Chapter 2

The Five Families of DNA Repair Proteins and their Functionally Relevant Ubiquitination

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Additional information is available at the end of the chapter

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Abstract

The process of DNA repair, be it a response to replication dysfunction or genotoxic insult, is critical for the resolution of strand errors and the avoidance of DNA mismatches that could result in various molecular pathologies, including carcinogenic development. Here, we will describe the five main mechanisms by which DNA avoids mutation, namely the processes of base excision repair, mismatch repair, nucleotide excision repair, homologous recombination, and nonhomologous end joining. In particular, we will dissect the functional significance of various posttranslational modifications of the essential proteins within these pathways, including but not limited to ubiquitination, acetylation, and phosphorylation.

Keywords: base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), homologous recombination (HR), nonhomologous end joining (NHEJ), posttranslational modification

1. Introduction

The mammalian genome is under constant barrage by exogenous and endogenous insult that can beget damage and instability. Exogenous insults include exposure to UV radiation and chemical carcinogens found in the environment, while endogenous factors include ROS produced by cellular metabolism, spontaneous chemical reactions like base deamination and mistakes made during the replicative process. It is critical to the survival of the organism that each cell have the ability to resolve the damage induced by this wide variety of insults, and that the machinery responsible for responding to damage must be equally diverse.

There are five main mechanisms responsible for repairing damaged DNA, and their conservation from bacteria all the way to humans exemplifies their critical role in the maintenance of

an organism's genome. These mechanisms consist of base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, and nonhomologous end joining.

2. Ubiquitination and the proteasome degradation pathway

The ubiquitin proteasome pathway (UPP) is a mechanism used for the maintenance of proper levels of cellular proteins and the destruction of old or misfolded proteins by targeting them for degradation. This targeting comes in the form of ubiquitination, the process of covalently linking a polyubiquitin chain to the protein that is recognized and bound by the 26S proteasome, which degrades the protein and releases the ubiquitin. Ubiquitin is a highly conserved 76-amino acid protein that serves as the subunit of the polyubiquitin chain. Ubiquitin is covalently linked to its target in a three-step cascade conducted by a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-ligating enzyme (E3) [1]. Apart from protein degradation, ubiquitination can also mediate protein-protein interaction.

3. Detection and repair within the DNA strand

3.1. Base excision repair

Base excision repair enzymes are responsible for correcting lesions induced by a wide variety of both endogenous and exogenous insults, including sites of base loss, nonbulky base lesions, and DNA single-strand breaks (SSBs) [2]. DNA glycosylases are responsible for the first step of base excision repair (BER) by initially detecting the damage and excising the base via hydrolyzing the N-glycosylic bond linking the DNA base to the sugar phosphate backbone. This process generates an abasic site (AP site) that AP endonuclease 1 recognizes and acts upon by cleaving the phosphodiester bond 5′ to the AP site, leaving a SSB with a 5′-sugar phosphate. A DNA repair complex composed of DNA pol β, XRCC1, and DNA ligase IIIα can recognize this SSB and remove the 5′-sugar phosphate through its AP lyase activity, and add a single nucleotide to the 3′-end through its DNA polymerase activity. The damage is finally resolved when Lig 3 seals the DNA ends together, thus completing what is referred to as short patch BER, the process by which human cells conduct the majority of their BER [3, 4].

At the moment, much of the work focusing on ubiquitination of the proteins involved in BER has been pursued by Dianov et al. [5]. This group has been able to demonstrate that, under normal conditions, BER components are targeted for destruction by the E3 ubiquitin ligase CHIP. When DNA damage occurs in cells, the BER components undergo stabilization to increase their ability to correct the damage. Specifically, pol β, XRCC1, and DNA ligase III are polyubiquitinated by CHIP and Mule when not bound to chromatin, and thus targeted for degradation [6].

