Chapter 3
MicroRNA, DNA Repair, and Cancer

Hailiang Hu

Abstract Genomic instability is a hallmark of cancer cells and one of the underlying mechanisms is probably caused by the failure to repair DNA damages that have been passed on to the progeny cells. Cells have evolved many types of DNA repair mechanisms to counteract the DNA damages induced by exogenous insults, such as ionizing radiation, ultraviolet radiation, and chemical reagents, or endogenous stimuli-like reactive oxygen species (ROS). These repair mechanisms constitute an elaborate genome maintenance system to protect genomic integrity and therefore defend tumorigenesis. Most recently, microRNAs (miRNAs) have been reported to be a new class of regulators that modulate the DNA damage response pathways by targeting the protein components of response machinery. Here, we summarize and highlight the miRNAs that have been shown to regulate the different DNA repair pathways and discuss their roles in carcinogenesis and implications in cancer therapy.

Keywords MicroRNA · DNA damage response · DNA repair · Double-strand breaks

1 Introduction

Genomic instability is a hallmark of cancer cells and is thought to be an underlying factor responsible for the other six acquired hallmarks of cancer [1, 2]. Cells have evolved an elaborate genome maintenance system to protect genomic integrity and resolve the defects in DNA [3, 4]. DNA repair mechanisms lie in the core of this genome maintenance system. Different DNA repair mechanisms have been developed by cells to counteract various types of DNA lesions. For example, base excision repair (BER) fixes the small chemical alterations of DNA bases; mismatch repair (MMR) replaces the mispaired DNA, while nucleotide excision repair (NER) removes an oligonucleotide containing the damaged bases [5, 6]. Single-strand breaks (SSBs) are repaired by single-strand break repair (SSBR), whereas double-strand breaks (DSBs) are processed either by nonhomologous end joining (NHEJ) or homologous recombination repair (HRR) [7, 8]. These repair mechanisms constitute a critical

H. Hu (✉)
Department of Pathology and Laboratory Medicine, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
e-mail: hhu@mednet.ucla.edu

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defense mechanism against genomic instability and subsequent tumorigenesis [9, 10]. The mutations in the genes encoding the protein components of the DNA repair machinery leading to cancer have been well documented. For instance, BRCA1 and BRCA2, two important DSB HRR proteins, are involved in hereditary breast cancers [11]. Most of these genes behave like tumor suppressors during tumorigenesis.

MicroRNAs (miRNAs) are single-stranded small RNAs of 19–25 nucleotides in length that have been known to be involved in many normal physiological or abnormal pathological processes, including cancers. miRNAs function either as oncogenes or as tumor suppressors during tumor development [12, 13]. More than 700 human miRNAs have been identified, but the function for most of them still needs to be characterized [14]. Around 20–30% of human genes are predicted to be regulated by miRNAs [14]. Therefore, one miRNA can regulate multiple genes and one gene can be controlled by multiple miRNAs. miRNAs are found within or near genomic fragile sites and more than 50% human miRNAs are found at or near the cancer-associated genomic regions [15, 16], implicating the involvement of miRNAs in cancer development. Recently, miRNAs are reported to be involved in DNA damage response (DDR) and modulate the response of cancer cells to cytotoxic treatments, including radio/chemotherapy [17–19]. Here, we summarized the DNA repair-associated miRNAs and discussed their potential role in maintaining the genomic integrity and cancer development.

2 MicroRNA Biogenesis, Regulation and Cancer

2.1 MiRNA Biogenesis: Core Components

MiRNAs are noncoding small RNAs that silence gene expression by either cleaving target messenger RNAs (mRNAs) or repressing translation [20]. The biogenesis of miRNAs comprises three steps: transcription, processing/maturation, and degradation (Fig. 3.1). First, miRNAs are transcribed by RNA polymerase II into pri-miRNAs with the aid of transcription factors [21–24]. Second, pri-miRNAs are 5′ capped and 3′ polyadenylated and further cleaved into pre-miRNA by the Drosha/DGCR8 microprocessor complex [25–27]. Pre-miRNA is then exported from nucleus to cytoplasm by exportin-5 and Ran–guanine triphosphate (GTP) [28, 29]. In the cytoplasm, pre-miRNA is cleaved by Dicer/transactivation response (TAR) RNA-binding protein (TRBP) to an imperfect miRNA/miRNA* duplex of around 20–25 nt in size [30]. Only one strand of the duplex is incorporated into RNA-induced silencing complex (RISC/Argonaute (AGO) 1–4) to bind to 3′-untranslated region (UTR) of target gene and suppress its expression while the other strand is normally degraded. The RISC-loaded mature miRNA is protected from degradation by AGO proteins [31–33]. Finally, after finishing its task, the mature single-strand miRNA will be degraded by the 5′-3′ exoribonuclease XRN2 [34] or the 3′-5′ exoribonucleases, such as human polynucleotide phosphorylase (hPNPase) [35] and nuclear exosome [36].

