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Review Article

Linc'ing' RNA to DNA Repair

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To maintain genome stability cells have evolved complex DNA repair pathways to correct DNA damage caused by exogenous and endogenous factors. The role of proteins and small non coding RNAs in DNA repair is well known. Recently, several long non coding RNAs (lncRNAs) have emerged as critical players in regulating DNA double strand break repair by Homologous Recombination (HR) and Non Homologous End Joining (NHEJ). LncRNAs interact with regulatory proteins or coding and other non-coding transcripts involved in DNA repair to modulate DNA repair. This review summarizes the role of lncRNAs in DNA repair by HR and NHEJ pathways.

Keywords: Double Strand Breaks; Non Homologous End Joining; Homologous Recombination; Long Non Coding RNAs

Introduction

DNA Double Strand Break (DSB) Repair

DNA in the cells is susceptible to damage from both endogenous metabolic products such as reactive oxygen species and exogenous toxins such as ionizing and UV radiations. DNA damage occurs as base lesions or single strand breaks or as DSBs and their repair involves following steps (i) detection of kind of DNA damage (ii) choice and recruitment of repair factors and (iii) repair by effector molecules (Ciccia and Elledge, 2010; Jackson and Bartek, 2009). Among the different kinds of DNA damage, DSBs are the most difficult to repair DNA lesions (Mehta and Haber, 2014; Srivastava and Raghavan 2015). For repair of DSBs two major pathways exist namely: homologous recombination (HR) and non-homologous end joining (NHEJ). These two pathways differ with respect to template strand requirements, kinetics, fidelity and phases of cell cycle in which they occur (Filippo *et al.*, 2008; Lieber, 2008; Branzei and Foiani, 2008).

HR operates during the S and G2 phases of cell cycle because it requires homologous sister chromatids. Repair by HR requires recognition and processing of DSBs resulting in a 3' hydroxyl overhang by DNA end resection. The MRE11-RAD50-NBS1 (MRN) complex is involved in recognizing the DSBs which need to be repaired by HR. The detection of DSBs by MRN complex is followed by auto phosphorylation of ATM, this is followed by phosphorylation of DNA repair factors namely BRCA1, CtIP, H2AX and exonuclease EXO1 by ATM. The resection is completed with the action of the proteins MRE11, CtIP, EXO-1 and BRCA1 (Filippo *et al.*, 2008). End resection is followed by strand invasion between the duplex of homologous donor sequence and the resected overhangs of the DSB. The 3' overhangs are bound by RPA which is a single strand DNA (ssDNA) binding protein that enhances strand exchange and destabilizes the formation of secondary structures (Nimonkar *et al.*, 2011). The identification of homologous template strand to repair the DSBs is performed by RAD51 recombinase (Mehta and Haber, 2014). With the help of BRCA2 and other recombinase accessory factors, RPA is replaced by RAD51 in the ssDNA forming a presynaptic filament leading to D loop formation (Seong *et al.*, 2009). The functional strand invasion and D loop formation is facilitated by RAD51 and RAD52

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(Mehta and Haber, 2014). Processing of D loop intermediate can occur in any of the following ways: by the formation of a holiday junction which could be processed by resolvase or by synthesis-dependent stand annealing (SDSA) or by break induced replication (BIR) (Filippo *et al*., 2008; Srivastava and Raghavan 2015). Repair of DSBs in the pre-replicative phases (G0 and G1 phases) is dominated by the error prone NHEJ pathway. The entire process of NHEJ occurs without any requirement of a template strand and without base pairing and it occurs throughout the cell cycle (Waters *et al*., 2014). It involves the recognition of broken DNA ends by Ku70, Ku80 proteins and activation of kinase DNA PKCs (Waters *et al*., 2014; Balestrini *et al*., 2013). This is followed by recruitment and phosphorylation of Artemis by DNA PKCs producing clear overhangs in the DSB sites. NHEJ often requires DNA processing prior to ligation, which may involve DNA polymerases to fill the gaps generated after alignment of the ends (Waters *et al*., 2014). However, conventional DNA polymerases are mostly inactive during NHEJ and DNA synthesis for filling gaps is done by specialized polymerases such as DNA Polμ and Polë (Moreno *et al*., 2017; Waters *et al*., 2014). Ligation of the broken ends is performed by the XRCC4/XLF/DNA ligase IV enzyme complex (Mladenov and Iliakis, 2011; Chiruvella *et al*., 2013).