DNA pol λ can also be targeted by posttranslational modifications. Pol λ contains four distinct phosphorylation sites, but phosphorylation of the Thr553 has the strongest impact on the stability of the protein. Pol λ can be phosphorylated on all of these sites by the Cdk2/cyclin A

complex, but its levels of phosphorylation are reduced when it is interacting with proliferating cell nuclear antigen (PCNA) [7], a sliding clamp that associates with DNA polymerases and ensures accurate and possessive DNA synthesis [8]. Increased phosphorylation of Thr553 on pol λ positively correlates with its protein levels in the cell, likely due to the fact that phosphorylation at this site protects pol λ from ubiquitination and degradation. This stabilization occurs in the late S and G2 phase. Pol λ is closely related to pol β, and indeed both polymerases can be ubiquitinated by CHIP and Mule. It is thought that pol λ is needed in late S and G2, specifically whenever oxidative DNA damage presents at this phase and induces 8-oxo-G lesions [9, 10]. When these lesions occur, it is Mule that is responsible for regulating protein levels of pol λ. When Mule is able to ubiquitinate pol λ, this action targets both pol λ for degradation and decreases its enzymatic activity. Mule is responsible for the monoubiquitination of pol β, which can be further polyubiquitinated by CHIP and targeted for degradation [11]. While pol β is continuously expressed in unstressed cells, it is almost immediately targeted by Mule and CHIP in the absence of damage. Upon DNA damage detection, alternative reading frame (ARF) begins to accumulate and eventually inhibits Mule activity [12], allowing for pol β accumulation and activation of further BER proteins. Once the lesion(s) have been resolved ARF levels drop, Mule activity is restored, and pol β will once again be ubiquitinated and degraded. ARF is a BER protein frequently mutated in cancer cells; it functions by responding to DNA damage by directly inhibiting Mule, as well as regulating p53. The amount of ARF produce in response to DNA damage is dependent on the extent of the damage; and by inhibiting Mule activity, it allows p53 to halt replication while pol β complexes conduct repair [13]. Without ARF, both Mule and Mdm2 repress p53 activity. ARF is a 482 kDa protein belonging to the homologous to E6-AP carboxyl terminus (HECT) family of E3 ubiquitin ligases [14], named such due to their ubiquitous presence of a C-terminal HECT domain of ~350 amino acids that house their E3 catalytic activity. The HECT domain of Mule contains two subdomains connected by a flexible linker allowing these domains to undergo ubiquitin chain transfer [15]. Aside from allowing for p53 accumulation and activation of the DNA damage response in damaged cells, ARF also plays a p53-independent role in tumor suppression due to its ability to induce proliferation delay in cells lacking functional p53 and p21 [16, 17].

3.2. Nucleotide excision repair

Nucleotide excision repair is a process undertaken in both prokaryotes and eukaryotes to enzymatically remove bulky, helix-distorting base adducts from DNA. This process is the predominant method of DNA repair in mammals, especially when resolving damage induced by ultraviolet light from the sun. About 30 proteins are involved in eukaryotic nucleotide excision repair (NER), including nine major proteins identified by their mutation in humans and the development of UV-hypersensitivity as a result. Seven of these proteins, when mutated, lead to the development of Xeroderma pigmentosum syndrome (XPA to XPG) and two lead to the development of Cockayne's syndrome (CSA and CSB) [18]. Additional players in the process of NER include excision repair cross-complementing 1 (ERCC1), replication protein A (RPA), and Rad23 homologs (HR23A and HR23B) [19, 20]. The Rad23 homologs share redundancy with the function of Rad23 in yeast during the recognition of the lesion in NER. Upon initial recognition of a lesion, eukaryotic NER can continue by either the process of global

genome NER (GG-NER) or transcription-coupled NER (TC-NER). GG-NER removes DNA from untranscribed regions of DNA; XPC-HR23B and UV-DDB (damaged DNA-binding protein) can recognize UV damage and recruit XPA to this resultant lesion [21]. In TC-NER, RNA polymerase II recognizes the lesion when it is a mediating transcription but finds its progress blocked by the break. This stalling of RNA pol II is recognized by CSA and CSB, which will localize to the lesion and load XPA on the site to initiate NER. After initial lesion recognition, GG-NER and TC-NER follow the same pathway to resolve the damage. XPA further recruits XPB (5′ to 3′ helicase) and XPD (3′ to 5′ helicase) to unwind the DNA at the damage site and allow for incisions on the 3′ and 5′ sides of the gap to be made by XPG and XPF-ERCC1 endonucleases, respectively [22–24].