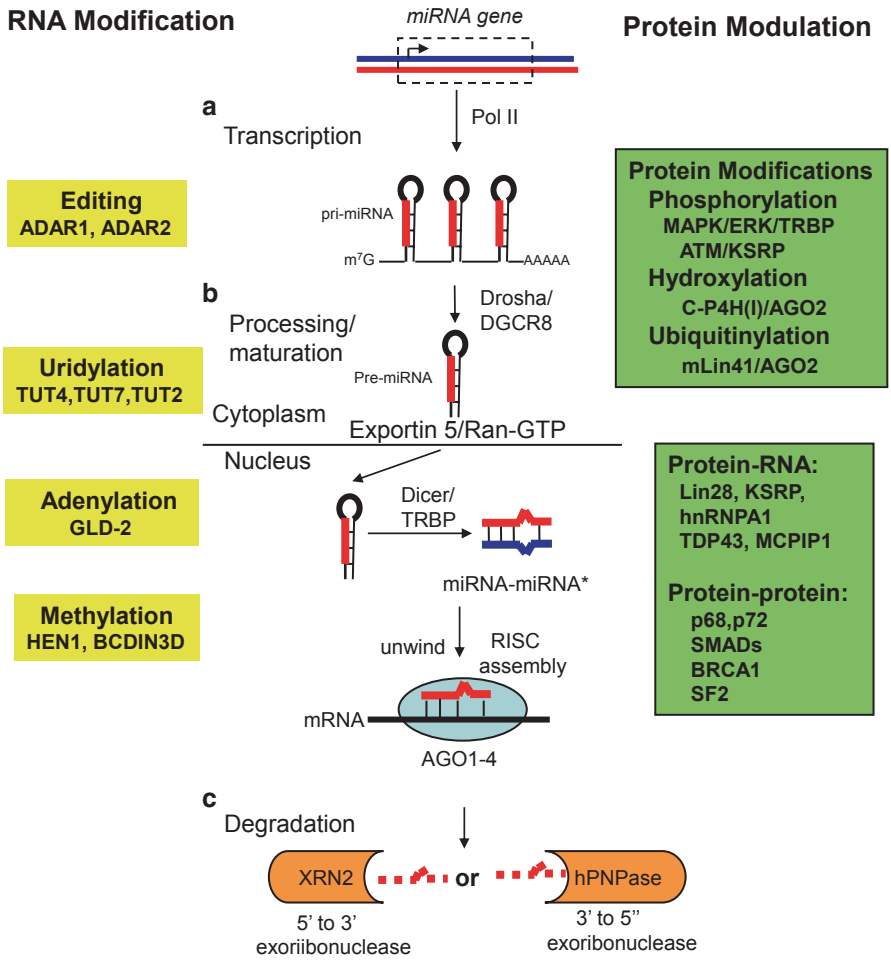

Fig. 3.1 miRNA biogenesis and regulation. The biogenesis of miRNA comprises three steps: transcription (**a**), processing and maturation (**b**), and degradation (**c**). The proteins involved in these steps constitute the core machinery of miRNA biogenesis [17]. RNA modification, including A-to-I editing, uridylation, adenylation, and methylation on pri-mRNAs, pre-miRNAs, or mature miRNAs, changes the miRNA stability and/or target specificity. Protein modulation, such as protein modification (phosphorylation, hydroxylation, ubiquitinylation), protein–RNA interaction, or protein–protein interaction, regulates the core miRNA biogenesis and alters the miRNA expression levels

The core components of miRNA biogenesis machinery include Drosha/DGCR8, exportin5/Ran5–GTP, Dicer/TRBP, AGO1–4, and XRN2/hPNPase [37]. Loss of expression or gain of function caused by mutations in these core genes results in dysregulation of many miRNAs, therefore contributing to the development of tumors. For example, loss of Dicer and Drosha expression is found in breast cancer samples [38], implying that Dicer and Drosha function as suppressors of breast cancer progression. Furthermore, loss of Dicer is a predictor of better response for

breast cancers to chemotherapy and to endocrine therapy [39]. Ovarian cancer patients with both high Dicer expression and high Drosha expression are associated with increased median survival [40]. A second example is that reduced TRBP protein expression caused by mutations in the *TARBP2* gene is found in sporadic and hereditary carcinomas with microsatellite instability and results in the defect of the processing of miRNAs. Reintroduction of TRBP in the deficient cells restores the efficient production of miRNAs and inhibits tumor cell growth [41]. The mutant exportin-5 protein traps pre-miRNAs in the nucleus and reduces miRNA processing, while the restored exportin-5 protein reverses the impaired export of pre-miRNAs and shows tumor suppressor features [42].

In addition to these core components, many other protein factors are reported to regulate miRNA expression by modulating the miRNA biogenesis machinery. Two modes of regulation have been reported: RNA modification, in which pri-miRNAs, pre-miRNAs, or mature miRNAs are modified and therefore their stability is changed accordingly, and protein modification/interaction, in which protein factors change the miRNA stability or target specificity by directly interacting with the RNAs or with the biogenesis machinery protein components.

2.2 RNA Modifications

Adenosine deaminases (ADAR1 and ADAR2) edit some human pri-miRNAs by switching A (adenosine) to I (inosine), which can block the maturation of pri- or pre-miRNAs and/or change their target specificity [43–45] (Fig. 3.1). For example, A-to-I editing of pri-miR-142 prevents its processing by Drosha but promotes its degradation by Tudo-SN, a ribonuclease, while A-to-I editing of pre-miR-376 in the "seed" sequence changes its target specificity [46]. A 3′ end of pre-miRNAs can be modified by oligouridylation or monouridylation. Some pre-miRNAs, including let-7, miR-107, miR-143, and miR-200c, recruit terminal uridylyltransferase 4 (TUT4) together with Lin28 to facilitate the 3′-end oligouridylation and subsequent degradation in stem cells [47]. Prototypic pre-miRNAs usually have a 2-nt 3′ overhang that can be recognized by Dicer for processing, while some pre-miRNAs (let-7 and miR-105) only acquire a 1-nt 3′ overhang from Drosha processing and therefore require a 3′-end monouridylation for Dicer processing. TUT7, TUT4, and TUT2, as the terminal uridylyl transferases, are responsible for pre-miRNA monouridylation. Monouridylation occurs in somatic cells to promote let-7 biogenesis, while oligouridylation inhibits let-7 in embryonic stem cells [48]. In addition to uridylation, the 3′-end of pre-miRNA can also undergo adenylation. A liver-specific miRNA, miR-122, is added to an adenosine at the 3′-end by GLD2, a regulatory cytoplasmic poly(A) polymerase, which is required for the selective stabilization of miR-122 in the liver [49, 50]. Interestingly, miR-122 is found to downregulate cytoplasmic polyadenylation element-binding protein (CPEB), which promotes polyadenylation/translation on the 3′-UTR of p53 mRNA by recruiting Gld4, a second noncanonical poly(A) polymerase. This newly identified pathway Gld2/miR-122/CPEB/Gld4/p53 results in cellular senescence of primary human diploid fibroblasts