Choice between HR and NHEJ in a cell is governed by various factors like: phase of cell cycle, DSB structures and end resection (Schipler and Iliakis 2013). Due to availability of homologous sister chromatids HR is predominant in S and G2 phases of cell cycle. Degradation of 5' end of DSBs gives rise to 3' overhangs committing the cell to HR since 3' overhangs do not favor the binding of Ku proteins. 53BP1 is a crucial player which controls DDR by inhibiting HR through inhibition of 5' end resection and promotes NHEJ (Zimmermann *et al*., 2013; Diaz *et al*., 2013). Phosphorylation of 53BP1 inhibits BRCA1 recruitment and end resection in HR (Zimmermann *et al*., 2013). Rif1 is the major factor used by 53BP1 to inhibit the 5' resection by limiting the function and accumulation of CtIP, Exo1 and BRCA1 complexes at the site of DSB (Zimmermann *et al*., 2013; Diaz *et al*., 2013). The repair of DNA lesions by the proteins requires appropriate chromatin modifications such as histone acetylation, methylation,

Table 1: List of lncRNAs regulating HR and NHEJ

S.No.	lncRNA, Chr. location	Repair pathway (HR/NHEJ)	Interacting or regulated proteins	Function	Reference
1	ANRIL, 9p21.3	Regulates HR; Effect on NHEJ is unknown	p15, p16, p14	Regulatory effects on cyclin dependent kinase inhibitors; accounts for DDR by controlling cell cycle check points, apoptosis and DNA repair by HR	Wan *et al*. (2013)
2	*PCAT-1,* 8q24.21	Regulates HR; Does not affect NHEJ	BRCA2	Controls cellular responses to genotoxic stress; has unique sequences necessary for the post transcriptional silencing of BRCA2 expression	Prensner *et al*. (2014)
3	*TODRA*1, 5q15.1	Regulates HR; Effect on NHEJ is unknown	TPIP	Promotes HR by acting on RAD51	Gazy *et al*. (2015)
4	*DDSR1,* 12q23.3	Regulates HR; Does not affect NHEJ	hnRNPUL1, BRCA1/ RAP80 complex	Controls recruitment of BRCA1 at DSBs along with hnRNPUL1	Sharma *et al*. (2015)
5	*LINP1,* 10p14	Regulates NHEJ; Effect on HR is unknown	Ku80, DNA-PKCs	Acts as molecular scaffold for Ku80 and DNAKCs during NHEJ	Zhang *et al*. (2016)
6	CUPID1& CUPID2, 11q13	Regulates HR and has mild effects on NHEJ	pRPA, RAD51	Knockdown of CUPID 1 and 2 alters recruitment of pRPA and RAD51 and inhibits HR	Betts et al. (2017)
7	*Lnc-RI,* 7p22.3	Regulates HR; No significant effect on NHEJ	RAD51	Enhances stability of RAD51 by acting as a ceRNA and binding with miR-193a-3p	Shen *et al*. (2017)