The first association between the ubiquitin proteasome pathway (UPP) and nucleotide excision repair (NER) was due to the identification of the ubiquitin-like domain present at the N-terminus of Rad23 [25, 26] that can serve as a ubiquitin receptor, similar to the subunit of the 26S proteasome Rpn1 [27]. Both can recognize polyubiquitinated chains and transport the target proteins to the proteasome [28]. The Rad23 ubiquitin-like domain is required for sufficient NER activity, and deletion of this domain can result in UV radiation sensitivity [29]. Russell et al. demonstrated that complete inhibition of the proteasome does not affect NER, while specifically targeting 19S activity does. Further, 19S influence on NER is mediated by the Ubl domain of Rad23, suggesting to them that 19S may be acting as a molecular chaperone in the context of NER by altering the conformation of certain NER proteins [29, 30]. Rad23 avoids proteasomal degradation due to its ubiquitin-like domain via a C-terminal ubiquitin-associated (UBA) domain [31] and can impart this protection on its binding partner XPC in a mechanism that will be detailed later.

XPE-deficient cells lack the ability for UV-damaged DNA-binding component (DDB), composed of DDB1 (p127) and DDB2 (p48), to bind DNA. DDB2 and CSA are present in separate but nearly identical molecular complexes, both associated by their interaction with DDB1 [32]. Both complexes contain CUL4A and ROC1, both ubiquitin ligase subunits, as well as the constitutive photomorphogenesis 9 (COP9) signalosome (CSN). When the complex is devoid of CSN, they are able to display robust ubiquitin ligase activity. After UV exposure, CSN rapidly dissociates from the DDB2 complex and CUL4A is modified by NEDD8 (via neddylation and polyubiquitination) [33], leading to ubiquitin ligase activity from the complex. This complex ubiquitinates XPC (which is bound to HR23, the specifics of this complex detailed later), allowing both of these complexes to bind the damaged DNA. DDB2 itself is also polyubiquitinated, causing it to dissociate from the complex, and get degraded by the proteasome. Ubiquitinated XPC and HR23 remain on the DNA, where they activate the process of NER. The CSA complex is not as well characterized as the DDB2 complex. What is known is that unlike the DDB2 complex, UV-induced damage stimulates the rapid association of CSN with CSA, suppressing all ubiquitin ligase activity from the complex. A target of DDB2 complex ubiquitination is XPC, which is required for GG-NER at the damage site [34]. In undamaged cells, XPC exists in a heterotrimeric complex with either mammalian homolog of Rad23, HR23A, or HR23B. XPC is normally bound to HR23B, but in its absence HR23A is sufficient [35, 36]. This XPC complex recognizes physical aberrations in the structure of DNA rather than the lesions themselves, and is recruited after ubiquitination by the DDB2 complex.

The ubiquitination appears to be protective, as XPC is not a target of proteosomal degradation and is further stabilized through its interaction with the ubiquitin-associated (UBA) domain of the HR23 protein it is bound to [37, 38]. It is of great interest that both DDB2 and XPC are ubiquitinated and this ubiquitination yields drastically different outcomes, yet there are still parts of this mechanism that have not been defined. The specific ubiquitination sites on these two proteins have not been mapped, and the factors that specifically interact with these two proteins upon ubiquitination have yet to be defined.