[51]. A 2′-O-methylation on the 3′-terminal ribose is another major mechanism that increases the stability of small RNAs. HUA ENHANCER1 (HEN1), an RNA methyltransferase, has been reported to methylate miRNAs and small interfering RNAs (siRNAs) in plants and *Drosophila*, Piwi-interacting RNAs (piRNAs) in animals [52–55]. Another RNA methyltransferase, BCDIN3D, can O-methylate 5′-monophosphate of pre-miRNAs and negatively regulates miRNA maturation. Specifically, BCDIN3D phospho-dimethylates pre-miR-145 and inhibits the Dicer processing for pre-miR-145 [56].

2.3 Protein Modifications

The core protein component TRBP can be phosphorylated by the mitogen-activated protein kinase (MAPK) Erk, and this phosphorylation enhances miRNA production by increasing stability of the Dicer/TRBP complex [57] (Fig. 3.1). KH-type splicing regulatory protein (KSRP) has been shown to be serine phosphorylated by ataxia telangiectasia mutated (ATM) protein in response to DNA damage, and this phosphorylation facilitates the miRNA processing for a subset of miRNAs [58]. These studies suggest a general principle wherein signaling pathways can achieve their biological outcome through regulating the miRNA machinery. AGO proteins are essential components of the RISCs. It is reported that AGO2 can be hydroxylated by the type I collagen prolyl-4-hydroxylase (C-P4H(I)) and this hydroxylation is important for AGO2 stability and efficient RNA interference [59]. AGO2 can also be ubiquitinylated by an E3 ubiquitin ligase Mouse Lin41 (mLin41) and destined for degradation. Therefore, mLin41 acts as an inhibitor of the miRNA pathway by targeting AGO2 for ubiquitinylation. But mLin41 also cooperates with Lin28 in suppressing let-7 activity independent of its E3 ligase activity, revealing a dual control mechanism regulating let-7 in stem cells [60].

2.4 Protein–RNA Interactions

Lin28 Lin28 is a pluripotency factor with two isoforms Lin28A and Lin28B. Both Lin28A and Lin28B can downregulate let-7 miRNA expression but with different mechanisms (Fig. 3.1). Lin28A recognizes a tetranucleotide sequence motif (GGAG) in the terminal loop and recruits TUT4 to add an oligouridine tail to the pre-let-7, which blocks Dicer processing [47]. Lin28A also uses TUT7 as an alternative TUTase that redundantly controls let-7 biogenesis in embryonic stem cells with TUT4 [61]. Lin28B represses let-7 processing through a TUT4-independent mechanism. Lin28B functions in the nucleus by sequestering pri-let-7 transcripts and inhibiting their processing by Drosha/TRBP complex. Furthermore, Lin28A and Lin28B are exclusively expressed in human breast tumors: Lin28A is overexpressed in HER2-overexpressing breast tumors, whereas Lin28B is overexpressed in triple-negative breast tumors, suggesting that the different mechanisms that Lin28A and Lin28B employed may contribute to the different types of breast tumors [62].

KSRP KSRP is a key mediator of mRNA decay and can regulate the biogenesis of a subset of miRNAs by forming complexes with Drosha or Dicer [63]. KSRP binds to the terminal loop of pre-miRNAs and promotes their maturation. The target mRNAs by KSRP-induced miRNAs have been shown to be involved in specific biological programs, including proliferation, apoptosis, and differentiation [63]. Interestingly, KSRP is also a key player that transduces DNA damage signaling to miRNA biogenesis. The ATM kinase directly binds to and phosphorylates KSRP, leading to enhanced interaction between KSRP and pri-miRNAs and increased KSRP activity in miRNA processing, suggesting a novel mechanism by which DNA damage signaling is linked to miRNA biogenesis [58].

hnRNP A1 Heteronuclear ribonucleoprotein A1 (hnRNP A1) can negatively regulate let-7a by binding to the terminal loop of pri-let-7a and inhibiting its processing by Drosha. The binding of hnRNP A1 to let-7a interferes with the binding of KSRP, thereby having an antagonistic role in the regulation of let-7a expression [64, 65]. Additionally, hnRNP A1 not only binds to the pri-miR-18a and reduces its processing by Drosha but also involves in the mature miR-18a-mediated repression of target genes, suggesting the new role for general RNA-binding proteins as auxiliary factors to facilitate the processing of specific miRNAs [66].

TDP-43 TAR DNA-binding protein-43 (TDP-43), a homolog to hnRNPs, is known to be involved in RNA processing and its mutation and abnormal cellular distribution is a key feature of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), two neurodegenerative diseases [67]. TDP-43 facilitates the production of a subset of pre-miRNAs by interacting with the Drosha complex and binding directly to the relevant pri-miRNAs. Furthermore, cytoplasmic TDP-43, which interacts with the Dicer complex, promotes the processing of some pre-miRNAs via binding to their terminal loops. The involvement of TDP-43 in miRNA biogenesis is indispensable for neuronal outgrowth [68]. The *Drosophila* TDP-43 (dTDP-43) has also been reported as controlling the precision of sensory organ precursor (SOP) specification through acting on miR-9a, suggesting a novel role for endogenous TDP-43 in neurodegeneration diseases via miRNAs [69].

