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RNA-directed repair of DNA double-strand breaks

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ABSTRACT

DNA double-strand breaks (DSBs) are among the most deleterious DNA lesions, which if unrepaired or repaired incorrectly can cause cell death or genome instability that may lead to cancer. To counteract these adverse consequences, eukaryotes have evolved a highly orchestrated mechanism to repair DSBs, namely DNA-damage-response (DDR). DDR, as defined specifically in relation to DSBs, consists of multi-layered regulatory modes including DNA damage sensors, transducers and effectors, through which DSBs are sensed and then repaired via DNAprotein interactions. Unexpectedly, recent studies have revealed a direct role of RNA in the repair of DSBs, including DSB-induced small RNA (diRNA)-directed and RNA-templated DNA repair. Here, we summarize the recent discoveries of RNA-mediated regulation of DSB repair and discuss the potential impact of these novel RNA components of the DSB repair pathway on genomic stability and plasticity.

1. Introduction

Eukaryotic DNA sustains constant threats from deleterious endogenous and environmental agents. Double-strand DNA breaks (DSBs) are arguably the most toxic DNA lesions [1]. If left unrepaired, one lesion can induce cell death or oncogenic chromosomal translocations. Cells have evolved a highly conserved and orchestrated DNA damage response (DDR) network to sense DSBs and mediate repair [1,2]. There are prominent, conserved DSB repair mechanisms: error-prone non-homologous end-joining (NHEJ), faithful homologous recombination (HR), alternative NHEJ and single strand annealing [3]. Dynamic spatial, physical and temporal organizational properties of the protein-based multiple regulatory layers of DSB repair have been reviewed in detail elsewhere [1,4–9]. Interestingly besides the involvement of proteins, efficient DSB repair also requires RNA elements including microRNAs, diRNAs and template RNA [10–14]. In this review, we focus upon recent discoveries of RNA-based regulatory layers of DSB repair and discuss their potential impacts on genome stability.

2. MicroRNAs regulate DNA repair genes

MicroRNAs (miRNAs) are a family of small non-coding RNAs that destabilize and repress translation of their target messenger RNAs. MiRNAs play an important regulatory role in various biological processes. Emerging evidence indicates that miRNAs can regulate the expression of central components of the DDR machinery, which may have a role in feedback regulation of miRNA expression. There are excellent reviews about modulation of miRNA expression during DDR [15–17]. Here, we summarize recent studies on the functions of miRNAs in the DDR to DSBs.

MiRNAs have been shown to target cell cycle checkpoint genes, thereby influencing DNA damage repair. Multiple DNA damage agents can induce the expression of several miRNA families including miR-15a/b, miR-16, miR-21, miR-34, let-7 and miR-24 [18–23]. miR-21 and miR-16 repress the expression of critical cell cycle checkpoint phosphatases CDC25A and WIP1 and then modulate cell cycle checkpoint activation [19,24–26]. The p53-dependent miR-34 can regulate G1/S and S-phase checkpoints by modulating multiple genes including E2F, cyclinE2, CDK4/CDK6, and c-MYC [27–31]. let-7 regulates G1/S and G2/M cell cycle checkpoints by targeting E2F2, cyclin D2, Cdc34/cyclins D1/D3/A and Cdk4, respectively [23,32–34], while miR-24 activates the G1/S cell cycle checkpoint mainly by targeting E2F2 [35]. DNA damage can also down-regulate the expression of some miRNA families including miR-106b, miR-

Abbreviations: DSBs, DNA double-strand breaks; DDR, DNA-damage-response as defined specifically in relation to DSBs; diRNA, DSB-induced small RNA; NHEJ, non-homologous end-joining; HR, homologous recombination; Ago2, Argonaute2; RISC, RNA-induced silencing complex.

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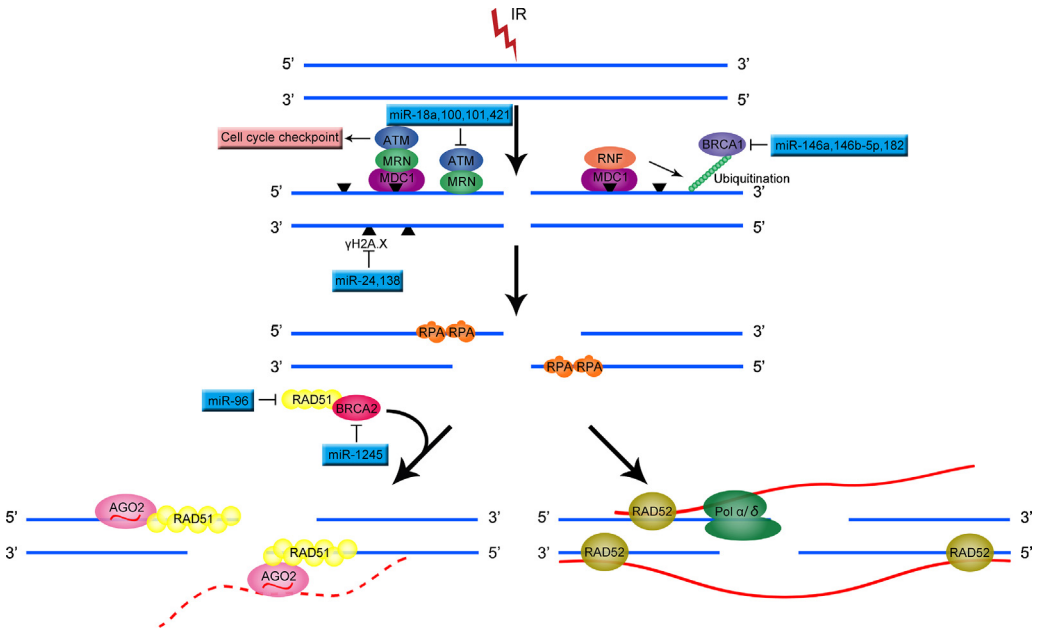