Returning to the CSA complex, after UV exposure, it rapidly associates with CSN and its ubiquitin ligase activity is suppressed. This action has implications on the function of RNA polymerase II, which stalls on DNA strands during transcription when it encounters a break or adduct (any transcriptional blockade) and signals for the assembly of TC-NER machinery. Reports have indicated that UV exposure can activate CSA- and CSB-dependent polyubiquitination of RNA pol II [39], an observation that contrasts with the previously discussed reports of CSN inhibiting the CSA complex's ubiquitin ligase activity. Groisman et al. have suggested that CSN can differentially regulate the activity of DDB2 and CSA complexes, and that its interaction with CSA may not in fact be inhibitory [32]. It is also possible that there is an additional member of the CSA complex, or a separate complex is mediating ubiquitination of RNA pol II. Svejstrup et al. have argued that RNA pol II ubiquitination is conducted by a Rad26-Def complex [40]. Def1 is a protein discovered in yeast that complexes with Rad26 on chromatin, and when this protein is deleted in yeast, these cells are unable to degrade stalled DNA pol II in response to DNA lesions [40]. RNA pol II stalling has been reported to induce ubiquitination and degradation of Rpb1, the largest RNA pol II subunit, in a Def1-dependent manner [41]. When RNA pol II is polyubiquitinated after UV-induced damage (an additional E3 ligase is BRCA1/BARD1 of the homologous recombination pathway), it is either degraded or bypasses the transcriptional block, allowing mRNA synthesis to continue [42] and the damage is to be resolved later by GG-NER [43].

UV radiation has often been used to elucidate the mechanisms of NER components, as helix-distorting damage (cyclobutane pyrimidine dimers, 6-4 photoproducts) is repaired by NER [44]. These studies have also revealed the posttranslational modifications necessary for the functional relevance of these proteins. UV radiation experiments were responsible for the initial observation that genes encoding certain components of the UPP influenced the ability of cells to survive after being irradiated, and the researchers interpreted this data in a manner that highlights the proteolytic activity for the proteasome in NER [45–47]. After these initial observations, Rad23 was investigated and determined not to be targeted for ubiquitination, and Rad4 (yeast homolog of XPC) became the next potential target for ubiquitination. This focus was based on the observation that Rad4 overexpression can increase NER activity [47]. Further studies in human cells revealed that XPC also accumulated after DNA damage, and like their yeast counterparts, increased NER activity [37]. This accumulation was correlated to hHR23 in mouse cells, and it was found that Rad23 could use its UBA domains to stabilize Rad4/XPC by acting in trans [48] as well as controlling its own turnover by acting in cis [49]. The C-terminal tail of H2A is a target for posttranslational modification, with as much as 5–15% of H2A being monoubiquitinated in mammals [50]. Ubiquitinated H2A is associated with condensed DNA and gene repression, and Ring2 is the predominant

E3 ubiquitin ligase responsible for this modification [51, 52]. UV-induced DNA damage can induce monoubiquitination of H2A in close proximity to the lesions [53] in a manner very similar to its phosphorylation. Both of these histone modifications occur in UV treated, non-S-phase cells and are dependent on functional NER, and ATR signaling is required for the tail modification to occur [54, 55]. Ubiquitination of XPC, DDB2, and PCNA can still occur in NER-deficient XP-A cell lines, but H2A ubiquitination relies upon NER-sufficiency. Ubc13 and RNF8 are responsible for perpetuating sustained H2A ubiquitination so that NER can occur, but do not initially ubiquitinate H2A [56].

3.3. Mismatch repair

The DNA mismatch repair pathway is responsible for correcting mispaired nucleotides and insertion/deletion loops (IDLs) that are a consequence of replication, recombination, and repair errors [57].