MCPIP1 Monocyte chemotactic protein-induced protein 1 (MCPIP1) is a ribonuclease that acts as a broad suppressor of miRNA activity and biogenesis. MCPIP1 counteracts with Dicer in miRNA processing and suppresses miRNA biosynthesis via cleavage of the terminal loops of pre-miRNAs. The balance between Dicer-mediated processing and MCPIP1-mediated destroying modulates miRNA biogenesis and potentially affects the normal and pathological miRNA regulation [70].

2.5 Protein–Protein Interactions

p68 (DDX5) and p72 (DDX17) Both p68 and p72 are DEAD-box RNA helicase subunits that are required for efficient RNA splicing and miRNA processing. They are found in the Drosha complex and are required for the recognition of a subset of

pri-miRNAs in Drosha-mediated processing [71]. In particular, in response to DNA damage, p53 interacts with the Drosha-processing complex through the association with p68 and facilitates the processing of pri-miRNAs to pre-miRNAs, including miR-16–1, miR-143, and miR-145. Inactive p53 mutants interfere with the p68/Drosha interaction, leading to attenuation of miRNA-processing activity [72]. miR-143 and miR-145 belong to a subset of miRNAs whose expression is controlled by p53 and p68/p72. The combination of miR-143 and miR-145 inhibits the expression of c-Myc, whereas miR-145 downregulates p72 expression, forming a feedback loop to prevent overproduction of a subset of tumor suppressive miRNAs by repressing their own modulators p68/p72 [73]. The overexpression of p68/p72 has been reported in three major human cancers (colon, breast, prostate), strongly suggesting their proto-oncoprotein properties [74].

SMADs Smads, the signal transducers of transforming growth factor (TGF)-β/bone morphogenetic protein (BMP), promote the expression of a subset of miRNAs by facilitating the cleavage by Drosha. A majority of TGF-β/BMP-regulated miRNAs (T/B-miRs) contain a consensus sequence Smad binding element (R-SBE) within the stem region of the primary transcripts of T/B-miRs (pri-T/B-miRs), to which Smads directly bind. Mutation of the R-SBE abrogates TGF-β/BMP-induced recruitment of Smads, Drosha, and DGCR8 to pri-T/B-miRs and impairs their processing, whereas introduction of R-SBE to unregulated pri-miRNAs is sufficient to recruit Smads and to allow regulation by TGF-β/BMP [75, 76].

BRCA1 BRCA1 accelerates the processing of pri-miRNAs. BRCA1 increases the expressions of both precursor and mature forms of let-7a-1, miR-16–1, miR-145, and miR-34a. BRCA1 binds directly to Drosha and p68 and also recognizes the RNA secondary structure and directly binds with pri-miRNAs via a DNA-binding domain. BRCA1 regulates miRNA biogenesis via the Drosha/DGCR8 complex, suggesting a novel function of BRCA1 in miRNA biogenesis, which may be linked to its tumor suppressor mechanism and maintenance of genomic stability [77]. BRCA1 can also epigenetically repress miR-155 expression via its association with HDAC2, which deacetylates histones H2A and H3 on the miR-155 promoter. The R1699Q variant of BRCA1, a potentially moderate-risk variant, does not impair DNA damage repair but abrogates the repression of miR-155. This demonstrates a new mode of tumor suppression by BRCA1 and suggests that miR-155 is a potential therapeutic target for BRCA1-deficient tumors [78].

SF2 The splicing factor serine/arginine-rich splicing factor 2 (SF2/ASF) is found to regulate about 40 miRNAs expression by miRNA deep sequencing [79]. SF2/ASF and one of its upregulated miRNAs (miR-7) form a negative feedback loop: SF2/ASF promotes miR-7 maturation, and mature miR-7 in turn targets the 3′-UTR of SF2/ASF to repress its translation. Direct interaction between SF2/ASF and pri-miR-7 facilitates Drosha cleavage and enhances its expression, which is independent of SF2/ASF's splicing function. Other miRNAs, including miR-221 and miR-222, may also be regulated by SF2/ASF through a similar mechanism [79].

Taken together, the miRNA biogenesis is subject to multiple levels of regulation to tightly control its activity (Fig. 3.1). Loss of activity of the core components

and other regulatory factors leads to the dysregulation of a subset of miRNAs or a specific miRNA. It will be interesting to see how the core miRNA biogenesis machinery is regulated by specific factors to achieve its specificity of regulation on a subgroup of miRNAs or a specific single miRNA. Of particular interest to the DNA repair is that the miRNA biogenesis regulatory proteins, p68, BRCA1, and ATM/KSRP, are already known as DNA repair factors, suggestive of an intrinsic link of DDR with miRNA biogenesis.

3 MicroRNAs in DNA Repair

It has been known that miRNA expression can be modulated by different types of DNA damage. For example, ionizing radiation (IR)-induced DNA damage (mainly the DSBs) induces miRNA expression. However, no obvious overlap of IR-responsive miRNAs has been found among different cell lines, suggesting that IR-responsive miRNA profiles might be cell type specific [17]. Different DNA-damaging agents, such as ultraviolet (UV) radiation, H_2O_2, and chemical compounds, have been reported to modulate miRNA expression but induce a different miRNA response [80–82]. In addition to being regulated by DNA damage, miRNAs can also regulate DDR by targeting the protein components of DDR pathways. Herein, we will summarize the miRNAs that modulate the DDR after briefly reviewing the different types of DNA repair mechanisms.

3.1 DNA Repair Mechanisms

Cells have developed different DNA repair mechanisms to deal with DNA damages caused by endogenous stimuli and exogenous environmental insults. The major endogenous DNA damage molecules are reactive oxygen species (ROS), which are the by-products of normal cellular metabolism and can cause oxidative stress. Oxidative stress has been demonstrated to play a significant role in the etiology of many diseases, including DNA repair deficiency disorders [83]. An excess of ROS may lead to the formation and accumulation of mutagenic, toxic, and genome-destabilizing DNA lesions. To repair and resolve such lesions, cells have developed several DNA repair pathways, such as NER, BER and MMR.