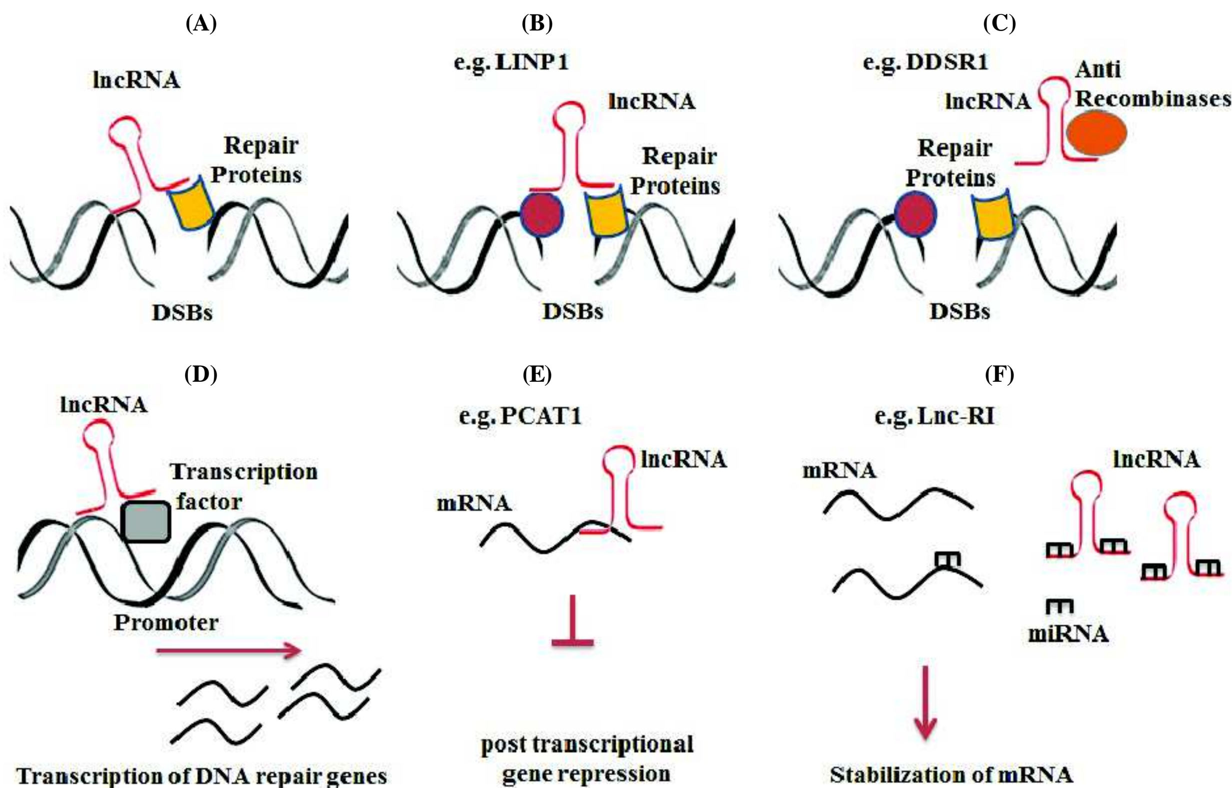

Fig. 1: Possible roles of lncRNAs in DNA repair. 1A) lncRNAs may serve as guides for recruiting DNA repair factors or chromatin modifying complexes at DSBs; 1B) lncRNAs act as molecular scaffolds for DNA repair proteins and/ or chromatin modifiers at the DNA repair foci, e.g. lncRNA LINP1 acts as a molecular scaffold to enhance interaction between the DNA repair protein Ku80 and DNA-PKCs at DSBs; 1C) lncRNAs act as decoys for proteins having anti recombinant or anti repair function, e.g. *DDSR1* binds to BRCA1 and prevents excessive recruitment of BRCA1 and RAP80 at DSBs to avoid inhibition of DNA end resection by BRCA1 and RAP80 complex; 1D) lncRNAs may act as regulators or recruiters of transcription factors involved in modulating expression of DNA repair genes; 1E). lncRNAs may bind to UTR (untranslated region) of DNA repair related transcripts and cause their post transcriptional repression, e.g.: lncRNA *PCAT-1* binds to BRCA2 and destabilizes it and 1F) lncRNAs serve as ceRNAs for DNA repair genes by binding to miRNAs involved in post transcriptional silencing of DNA repair genes and stabilize DNA repair gene mRNA, e.g. *Lnc-RI* acts as a ceRNA to stabilize *RAD51* mRNA via competitive binding with miR-193a-3p

phosphorylation and ubiquitylation (Branzei and Foiani, 2008; House *et al*., 2014). The role of small ncRNAs such as miRNAs in DNA damage and repair is well studied and has been reviewed elsewhere (Sharma and Misteli, 2013; Chowdhury *et al*., 2013; Arjumand *et al*., 2017). In this review, we discuss the role of lncRNAs in DNA double strand break repair.