The role of ubiquitination in the process of mismatch repair is relatively uncharacterized, compared with the rest of the DNA repair pathways detailed in this chapter. However, research conducted in our lab has identified that the stability of MutS protein homolog 2 (MSH2), an essential DNA mismatch repair protein, is regulated through ubiquitination by histone deacetylase 6 [58]. Ubiquitination of MutSα was first reported by Lautier et al. [59], although the enzyme responsible remained undetermined until our 2014 publication [58]. MSH2 forms two heterodimers, MSH2-MSH6 (MutSα) and MSH2-MSH3 (MutSβ). MutSα recognizes single base mismatches and 1-2 nucleotide insertions and deletions [60] while MutSβ recognizes bulky DNA adducts and larger insertions and deletions [61]. MutSα specifically recognizes DNA lesions induced by a wide variety of DNA-damaging agents (6-thioguanine, cisplatin, doxorubicin, etoposide) [62]. In the absence of MutSα, cells display resistance to these DNA-damaging agents and do not undergo apoptosis as a result of a futile repair cycle [63, 64]. Elucidation of the mechanism of MSH2 stability in cells is critical to the field of mismatch repair, as the initiation of MMR is controlled by the binding of MutSα and MutSβ to the mispair. These proteins subsequently signal the downstream effectors of MMR; MutLα (MLH1-PMS2), PCNA, and RPA, which can further lead to the recruitment of excision protein exonuclease 1 (EXO1). EXO1 excises the mismatched base, forming a gap that is filled by polymerase δ and a nick that is resolved by DNA ligase 1. When MSH2 is acetylated, it cannot be ubiquitinated, and thus is retained and is able to form MutSα and Mutsβ complexes. MSH2 turnover can be induced by HDAC6 activity, which subsequently deacetylates and ubiquinates MSH2 to target it for proteosomal degradation. This action is possible because of the E3 ubiquitin ligase activity HDAC6 possesses in its DAC1 domain (HDAC6 has two active sites: DAC1 and DAC2). HDAC6 can target MSH2 even when it is in its heterodimeric complex; MSH2 deacetylation causes it to dissociate from its stabilizing partner MSH6 [65], and as a free monomer MSH2 can be ubiquitinated [58]. MSH2 can be acetylated at four lysine residues (K845, K847, K871, and K892), and all of these sites can also be ubiquitinated. MSH2 can be protected from ubiquitination and degradation by protein kinase C (PKC), which can phosphorylate the MutSα complex [66].

Further research out of our lab has indicated that ubiquitin-specific peptidase 10 (USP10) also plays a role in MSH2 stability, but rather than targeting it for degradation like HDAC6, USP10 is responsible for stabilizing MSH2 by deubiquitinating it [67]. USP10 has recently been identified as a regulator of p53 in response to DNA damage in a tumor development context [68–70]; ATM phosphorylation of USP10 induces its translocation to the nucleus, where it stabilizes p53. However, we now know that USP10 can work in opposition to HDAC6 by interacting with the N-terminal region of MSH2, while HDAC6 interacts with the C-terminal region. Under stress conditions (IR, carcinogen treatment), USP10 phosphorylation is increased [68] suggesting enhanced translocation to the nucleus where it may increase stabilization of the MutSα complex.

MMR can respond to endogenous insult to genomic integrity as well as exogenous. Oxidative DNA damage, for example, can induce MutSα-dependent PCNA ubiquitination, a process dependent on the PCNA E3-ubiquitin ligase RAD18 [71] in a process of noncanonical MMR (ncMMR) described by Jiricny et al. [72]. Briefly, ncMMR is mostly independent of DNA replication, lacks strand directionality, and could potentially play a role in genomic instability. This type of MMR occurs outside of S-phase when the dNTP pool is limited and replicative polymerases are not present, and the activity of MutLα in this situation can result in nicks in either strand of the DNA. This noncanonical MMR activation can itself promote ubiquitination of PCNA, which is directly responsible for recruiting pol-η (an error-prone polymerase) to chromatin [72] in the absence of higher fidelity polymerases. ncMMR is currently considered a stress response to genotoxic agents that contribute to genomic instability.

4. Repair of DNA strand breakage

While base and nucleotide damage can occur both by mistakes of the replicative machinery and chemical carcinogens, more robust insults to genome stability can induce single-strand and double-strand DNA breaks. These breaks can be caused by chemical carcinogens operating by different mechanisms than the ones previously mentioned, as well as ionizing radiation.

4.1. Homologous recombination

Homologous recombination (HR) is a major DNA repair pathway in which a sister strand of DNA is used to accurately repair DSBs. DSBs generally occur in euchromatin (as heterochromatin is relatively protected in its condensed state), and must be sensed, identified, and stabilized so that repair machinery can be recruited to the site without further damage occurring. The initial sensing of these ends occurs via the joint effort of ATM, and to a lesser extent, the MRN complex. ATM is a resident protein of the nucleus, existing in its inactive dimerized form, but upon the detection of a lesion it can activate itself via autophoshorylation. ATM can recognize large-scale changes in the chromatin structure [73], RNF8- and CHFR-mediated chromatin relaxation by histone ubiquitination [74], and R-loops (RNA/DNA hybrids) at lesions blocking the transcriptional machinery [75]. Thus, begins the ATM signaling cascade, recruiting a wide variety of DNA damage response elements and break responders, as well