NER and BER are two pathways responsible for repair of the majority of DNA lesions induced by ROS. NER is a multistep repair pathway that specifically fixes the oxidative-modified DNA bases, such as 8-oxoguanine, thymine glycol (Tg), and cyclodeoxyadenosine. NER involves five steps: recognition, recruiting, incision, synthesis, and ligation. Basically, the DNA damage is recognized by the repair factors xeroderma pigmentosum complementation group A (XPA), replication protein A (RPA) and XPC, which recruit the helicases XPB and XPD to the damaged sites to unwind DNA. Then XPG and XPF–excision repair cross-complementing (ERCC1) nucleases are recruited for the 3′ and 5′ incisions, respectively, to remove

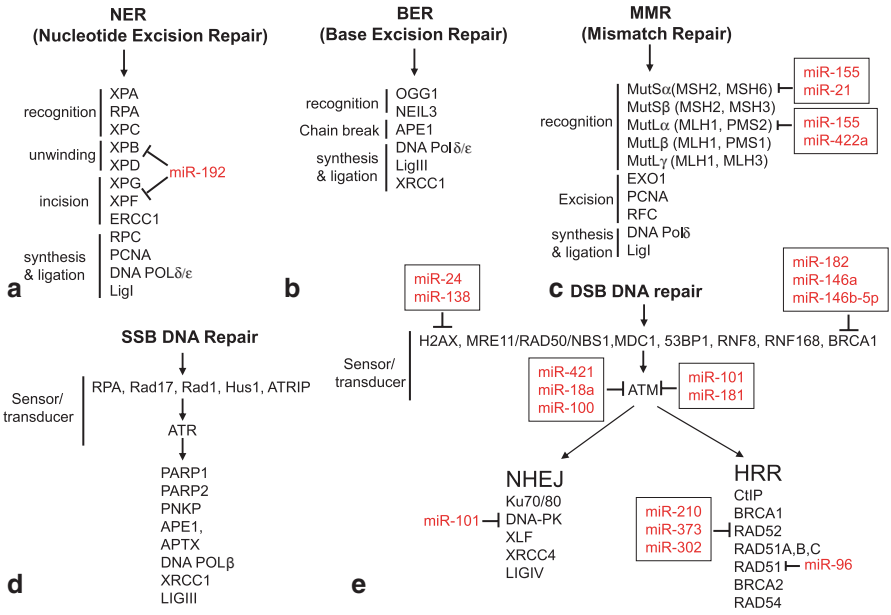

Fig. 3.2 DNA repair machinery and miRNAs**.** The core protein components of different repair pathways are summarized: **a** nucleotide excision repair, **b** base excision repair, **c** mismatch repair, **d** single-strand break DNA repair, and **e** double-strand break DNA repair. miRNAs that have been shown to regulate the core proteins of these different repair pathways are indicated in *red*

the damaged DNA. Then repair synthesis starts to fill the gap by replication factor C (RPC), proliferating cell nuclear antigen (PCNA) and DNA polymerases POLδ/ε using the other strand as a template and finally the newly synthesized strand is ligated by DNA ligase I (Fig. 3.2a) [84].

BER is a simpler version of NER in the case that the oxidative damage is confined to a base. BER essentially involves three steps: (1) Recognition: the modified base is recognized and cleaved by an appropriate DNA glycosylase, such as oxoguanine glycosylase (OGG1) for 8-oxo-G damage or endonuclease VIII-like 3 (NEIL3) for Tg damage. (2) Chain break: the AP endonuclease (APE)1 is recruited to the 5′ side of the base to break the chain. (3) Fill-up and ligation: DNA POLδ/ε comes to fill up one nucleotide gap and the DNA ligase III/XRCC1 complex arrives to seal the nick (Fig. 3.2b) [85, 86].

Mispaired bases of DNA are recognized by heterodimeric complexes of mutator S (MutS)-related proteins, which then recruit the downstream MutL-related proteins to facilitate MMR. Different heterodimeric complexes of MutS and MutL are reported to be responsible for recognition and repair of different types of mismatched DNA. For example, MutSα, composed of melanocyte-stimulating hormones (MSH)2 and MSH6, initiates the repair of base–base mismatches and small insertion–deletion loops (IDLs). MutSβ, a heterodimer of MSH2 and MSH3, repairs both small loops in addition to large loop mismatches of approximately ten nucleotides. MutLα (composed of MutL homolog 1 (MLH1) and postmeiotic segregation

2 (PMS2) proteins) is the primary complex for mismatch correction while MutLβ heterodimer (composed of MLH1 and PMS1 proteins) plays a minor role. Furthermore, MutLγ (MLH1 and MLH3) acts as a backup for MutLα in the repair of base–base mismatches and small IDLs. MutS/MutL forms a sliding clamp with PCNA and replication factor C (RFC) to allow the identification of the daughter strand, and exonuclease 1 (EXO1), a DNA exonuclease, enters the DNA structure to remove daughter-strand DNA. Once the mismatch is removed, the activity of EXO1 is suppressed by MutL, thus terminating DNA excision. Upon completion of this process, a DNA polymerase δ synthesizes DNA in place of the excised sequence with a DNA ligase I that joins any gaps in the DNA sequence (Fig. 3.2c) [5, 87].

SSB and DSB are usually induced by exogenous stimulators such as UV, IR or chemical compounds. ATM and Ataxia Telangiectasia and Rad3 related (ATR) are serine/threonine kinases that transduce these SSB and DSB DNA damage signals to downstream events. ATM is primarily activated in response to DSBs, whereas ATR is mainly involved in SSB and stalled replication forks. ATM/ATR coordinate downstream events such as cell cycle, DNA repair, and apoptosis by phosphorylating a wide set of protein substrates and impact a variety of cellular physiologies (Fig. 3.2d, e) [88, 89].