Long Noncoding RNAs (LncRNAs)

Transcriptome studies using tiling microarrays and next generation sequencing techniques have led to the identification of numerous pervasively transcribed but non-coding transcripts known as lncRNAs (Rinn and Chang, 2012; Bonasio and Shiekhattar, 2014; Guttman *et al*., 2009). LncRNAs range between 200 bp to 100 kb and do not code for any proteins (Rinn and Chang, 2012; Bonasio and Shiekhattar, 2014). Based on their relative position with respect to the protein coding genes, lncRNAs are classified into four types: antisense lncRNAs, intronic lncRNAs, divergent lncRNAs and long intergenic RNAs (lincRNAs) (Rinn and Chang, 2012). LncRNAs primarily contribute to cellular function by regulating gene expression. Control of gene expression by lncRNAs involves interacting with proteins or other coding and non-coding transcripts. LncRNAs function as decoys, scaffold, and guides to regulate protein/DNA interactions (Rinn and Chang 2012) or bind to other coding or non-coding RNAs and act as competing endogenous RNA (ceRNAs) to regulate mRNA

function (Cesana *et al*., 2011; Salmena *et al*., 2011). (Fig. 1). Expression of several lncRNA molecules is altered upon DNA damage (Sharma and Misteli, 2013; Zhang and Peng, 2015) and most of these lncRNAs contribute to changes in gene expression occurring due to DNA damage (Zhang and Peng, 2015). However, a handful of lncRNAs that are mis-regulated upon DNA damage have emerged as active participants in DNA double strand break repair (Table 1). We discuss below the role of these lncRNAs in DSB repair.

Role of lncRNAs in DSB Repair

Antisense Noncoding RNA in the INK4 Locus (ANRIL)

ANRIL was the first lncRNA that was shown to play a role in DNA damage response (Wan *et al*., 2013). It is strongly induced in response to DNA damage by radiomimetic drugs in human cells. ANRIL induction upon DNA damage is regulated by kinase ATM and transcription factor E2F1. It is transcribed in the opposite direction to INK4B-ARF-INK4A genes (Wan *et al*., 2013). The protein and RNA levels of these genes are reduced post DNA damage with ANRIL over expression and increased upon ANRIL knockdown post DNA damage. Thus, repression of p15, p16 and p14 by ANRIL upon DNA damage indicates a role in regulating DDR (Wan *et al*., 2013). Interestingly, silencing of ANRIL alone leads to about 50% decrease in DNA repair by HR; the effect of ANRIL knockdown on DNA repair by NHEJ is not known (Wan *et al*., 2013). There is no evidence available to suggest that ANRIL interacts with any DNA repair molecule. However, over expression or knockdown of ANRIL alters cell proliferation and DNA synthesis. Given the fact that ANRIL over expression or knock down affects cell cycle and ANRIL most likely does not interact with DNA repair proteins, it is plausible the effect of ANRIL knockdown on DNA repair by HR is not direct, but a consequence of changes in cell cycle. The physiological functions of ANRIL and the exact mechanism of regulation of HR by ANRIL is not known.

Prostate Cancer Associated Transcript 1 (PCAT-1)