as the proteins that modify these responders to activate or enhance their function. To open the damage site to this massive recruitment effort, ATM phosphorylates the methyltransferase MMSET to methylate the surrounding histones and promote 53BP binding [76, 77]. ATM can also phosphorylate MDC1, which leads to the recruitment of ubiquitin ligase RNF8 via its FAA domain [78], which subsequently ubiquitinates histones H2A and H2AX, and promotes the retention of the factors recruited by ATM until the damage has been fully resolved [79]. MDC1, once initially activated by ATM, can bind ATM as well as the MRN complex, thus stabilizing these critical responders at the site of damage and amplifying their continued colocalization with the breaks [80, 81]. Ubiquitinated H2A and H2AX in the presence of RNF8 can recruit a second ubiquitin ligase, RNF168, which amplifies the ubiquitination signal at these histones and ensures that BRCA1, Rap80, Rad18, and 53BP1 localize to the site of damage [82, 83].

BRCA1 is a crucial responder to DNA damage that plays roles in cell cycle checkpoints, DNA cross link repair, and replication fork stability at the sites of DNA damage. Mutations in this gene severely limit its function and force cells to repair their DSBs via the error-prone process of NHEJ, which can predispose individuals to developing breast or ovarian cancer. BRCA1 can recruit RAD51 to the sites of DSBs and is necessary for the cell to repair the damage via homologous recombination and subsequent progress through the G2/M checkpoint [84, 85]. BRCA1 can also form a complex with BRCA2, which contributes to DNA break resolution. One of the proteins that can recruit BRCA1 to the DSB site is Rap80, which directs BRCA1 to K63-linked ubiquitin chains present on postreplication repair effector and sliding clamp PCNA [86]. These ubiquitin chains are generated by RING type E3 ubiquitin ligases RNF8 and RNF168 previously recruited by ATM action [79, 87]. Depletion of RAP80 has been demonstrated to increase the frequency of HR in reporter cells, and these cells eventually developed large chromosomal rearrangements.

BRCA1 itself can also serve as an E3 ubiquitin ligase by forming an obligate RING heterodimer with binding partner BARD1 [88], and this dimerization is required for BRCA1 to exert its tumor suppressor function. BRCA1's RING domain is adjacent to a large sequence of α helices that interact with a similar α helix sequence on BARD1 [89], while the RING domain is left free to interact with E2 enzymes and exert its ubiquitin ligase activities on target proteins [90]. BRCA1-BARD1 is a type I dimeric RING E3 ubiquitin ligase, but is missing a conserved positive residue for these E3 ligases that is required for its binding activity, so this residue must be supplied by their binding partner [91]. The BRCA1-BARD1 heterodimer can target histones (H2A and H2AX), RNA polII, TFIIE, NPM1, CtIP, γ-tubulin, ER-α, and claspin [88]. BRCA1-BARD1 can also interact with 53BP1, and its ligase activity is thought to relocate 53BP1 to the periphery of the damage foci to allow for damage proteins like RPA and RAD51 to localize. E3 ligase-defective cells demonstrate reduced, but not entirely eliminated RPA and RAD51 foci in S-phase cells after being hit with a dose of IR [92]. However, in their normal S-phase counterparts, BRCA1 can counter the 53BP1-mediated stall on resection and allows HR to occur [93] by removing 53BP1 to the periphery and allowing RPA foci to form at the damage site [94]. These observations are thought to be mediated by the human homolog of the yeast SWI/SNF-like chromatin remodeler Fun30, SMARDCAD1, which is recruited by BRCA1-BARD1 to interact with BP531 and remove it from the vicinity of the break [95, 96].