To repair SSB, ATR has to be activated. Single-strand DNA (ssDNA) is stabilized by replication protein A (RPA) binding and then recruits Rad17 to load Rad9–Rad1–Hus1 (9–1–1) complex and ATR-interacting protein (ATRIP)–ATR complex onto DNA, during which ATR is activated. In SSBR, poly(ADP-ribosyl)ation of ssDNA by poly(ADP-ribose) polymerase (PARP)1/2 is the first step and thought to aid in recruiting other DNA repair proteins, such as XRCC1 and ligase III to the site and promote SSB repair following DNA end processing by XRCC1-interacting proteins such as DNA Polβ, polynucleotide kinase (PNK), apurinic/apyrimidinic (AP) endonuclease 1 (APE1), Aprataxin (APTX), and Aprataxin and PNKP like factor (APLF) (Fig. 3.2d) [90].

DNA DSBs are highly toxic to cells and can drive genomic instability [91]. Failure to properly execute DSB repair is known to accelerate tumorigenesis and is associated with several genetic disorders [92]. ATM activation is required for DSB repair. The MRE11-RAD50-NBS1 (MRN) complex acts as a sensor of DSB to recruit ATM to DNA damage site. The phosphorylation of histone variant H2A histone family, member X (H2AX) by ATM results in the recruitment of MDC1 as a scaffold to further recruit 53BP1 and a series of ubiquitin ligases ring finger protein 8 (RNF8), RNF168 and BRCA1 to initiate DSB DNA repair (Fig. 3.2e) [93].

NHEJ and HRR represent two major DSB repair pathways in different cell cycle phase. NHEJ occurs in the G0/G1 phase while HRR in the late S/G2 phase. Up to 90% of DSBs are repaired by NHEJ in G1 phase of the cell cycle by an ATM-independent mechanism. Six core proteins required for NHEJ have been identified to date, Ku70, Ku80, DNA-dependent protein kinase, catalytic subunits (DNA-PKcs), X-ray repair cross-complementing protein 4 (XRCC4), XRCC4-like factor (XLF), and DNA ligase IV (LIGIV), which are assembled as two steps: the Ku heterodimer (Ku70/80) binds to DSB ends and recruits DNA-PKcs and consequently coordinate end processing with rejoining by recruiting XRCC4, XLF, and LIGIV (Fig. 3.2e) [94]. DSBs can also be repaired by homologous recombination (HR)-mediated

pathways. Repair is initiated by CtBP-interacting protein (CtIP)–BRCA1 complex-mediated resection of a DSB to provide 3′ssDNA overhangs [95] and followed by strand invasion and strand displacement, which is mediated by RAD52, RAD51 paralogs (-A, -B, -C). DNA resynthesis of the broken portion with the undamaged sister molecules as a template is then mediated by RAD51–BRCA2 complex and RAD54 (Fig. 3.2e) [96].

3.2 Core MMR Proteins-miR-155, miR-422a, miR-21

Defects in MMR can lead to genomic instability and cause hereditary colorectal cancer as well as 10–40 % of sporadic colorectal and gastric cancers [97] (Fig. 3.2c). A human miRNA, miR-155, is reported to negatively regulate MSH2, MSH6, and MLH1, the three core MMR proteins that affect the recognition and repair of mismatch DNA. Overexpression of miR-155 induces the genomic instability, including elevated mutation rates and microsatellite instability (MSI), by targeting these MMR core proteins. The inversed correlation between miR-155 overexpression and downregulation of MLH1 or MSH2 protein expression is found in human colorectal cancer, suggesting that miR-155 modulation of MMR might be a mechanism of colorectal cancer pathogenesis [98]. Another miRNA, miR-422a, is found to downregulate MutLα (MLH1–PMS2 heterodimer) levels by suppressing MLH1 expression through its 3′-UTR. Interestingly, MutLα stimulates the conversion of pri-miR-422a to pre-miR-422a, thereby forming a feedback loop that regulates the level of both molecules [99]. MMR repair-deficient cells display a characteristic of reduced 5-fluorouracil (FU)-induced G2/M damage arrest and apoptosis. Overexpression of miR-21 in cells exhibits this cellular phenotype and miR-21 is found to downregulate the core MMR recognition protein complex, MSH2 and MSH6. A high level of miR-21 is inversely correlated with reduced MSH2 protein expression in a number of human tumors including colorectal cancer. Moreover, xenograft studies demonstrate that miR-21 overexpression dramatically reduces the therapeutic efficacy of 5-FU. These studies suggest that the downregulation of the MMR gene by miR-21 overexpression may be an important clinical indicator of therapeutic efficacy in colorectal cancer [100].

3.3 NER Proteins–miR-192

NER has been found to be inhibited by hepatitis B virus (HBV) [101] (Fig. 3.2a). miRNA expression profiling of HBV-infected hepatocellular cells identified that miR-192 is significantly upregulated in these infected cells. Furthermore, overexpressing miR-192 inhibits cellular NER by downregulating XPB and XPF, two key factors in NER. These results indicate that persistent HBV infection might trigger NER impairment in part through upregulation of miR-192, which suppresses the levels of XPB and XPF. It provides new insights into the effect of chronic HBV infection on NER and genetic instability in cancer [102].