Fig. 1. Working model for RNA-directed repair of DSBs.
MicroRNAs regulate DNA damage repair genes as indicated. DiRNAs regulate the recruitment of Ago2–Rad51 complexes to DSB site through base pairing between diRNAs and homologous DNA sequences surrounding the break site or scaffold RNA transcripts (red dashed line) generated from around the break site. Rad52-dependent template-RNAs direct DNA repair as indicated (template-RNAs as red solid line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

17 miR-421, miR-101 and miR-100, which then activate cell cycle checkpoint [36–39]. The down-regulation of miR-106b regulates p21-dependent G2/M cell cycle arrest [40]. miR-17-92, miR-106a and miR-106b-25 actively participate in cell cycle control by targeting E2F1-associated G1 checkpoint activation [41–43]. miR-17-5p targets specific genes required for the G1/S transition [44]. These findings suggest that miRNAs have complex roles in targeting either negative or positive regulators of the cell cycle checkpoint upon DDR.

miRNAs can also target DNA repair or damage response genes and modulate levels of DNA repair proteins. Several miRNAs including miR-421, miR-101 and miR-100 repress ATM expression [45–47]. miR-101 also targets DNA-PKcs miR-124 regulates Ku70 expression [48,49], and miR-138 and miR-24 both targets histone H2AX [50,51]. miR-182 suppresses BRCA1, whereas miR-96 represses RAD51 and REV1 [52–54]. Expression of these miRNAs turns out to be down-regulated in response to DNA damage. Therefore, down-regulation of these miRNAs may release their inhibitory effects and allow DNA repair proteins to rapidly accumulate upon DNA damage. In contrast to the above down-regulated miRNAs, some miRNAs targeting DNA repair or DDR genes are up-regulated upon DNA damage. These include miR-146a and miR-146b-5p that targets BRCA1 [55] and miR-155 that targets mismatch repair genes MLH1 and MSH2 [56,57]. These miRNAs may be required for appropriate activation of DDR and DSB repair.

Collectively, miRNAs provide a new regulatory layer of DDR and DSB repair genes at the post-transcriptional level (Fig. 1). Detailed mechanisms underlying their cooperative roles will require further investigation.

3. DiRNAs recruit DNA repair factors

Recent studies have shown that a new class of 21 nt-long small RNAs can be induced by DSBs from the sequences in vicinity of DSB sites in several species [58–60]. The generation of these small RNAs (namely DSB-induced small RNAs, or diRNAs) is Dicer-dependent. diRNAs are bound by Argonaute2 (Ago2), the core component of RNA-induced silencing complex (RISC) in RNAi. Consistently, depletion of Dicer or Ago2 but not other Agos decreases HR repair efficiency of DSBs [61]. Several possible mechanisms through which diRNA mediates DSB repair can be envisioned: (1) Ago2–diRNA may modulate the activity of DNA damage response kinases, including ATM and ATR, and then influence DSB lesion sensing; (2) Ago2–diRNA may regulate cell-cycle progression and affect the expression of DNA repair proteins; (3) Ago2–diRNA may recruit chromatin modifying complexes to alter chromatin status, which in turn regulates the access and/or dissociation of repair factors to/from chromatin near DSBs; (4) Ago2–diRNA may guide repair factors to DSB sites to facilitate repair; (5) Ago2–diRNA may function in Rad51-mediated homology search or at a later stage of HR. Depletion of Dicer or Ago2 did not alter cell cycle distributions and major histone modifications (our unpublished observations), suggesting that diRNAs are unlikely involved in regulating cell cycle progression and chromatin modification. Moreover, indirect fluorescence and Western-blotting approaches revealed that both phosphorylation of ATM/ATR and their targets Chk2, Chk1 and RPA, as well as focus formation of MDC1 and 53BP1 appeared to have no significant changes in Dicer- and Ago2-deficient cells [61], excluding potential participation of diRNAs in ATM-mediated DNA damage sensing. Rad51 forms repair foci at DSB sites upon DNA damage and plays a critical role in the exchange of single-strand DNAs during HR repair of DSBs [61]. Intriguingly, Ago2 interacted with Rad51 and such interaction was enhanced following ionizing-radiation (IR). This raised a possibility that Ago2–diRNA may regulate Rad51 focus formation. Indeed, the recruitment of Rad51 but not other DDR proteins including γ-H2AX, RPA, 53BP1 and MDC1, was severely compromised at IR-induced DSB sites in Dicer- or Ago2 or Dicer-depleted cells. In addition, mutant Ago2 proteins that are deficient in either small RNA binding or Slicer activity could neither retain the recruitment of Rad51 to DSB sites nor restore HR repair in Ago2-deficient cells, albeit they were still able to interact with Rad51 [61].

Based on current data, it is appealing to propose that the Ago2/diRNA complex recruits Rad51 and then targets it onto DSBs