Prensner *et al.* (2014) characterized *PCAT-1* as a prostate cancer specific lncRNA implicated in the regulation of DSB repair by HR. Interestingly, expression of *PCAT-1* is not induced by DNA damage and neither the transcription factors involved in its expression are known. *PCAT-1* overexpression in prostate cancer tumor samples is associated with low levels of *BRCA2*. Overexpression of *PCAT-1* in Du145 and RWPE cancer cells results in the down regulation of *BRCA2*. Since *BRCA2* inactivation impairs DNA repair of DSBs by HR, over expression of *PCAT-1* leads to significant reduction in DNA repair by HR (Prensner *et al*., 2014). This is accompanied by decreased RAD51 foci formation and enhanced γH2AX foci formation. However, the expressions of other effectors of DNA repair: XRCC1, XRCC3, XRCC4, Ku70, Ku80 and *BRCA1* is unaffected by *PCAT-1* over expression. Thus, *PCAT-1* expression decreases HR by specific downregulation of *BRCA2* (Prensner *et al*., 2014). In addition, PARP1 inhibition in *PCAT-1* over expressed cells resulted in increased cell death compared to control cells. *PCAT-1* does not regulate transcription of *BRCA2* by interacting with chromatin or transcription factors but rather it is involved in post transcriptional repression of *BRCA2* (Prensner *et al*., 2014). *PCAT-1* overexpression is able to directly repress the activity of the *BRCA2* 32 untranslated region (UTR) and that this repression requires the 52 end of *PCAT-1*. The authors speculate that that alternative mechanism of miRNA-like mismatch base pairing may contribute to *PCAT-1*-mediated regulation in a manner similar to competing endogenous RNAs (Fig. 1E) (Prensner *et al*., 2014; Salmena L *et al*., 2011).

Transcribed in the Opposite Direction of RAD51 (TODRA)

Gazy *et al*. characterized a divergent lncRNA, named as *TODRA* (Transcribed in the Opposite Direction of *RAD51*), that is transcribed 69bp upstream to *RAD51*, in the opposite direction and regulates DNA repair by HR. The intergenic RAD51 promoter region facilitates transcription of *TODRA*. Transcription factor E2F1 binding results in RAD51 expression and *TODRA* downregulation. *TODRA* overexpression promotes DNA repair by HR in a RAD51-dependent manner, and also increases formation of DNA damage-induced RAD51-positive foci (Gazy *et al*., 2015). *TODRA* overexpression induces TPIP expression, which in turn functions as

co-factor for E2F1 to induce RAD51 expression. *RAD51* expression in breast tumors was positively correlated with *E2F1* expression and negatively correlated with *TODRA* levels (Gazy *et al*., 2015). The exact mechanism how *TODRA* contributes to DNA repair by regulating RAD51 is not clear and needs further investigation.

DNA Damage Sensitive RNA1 (DDSR1)

Using a genome-wide microarray screen we identified a novel ubiquitously expressed lncRNA, *DDSR1* (DNA damage-sensitive RNA 1), which is induced upon DNA damage by several DNA double-strand break (DSB) agents. *DDSR1* is a 1.6-kb transcript induced with intermediate kinetics by the ATM-NF-κB pathway upon DNA damage but independent of p53 (Sharma *et al*., 2015). Loss of *DDSR1* impairs DDR signaling and reduces DNA repair capacity by HR. *DDSR1* is induced relatively late upon DNA damage (~3 h), at a time when recruitment of repair factors to DSBs and initial phases of DNA repair have already occurred. Yet, we find that loss of *DDSR1* acutely reduces DNA repair efficiency by HR, indicating that the basal levels of *DDSR1* are important for DNA repair (Sharma *et al*., 2015). Interestingly HR defect upon DDSR1 knockdown is characterized by increased BRCA1 and RAP80 accumulation at DSB sites. These observations, coupled with the fact *DDSR1* interacts with BRCA1 and this interaction is reduced upon DNA damage, suggest that *DDSR1* sequesters the excess BRCA1-RAP80 complex and prevents it from aberrant DNA binding. *DDSR1* by binding to BRCA1 limits its availability at DSB sites and prevents the HR limiting activity of BRCA1 and RAP80 complex (Fig. 1C). This explanation agrees well with our finding that *DDSR1* knockdown results in reduced DNA end resection a characteristic of excess BRCA1 and RAP80 recruitment to DSB sites (Sharma *et al*., 2015). Interestingly, lncRNA *DDSR1* also interacts with hnRNPUL1, an RNA-binding protein involved in modulating HR by regulating DNA end-resection. As in the case with *DDSR1* depletion, loss of hnRNPUL1 also results in aberrant BRCA1 and RAP80 recruitment at DSB sites. Our results indicate that *DDSR1*/hnRNPUL1 depletion results in HR inhibition due to reduced end resection caused by aberrant accumulation of BRCA1 and RAP80 at DSBs. These results establish a role for lncRNA *DDSR1* in maintaining genome stability (Sharma *et al*., 2015). In addition to HR defect caused by *DDSR1* depletion, loss of *DDSR1* leads to mis-regulation of numerous genes involved in DNA damage and repair such as *CENPW, MCM6, ANP32E, HELLS, HIST1H2A* and up-regulation of p53 target genes, suggesting that *DDSR1* negatively regulates p53-mediated gene expression (Sharma *et al*., 2015).

