an important role in maintaining genomic stability. Under normal conditions, p53 levels are low due to rapid degradation through the UPS. In response to DNA damage, p53 is stabilized and acts to halt cell division and allows repair of DNA lesions prior to DNA synthesis. The E3 ubiquitin ligase mouse double minute 2 (MDM2) homolog is the most prominent E3 ligase involved in the negative regulation of p53 [53]. MDM2 can regulate p53 with both mono- and polyubiquitination to regulate both its stability and cellular location. Monoubiquitination of p53 triggers nuclear export, whereas K48-linked polyubiquitination targets p53 for proteasomal degradation. Overexpression of MDM2 has been reported in a number of malignancies, including MM, and can act to suppress p53 levels, even under stress conditions [54]. Numerous pharmacological approaches have been developed to disrupt MDM2-p53 binding, thus stabilizing p53 levels and increasing p35 activity. The nutlins were the first small molecule inhibitors of MDM2 to be developed and have been demonstrated to stabilize p53 and its substrates, resulting in increased apoptosis and cell cycle arrest in MM cell lines and primary cells [55]. Similar effects have been reported with a number of other MDM2 inhibitors. In addition to targeting MDM2 directly, another promising approach is to inhibit the deubiquitinating enzyme ubiquitin-specific protein 7 (USP7). USP7 deubiquitinates and stabilizes levels of MDM2. Expression of USP7 is elevated in MM and small molecule inhibition of this DUB leads to decreased levels of MDM2 and accumulation of p53 [56]. Early phase clinical trials are in preparation for two MDM2 inhibitors in MM (AMG-232, NCT01723020; DS-3032b, NCT02579824), highlighting the potential of disrupting the DDR through inhibition of this E3 ligase in MM.

7.3. USP1 as a therapeutic target

USP1 is the most widely characterized DUB known to be involved in the DNA damage response. USP1, along with its binding partner USP1-associated factor (UAF1), is important for the regulation of the FA pathway and translesion synthesis (TLS) [57, 58]. Monoubiquitination of FANCD2-FANCI directs this complex to DNA damage foci to activate the FA repair pathway. USP1 in conjunction with UAF1 is the DUB responsible for deubiquitinating FANCD2 and FANCI. USP1-UAF1 is also responsible for the deubiquitination of proliferating cell nuclear antigen (PCNA), a central regulator of TLS. Elevated expression of USP1 has been reported in MM and is associated with poor prognosis. A recent study demonstrated that both siRNA

knockdown and small molecule inhibition of USP1 in MM cells results in increased levels of ubiquitinated FANCD2, FANCI and PCNA and decreased RAD51 formation, ultimately leading to inhibition of the FA pathway and HR [59]. Inhibition of USP1 was also found to trigger synergistic cytotoxicity with a number of MM therapies highlighting its potential as a therapeutic agent in MM.

8. Concluding remarks

Chromosomal translocations and genetic abnormalities are a hallmark of MM, contributing to the initiation and progression of the disease. Genomic instability is largely beneficial to MM cells by providing a growth advantage or contributing to drug resistance; however, an understanding of the mechanisms driving this can create therapeutic opportunities to exploit vulnerabilities within the malignant cells. Alterations in DNA damage repair pathways are implicated as one mechanism contributing to genomic instability. The UPS plays a central role in the regulation of DNA damage repair through ubiquitination and degradation of key proteins. The UPS is already recognized as an important therapeutic target in MM, through the clinical success of proteasome inhibitors. Proteasome inhibitors have recently been demonstrated to impair HR and FA DNA repair pathways, leading to increased sensitivity of MM cells to a number of DNA damage-inducing agents. In addition to the proteasome, there is mounting interest in the therapeutic potential of targeting ubiquitination and deubiquitination enzymes. A number of these enzymes involved in regulating DDR are also deregulated in MM. Expression of the E3 ligase RNF168 in MM has implications for treatment response to DNA damage-inducing agents in MM, whereas the E3 ligase MDM2 and DUBs USP7 and USP1 are under investigation as therapeutic targets. As our knowledge of the role of the UPS in regulating DNA damage repair increases, it is likely that further opportunities for targeted therapies will emerge.

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