53BP1 appears to serve as a regulator of end resection and DSB resolution based on its associations with factors implicated in transcriptional silencing as well as its previously discussed functions. It can control the length of the resected ends in HR, and serves to prevent aberrant resection that can lead to RAD52-mediated ssDNA annealing and subsequent chromosomal rearrangements [97]. Further evidence for the interplay between BRCA1 and 53BP1 comes from mouse studies, where researchers found that lacking BRCA1 exon two (but expressing a RING-less BRCA1) is an embryonic lethal condition that can be rescued if the deletion occurs in a 53BP−/− embryo, suggesting that murine embryos lacking RING die because of the presence of 53BP [98]. BRCA1 and BARD1 interact with cyclin-dependent kinase 9 (CDK9) via their RING finger and BRCT domains, and localize to γ-H2AX foci indicative of damage to induce the process of HR over NHEJ [99].

Neddylation is a form of posttranslational modification similar to ubiquitination that has also been implicated in the process of double-strand break repair. Neural precursor cell expressed developmentally down-regulated 8 (NEDD8) is a ubiquitin-like protein involved in regulating cell growth, viability, and development [100]. Neddylation can serve as yet another layer of regulation in the function of DNA repair in damaged cells, and targeting this process has demonstrated some efficacy in preclinical models. Given the ubiquitous nature of BRCA1 in HR, it makes sense that this protein is a target of neddylation. In order for a cell to undergo HR, it must recognize the damage and be in the correct stage of the cell cycle (in this case, S/G2) so that a sister chromatid is present for the repair machinery to use as a template. This process of choice can be mediated by BRCA1 in complex with CtIP (RBBP8) in a number of different ways. For instance, CtIP must be phosphorylated on serine residue 327 for the cell to undergo HR, otherwise repair will be conducted via the error-prone process of microhomology-mediated end joining [101]. If this complex undergoes RNF111/UBE2M-mediated neddylation, the complex is rendered unable to perform its 5′→3′ nucleolytic end resection at the DSB, and without the ssDNA overhang tails HR cannot occur [102]. The COP9 signalosome is an additional mediator of the choice between types of DSB repair mechanisms [103]. COP9, the constitutive photomorphogenesis 9 signalosome, has significant homology with the 19S lid complex of the proteome and functions by deneddylating cullin-RING ubiquitin ligases, which may subsequently coordinate CRL-mediated ubiquitination of downstream protein targets [104]. COP9 is recruited to sites of DNA damage in a neddylation-dependent mechanism, and once there mediates deep end resection of the breaks, the first step of HR.

Targeting the process of neddylation as a preclinical strategy to sensitize tumors to chemotherapy is an avenue that has just recently began to garner attention. In a model of nonsmall cell lung cancer, neddylation inhibitor MLN4924 was able to inhibit the recruitment of members of the BRCA1 complex to sites of DNA damage. Examining expression of NEDD8, BRCA1, and PARP via Kaplan–Meier survival analysis revealed that high expression of these three factors correlated with a poor overall survival [105].

4.2. Nonhomologous end joining

The first step of nonhomologous end joining is the detection of the DSB by the Ku70/80 heterodimer, a 150 kDa Ku forms a ring-like structure that surrounds a single-strand of DNA

with its central channel, and threads the broken DNA ends through this channel [106]. Because this protein can only accommodate one strand of DNA, in order for DNA replication to continue after resolution of the DSBs, Ku70/Ku80 must be removed [107]. The E3 ubiquitin ligase RING finger protein 8 (RNF8) has been found to down-regulate Ku80 at sites of DNA damage. Depletion of RNF8 leads to prolonged retention of Ku80 at damage sites and impairs NHEJ [108].

DNA-PK plays a central role in NHEJ of DNA DSBs largely during the G1 phase of the cell cycle as well as in V(D)J recombination [109, 110]. A poorly characterized ringer finger protein RNF144A has been reported as an E3 ubiquitin ligase for DNA-PK catalytic subunit (DNA-PKcs). RNF144A induces ubiquitination of DNA-PKcs in vitro and in vivo and promotes its degradation. Depletion of RNF144A results in an increased level of DNA-PKcs and resistance to DNA damaging agents [111]. Overall, there is no doubt that ubiquitination – either by regulating protein degradation or protein-protein interaction- plays a critical role in all five DNA repair families. Futures studies to better understand the role of ubiquitination, ubiquitin-like modifications, and enzymes responsible for these modifications in DNA repair pathways will be warranted.

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