3.4 H2AX-miR-24, miR-138

The phosphorylation of H2AX (γ-H2AX) is the initial step for a cascade of DSB response, functioning to link the damaged DNA to the DNA repair machinery. γ-H2AX foci formation is also an indicator for DSB. Modulation of H2AX expression is, therefore, important for the DSB detection and repair. miR-24 is identified by miRNA array to be upregulated during postmitotic differentiation of hematopoietic cell lines and downregulates the expression of H2AX. miR-24-mediated suppression of H2AX renders hematopoietic cells hypersensitive to γ-irradiation and genotoxic drugs, which might account for the reduced capacity to repair DSB in terminally differentiated hematopoietic cells [103]. By screening a library of human miRNA mimics to inhibit γ-H2AX foci formation, miR-138 directly targets the histone H2AX 3′-UTR to reduce H2AX expression and induces chromosomal instability after DNA damage. Overexpression of miR-138 inhibits HRR and enhances cellular sensitivity to multiple DNA-damaging agents. Reintroduction of H2AX in miR-138 overexpressing cells abrogates miR-138-mediated hypersensitivity. This study suggests that miR-138 is an important regulator of genomic stability and a potential therapeutic agent to improve the efficacy of radiotherapy and chemotherapy with DNA-damaging agents [104].

3.5 BRCA1-miR182, miR-146a

BRCA1 is a strong breast cancer susceptibility gene, and germline mutations in the BRCA1 gene predispose women to breast cancer. The BRCA1 protein plays a critical role in DSB DDR by detecting the DNA damage and promoting HRR [105]. BRCA1 deficient cells show genome instability and are intrinsically sensitive to PARP inhibitors [106]. BRCA1 modulates the miRNA biogenesis by interacting with Drosha and p68 (Fig. 3.1). However, BRCA1 per se is subjected to miRNA modulation. In a pull-down assay, BRCA1 transcripts are reported to be enriched in the AGO/miR-182 complex. Overexpression of miR-182 leads to the hypersensitivity of cells to IR and impairs HRR. These impaired DNA repair phenotypes can be rescued by introducing back BRCA1 protein, suggesting that BRCA1 mediates the effects of miR-182 on DNA repair. On the other hand, inhibition of miR-182 increases BRCA1 protein levels and protects cells from IR-induced cell death. miR-182-overexpressing breast tumor cells are hypersensitive to PARP1 inhibitors, similar to the BRCA1 deficient cell. Conversely, inhibiting miR-182 enhances BRCA1 levels and induces resistance to PARP1 inhibitors. These results suggest that miR-182-mediated downregulation of BRCA1 affects DNA repair and may impact breast cancer therapy [107].

Low levels of BRCA1 protein is also found in about one-third of sporadic breast cancers. Two miRNAs, miR-146a and miR-146b-5p, have been shown to bind to 3′-UTR of BRCA1 and downregulate BRCA1 expression. The miR-146a/miR-146b-5p-mediated BRCA1 increases cell proliferation and reduces HRR. Furthermore, the highest levels of miR-146a and/or miR-146b-5p are found in basal-

like mammary tumor epithelial cell lines, and in triple negative breast tumors, which are the closest to tumors arising in carriers of BRCA1 mutations. This work provides further evidence for the involvement of miRNAs in sporadic breast cancer through downregulation of BRCA1 [108].

3.6 ATM-miRNA421, miR-18a, miR-100, miR-101, miR-181

ATM is the chief transducer in the DSB signaling and mutations in this gene lead to a typical genomic instability disorder ataxia telangiectasia [109]. This disease also displays hypersensitivity to ionizing radiation, suggesting that modulation of ATM protein could alter cellular radiosensitivity [110, 111]. By using the target prediction program, miR-421 is reported to suppress ATM expression by targeting the 3′-UTR of ATM transcripts. Ectopic expression of miR-421 results in S-phase cell-cycle checkpoint changes and an increased sensitivity to ionizing radiation. This is the first study to show that ATM is subject to miRNA regulation and miR-421–ATM pathway might contribute to the DDR in a variety of cells given the broad expression pattern of ATM [112]. Interestingly, a squamous carcinoma cell line SKX exhibits a pronounced radiosensitivity after IR with enhanced levels of miR-421. Transfection of SKX cells with either anti-miR-421 inhibitor or a miRNA-insensitive ATM vector restores the ATM expression and abrogates the hyperradiosensitivity. This is the first report describing miRNA-mediated downregulation of ATM leading to clinically manifest tumor radiosensitivity [113].

Another miRNA, miR-18a, is found to be overexpressed in breast cancer cell lines and breast cancer patients' tissue samples. The overexpression of miR-18a reduces HRR and sensitizes breast cancer cells to IR treatment in a similar way to ATM siRNA. Ectopically expressing miR-18a downregulates ATM expression by directly targeting the ATM–3′-UTR and abrogates the IR-induced cell-cycle arrest. On the other hand, inhibition of miR-18a leads to augmentation of DNA damage repair, increase of HRR efficiency and reduced cellular radiosensitivity [114]. This work provides a second miRNA that regulates cellular radiosensitivity through modulation of ATM protein level.

A low level of ATM is found in a human malignant glioma cell line with hyperradiosensitivity while miR-100 is highly expressed in this cell line. The 3′-UTR of ATM contains a binding site for miR-100. Knocking down miR-100 promotes ATM expression while overexpressing miR-100 reduces ATM expression and sensitizes these cells to IR. These results indicate that miR-100 could be another miRNA to target ATM and sensitize tumor cells to IR [115].

By combining the program prediction and the experiment validation, miR-101 could efficiently target DNA-PKcs and ATM via binding to the 3′-UTR of DNA-PKcs or ATM mRNA. Upregulating miR-101 efficiently reduced the protein levels of DNA-PKcs and ATM in the tumor cells and, most importantly, sensitized the tumor cells to radiation both in vitro and in vivo [116].

Reduced ATM expression has been found in TGF-β-induced breast cancer mammospheres and this is thought to be mediated by miR-181, which is upregulated by

TGF-β at the posttranscriptional level. Overexpression of miR-181 or depletion of ATM is sufficient to induce sphere formation in breast cancer cells, suggesting that the miR-181–ATM pathway is involved in the TGF-β-induced cancer stem cells [117].