LncRNA in NHEJ pathway 1 (LINP1)

LINP1 is a lncRNA that is over expressed in triple negative breast cancer (TNBC) and regulates DNA repair by NHEJ (Zhang *et al*., 2016). To identify the lncRNAs associated with TNBC, Zhang *et al*. analyzed lncRNA expression in distinct pathological and molecular subtypes of breast cancers in the Cancer Genome Atlas (TCGA) dataset and breast cancer cell lines and discovered that LINP1 is significantly over expressed in TNBC samples and cell lines. EGF signaling mediated activation of the RAS-MEK-JNK pathway is involved in expression of LINP1 expression in TNBC and p53 pathway inhibits LINP1 expression via miR-29 expression (Zhang *et al*., 2016). Enhancement of LINP1 and NHEJ activity occurs immediately after DNA damage; while miR-29 mediated downregulation of LINP1 occurs at the later stages of DDR. Thus p53-miR-29 mediated LINP1 regulation is speculated to act as a negative feedback mechanism to reduce NHEJ in cells during the completion of DDR. LINP1 expression is positively correlated with the RNA expression of *EGFR* and *CDKN2A*, but negatively with *RB1*. Loss of LINP1 sensitized breast cancer lines to cell death by Doxorubicin and causes a significant decrease in DNA repair by NHEJ (Zhang *et al*., 2016). In line with its role in regulating NHEJ, LINP1 interacts with key regulatory proteins Ku80 and DNA-PKcs involved in NHEJ. N-terminal of LINP1 (1-300 nt) is essential for its interaction with Ku80 and region from nucleotides (600-917) is essential for interacting with DNA-PKCs (Fig. 1B). LINP1 knockdown also results in reduced association between Ku80 and DNA-PKcs after IR treatment suggesting LINP1 serves as an RNA scaffold to enhance the molecular interaction between Ku80 and DNA-PKcs in the NHEJ pathway (Zhang *et al*., 2016). Since cells without LINP1 expression (e.g. MCF7) can still repair DNA via the NHEJ pathway, LINP1 does not appear to be prerequisite for the

NHEJ process but over expression of LINP1 in non-LINP1 expressing cells enhances NHEJ mediated DNA repair activity suggesting LINP1 plays an important role in increasing the NHEJ mediated DNA repair activity.

CCND1-upstream Intergenic DNA Repair 1 and 2 (CUPID1 and CUPID2)

Betts *et al*. identified two lncRNAs named CUPID1 (transcribed from the positive strand) and CUPID2 (transcribed from the negative strand) that share a bidirectional promoter and are transcribed 20 kb away from transcriptional enhancer PRE1. RNA sequencing data suggests that CUPID2 is widely expressed in multiple tissues; but CUPID1 is predominantly expressed in ER^+ breast cancer cell lines (Betts *et al*., 2017). Both CUPID1 and CUPID2 are highly expressed in hormone receptor positive breast cancers, specifically in luminal A and B subtypes (Betts *et al*., 2017). The expression of CUPID1 and CUPID2 is regulated by the enhancer PRE1 by acting on the bidirectional promoter. Knockdown of CUPID1 and CUPID2 in DR-GFP MCF-7 cells significantly reduced the DNA repair by HR. Loss of CUPID1 and CUPID2 reduced the formation of phosphorylated RPA foci and hampered recruitment of RAD51 at DNA repair sites. This was accompanied by increase in 53BP1 recruitment at DSBs suggesting that the DNA repair occurs through NHEJ in the absence of CUPID1 and CUPID2. Consistent with this, NHEJ reporter assays demonstrated mild increase in DNA repair by NHEJ upon CUPID1 and CUPID2 depletion. Even though depletion of CUPID1 and CUPID2 alters recruitment of DNA repair factors such as RPA and RAD51 at DSB sites but it is not known if lncRNAs CUPID 1 and 2 interact with these repair proteins. How CUPID 1 and 2 alter recruitment of repair factors at DSBs needs to be further investigated.

Ionizing Radiation-inducible lncRNA- (lnc-RI)

Lnc-RI is a radiation-inducible lncRNA that was previously known to be involved in the control of mitosis by regulating *PLK1* expression. *Lnc-RI* promoter contains NF-κB binding sites and its induction upon DNA damage is NF-κB dependent (Shen *et al*., 2017). *Lnc-RI* expression is negatively correlated with micronucleus frequencies in the peripheral blood lymphocytes of healthy adults. Knock-down of *lnc-RI* expression significantly increases accumulation of spontaneous DSBs and impairs DNA repair by HR, this is accompanied by increased expression and stability of RAD51 mRNA (Shen *et al*., 2017). Since lncRNAs have been reported to bind miRNAs competitively to regulate mRNA stability, Shen *et al* searched for miRNAs that may bind to both Rad51 and *Lnc-RI.* Their analysis revealed that *Lnc-RI* functions as a ceRNA to relieve the inhibitory effects of miR-193a-3p on *RAD51* expression (Shen *et al*., 2017). Over expression of miR-193a-3p mimics inhibited the expression of both *lnc-RI* and *RAD51*, and enhanced DNA damage as indicated by increased γ-H2AX expression. Loss of *lnc-RI* expression leads to an enhanced interaction between miR-193a-3p and the *RAD51* mRNA 3'UTR, which accelerates the degradation of *RAD51* mRNA to suppress *RAD51* expression, eventually impairing DNA repair by HR (Fig. 1F). This results suggests *lnc-RI* as a novel regulator of the HR pathway that plays an important role in the maintenance of genome stability (Shen *et al*., 2017).

Conclusions

DNA Damage Response causes differential expression of several ncRNA species including lncRNAs (Sharma and Misteli, 2013; Zhang and Peng, 2015). The identification of lncRNAs in DSB repair pathways increases the diversity of molecular components involved in DDR and suggests that DNA repair is more complicated than currently understood. Majority of these small and long ncRNAs involved in DDR participate in regulating transcriptional responses involved in cell cycle arrest or apoptosis with no direct role in DNA repair. Till date, only seven lncRNAs have been shown to be involved in regulating DSB repair by HR or NHEJ using diverse mechanisms and have been discussed in this study (Table 1).

Following are the ways by which lncRNAs contribute to DNA repair by HR and NHEJ (i) lncRNAs may function as scaffolds for DNA repair proteins and/ or chromatin modifiers at the DSB sites to promote their interaction and to retain them at the site of DSB, e.g. lncRNA LINP1 acts as a molecular scaffold to enhance interaction between the sensor of DNA damage Ku80 and kinase DNA-PKCs at DSBs (Fig. 1B), (ii) lncRNA may function as decoys