3.7 Rad51–miR-96

The DNA repair protein RAD51 lies in the core of HRR by promoting DNA synthesis. MiR-96 is reported to target RAD51 on its coding region instead of 3′-UTR. Overexpression of miR-96 in human cancer cells reduces the levels of RAD51, decreases the efficiency of homologous recombination, and enhances sensitivity to the PARP inhibitor and to cisplatin. This study suggests that miR-96 can regulate chemosensitivity by repressing RAD51 and may serve as a therapeutic candidate to improve chemotherapeutic efficacy by increasing the sensitivity of cancer cells to DNA damage [118].

3.8 RAD52–miR-210, 373, miR-302

Two miRNAs, miR-210 and miR-373, are upregulated in a hypoxia-inducible factor-1 alpha (HIF1α)-dependent manner in hypoxic cells. Bioinformatics analyses suggested that these miRs could regulate factors implicated in DNA repair pathways. Overexpression of miR-210 is found to suppress the levels of RAD52, which is a key factor in HRR; the forced expression of miR-373 leads to a reduction in the NER protein, RAD23B, as well as in RAD52. Consistent with these results, both RAD52 and RAD23B are found to be downregulated in hypoxia, but in both cases, the hypoxia-induced downregulation could be partially reversed by antisense inhibition of miR-210 and miR-373. Importantly, luciferase reporter assays indicate that miR-210 is capable of interacting with the 3′-UTR of RAD52 and that miR-373 can act on the 3′-UTR of RAD23B. These results indicate that hypoxia-inducible miR-210 and miR-373 play roles in modulating the expression levels of key proteins involved in the HRR and NER pathways, providing new mechanistic insight into the effect of hypoxia on DNA repair and genetic instability in cancer [119]. miR-302 is downregulated in irradiated breast cancer cells. Additionally, the expression levels of miR-302a are inversely correlated with those of AKT1 and RAD52, two critical regulators of radioresistance. More promisingly, miR-302a sensitizes radioresistant breast cancer cells to radiation therapy and reduces the expression of AKT1 and RAD52. These data suggest that miR-302 is a potential sensitizer to radiotherapy [120].

4 miRNAs in Other DNA Damage Response Events

DNA repair is one of the most important events in DDR. There are some other events upstream or downstream of DNA repair, such as cell-cycle arrest to allow cells to repair damaged DNA or apoptosis if the DNA damage is not able to be fixed. miRNAs

are reported to be involved in these events. For example, overexpression of miR-106b promotes cell-cycle progression, whereas loss of function reverses this phenotype. The cyclin-dependent kinase inhibitor p21 is a direct target of miR-106b and the miR-106b-mediated p21 downregulation overrides a doxorubicin-induced DNA damage checkpoint [121]. miR-21 is induced by DNA damage, negatively regulates G1/S transition, and participates in DNA damage-induced G2/M checkpoint. This is achieved by the downregulation of CDC25A, a cell-cycle regulator. miR-21 suppresses CDC25A expression through a defined sequence in the 3′-UTR of CDC25A [122].

p53 plays an important role in the DNA damage-induced apoptosis. Computational predictions suggest that several miRNAs are involved in the posttranscriptional regulation of p53. miR-504 downregulates human p53 through its direct binding to two sites in the p53 3′-UTR. Overexpression of miR-504 decreases p53 protein levels and regulates p53 transcriptional activity, p53-mediated apoptosis, and cell-cycle arrest in response to stress [123]. miR-125b is another negative regulator of p53 by targeting the 3′-UTR of p53 mRNA. Overexpression of miR-125b represses the endogenous level of p53 protein and suppresses apoptosis [124]. miR-138 directly targets the 3′-UTR of p53, significantly decreasing the expression of p53 and its downstream genes. Interestingly, the ectopic expression of miR-138 significantly improves the efficiency of induced pluripotent stem (iPS) cell generation by downregulating p53 expression [125].

There are other reported miRNAs that regulate the expression of core protein components of the DDR pathways, including miR-449a/b and miR-16 both targeting CDC25A [80, 126], miR-195 targeting Wee1 [127], miR-124a targeting CDK2 [128], and miR-100 targeting PLK1 [129].

5 Conclusions and Future Prospective

Here, we reviewed the DNA repair mechanisms that cells have developed to process the different types of DNA damages and summarized the core protein components for these DNA repair machineries. Only a few proteins to date are known to be regulated by miRNAs and no miRNAs have been reported to regulate BER and SSBR (Fig. 3.2). It is estimated that 20–30 % of human proteins are regulated by miRNAs. Therefore, we envision that there will be more proteins in the DDR pathways that can be regulated by miRNAs.

Defects in these DNA repairs have been shown to cause different types of cancer with characteristics of genomic instability. Targeting DNA repair has already been used to treat cancers and the miRNA-mediated negative regulation of the DNA repair proteins is becoming a promising strategy to overcome the resistance developed by these cancer treatments, such as radiation therapy or chemotherapy. For instance, overexpression of miR-155 may induce colorectal cancer by modulating MMR protein expression [98] and miR-421 overexpression in squamous carcinoma tumors leads to clinical hyperradiosensitivity by targeting ATM expression [113]. These studies suggest that miRNAs are attractive therapeutic candidates to improve cancer treatment.

However, the challenge for using these miRNAs to treat cancers is how to deliver these miRNA mimics or anti-miRs specifically to tumors. Multiple technologies have been developed to achieve systemic delivery of miRNA mimics or anti-miRs, including the application of chemically modified oligonucleotides, lentiviral-based delivery, or nanoparticle-based delivery. However, each miRNA is known to target many transcripts and each target gene can be regulated by multiple miRNAs, as demonstrated by ATM, which can be regulated by multiple miRNAs (miR-421, miR-18a, miR-100, miR-101, and miR-181), and miR-101 can downregulate DNA-PK in addition to ATM (Fig. 3.2). Therefore, the safety of using miRNA for therapy needs to be extensively scrutinized and evaluated in animal model before clinical trials.

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