by binding to anti-recombinases and prevent their recruitment and action at DNA repair foci or may prevent excessive or aberrant accumulation of DNA repair molecules at DSBs, e.g., DDSR1 by binding to RNA binding protein hnRNPUL1 and DNA repair protein BRCA1, serves as a decoy to prevent excessive recruitment of BRCA1 and RAP80 complex at DSBs to avoid inhibition of DNA end resection by BRCA1 and RAP80 (Fig. 1C) (iii) lncRNAs act as ceRNAs for DNA repair genes by competing for binding with miRNAs involved in their post transcriptional silencing to prevent miRNA mediated downregulation of DNA repair genes, e.g., *Lnc-RI* acts as a ceRNA to stabilize *RAD51* mRNA via competitive binding with miR-193a-3p (Fig. 1F), iv) lncRNAs may directly bind to UTR of mRNA of DNA repair gene and cause their post transcriptional repression, e.g: lncRNA *PCAT1* binds to BRCA2 and destabilizes it by an unknown mechanism (Fig. 1E), (v) lncRNAs may serve as guides for recruiting chromatin modifying complexes or DNA repair factors at DSBs (Fig. 1A), although recruiting chromatin complexes at their site of action has emerged as the prominent function of lncRNAs while regulating gene expression, no lncRNA that contributes to chromatin recruitment at DSBs has been identified at this stage but lncRNAs CUPID1 and CUPID2 depletion results reduced recruitment of repair factors at DSBs but the mechanism by which CUPID1 and 2 controls recruitment of DNA repair factors at DSBs remains unclear and (vi) lncRNAs may interact with transcription factors involved in modulating expression of DNA repair genes and control their function in gene regulation (Fig. 1D). As of now there is no lncRNA has been identified that regulates direct transcription of DNA repair molecules but lncRNAs are involved in post-transcriptional regulation DNA repair genes.

DNA repair requires three major steps (i) sensing or recognition of DNA damage by proteins such as Ku80 or MRN (ii) recruitment of repair proteins such as BRCA1 and 53BP1 and (iii) repair by effector molecules such as DNA polymerases or DNA ligases. In principle, lncRNAs can regulate any of these steps involved in DNA Repair by interacting with different proteins specifically regulating these different aspects of DNA repair. Although there is no evidence to indicate that lncRNAs are directly present at the site of DSBs and are involved in DSB sensing, but because Ku80 is involved in sensing DSBs and localizes to DNA repair sites it is worth speculating that LINP1 that interacts with it, may transiently be present at the site of DSB and may be aiding in DNA damage recognition. To convincingly establish the role of lncRNAs in DNA damage sensing it will be necessary to demonstrate its localization to DSBs by RNA FISH along with co-immunostaining with DNA repair molecules that are present at DNA repair foci. The second stage of DNA repair involves recruitment of DNA repair molecules at DSBs sites and there is convincing evidence to suggest that lncRNAs are involved in regulating recruitment of DNA repair molecules at DSB sites. For example DDSR1 binds to BRCA1 and depletion of DDSR1 causes excessive recruitment of BRCA1 at DSBs suggesting DDSR1 is involved in sequestering excess BRCA1 from going to DSBs. The last stage of DNA repair involves either synthesizing a complementary strand in HR or direct joining of DNA ends in NHEJ, there is no evidence till date to indicate that lncRNAs are involved in this stage of DNA repair. Considering the kinetics of DNA repair and the currently known modes of action of lncRNAs in DNA repair, it is obvious that lncRNAs are involved in fine tuning the DNA repair process and are not master regulators of DNA repair.

Looking at the complexity with which identified lncRNAs function in DNA repair (Fig. 1), it is likely that many additional lncRNAs regulating DNA repair will be identified in near future, but it is important to determine if they play a direct role in DNA repair or contribute to DNA repair indirectly due to changes in cell cycle. The study of lncRNA function in DNA repair is still in its infancy and many important aspects still need to be addressed (1) Do lncRNAs play a role in determining the choice of repair between HR or NHEJ? (2) LncRNAs are involved in regulating recruitment of repair proteins at DNA repair foci but are there any lncRNAs that are directly present at DSB sites and do they play any role in preserving DNA repair foci integrity? (3) Is there any crosstalk among lncRNAs that regulate DNA repair? (4) How do lncRNAs coordinate DNA repair in time- and space-dependent manner? and (5) Over-expression and knockdown of repair related lncRNAs sensitizes cancer cells to chemotherapy or radiation in vitro but it remains to be seen if these strategies will be successful in clinical settings. Understanding the role of lncRNAs in DNA repair will increase our

understanding of mechanisms of maintaining genome stability and help us develop new targeted therapies for cancer.

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