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Noncoding RNA joins Ku and DNA-PKcs for DNA-break resistance in breast cancer

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Abstract

The noncoding RNA LINP1 acts as a scaffold that links Ku and DNA-PKcs and enables efficient DNA double-strand-break repair through nonhomologous end joining (NHEJ), thereby enhancing the resistance of triple-negative breast cancer cells to radiation and chemotherapies.

The American Cancer Society forecasted over 230,000 new cases of invasive breast cancer in 2015, of which ~12% were predicted to be triple-negative breast cancer (TNBC), owing to loss of expression of estrogen and progesterone receptors and human epidermal growth factor type II receptor (HER2). TNBC is an aggressive form of breast cancer with a relatively poor short-term prognosis, in part because there are no targeted therapies¹. In this issue, Zhang *et al.*² provide evidence that a long noncoding RNA (lncRNA) molecule that is highly expressed in TNBC cells acts as a scaffold that promotes DNA repair, thus promoting resistance to radiation and chemotherapeutic drugs in TNBC cells. The exciting finding that RNA molecules can directly regulate DNA-repair pathways offers possibilities for new therapeutic approaches for TNBC.

Less than 2% of the human genome comprises protein-coding genes, whereas ~70% of the genome is transcribed into thousands of noncoding RNAs³. Among these are ~16,000 lncRNAs, defined as >200-base transcripts that have no apparent protein-coding function⁴. lncRNAs form complex secondary structures and are highly regulated. Through their ability to bind proteins and nucleic acids, lncRNAs can modulate protein-protein and protein–nucleic acid interactions that control many cellular processes. Indeed, lncRNAs are emerging as key players in human disease, including cancer⁵. Zhang *et al.*² have identified over 100 lncRNAs that are enriched in TNBC and have found that depletion of one, the

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lncRNA ENSG00000223784—which they rename lncRNA in the NHEJ pathway 1 (LINP1)—enhances doxorubicin-induced cell death in TNBC cell lines. Like ionizing radiation (IR), doxorubicin treatment induces DNA double-strand breaks (DSBs), the most toxic form of DNA damage. In human cells, DSBs are mainly repaired by the NHEJ pathway. Thus, these studies suggest that the major pathway for the repair of DNA DSBs in human cells may be regulated by the lncRNA LINP1, whose dysregulation might promote therapeutic resistance in TNBC².

NHEJ is initiated when a DNA DSB is detected and bound by the Ku70–Ku80 heterodimer (Fig. 1). Ku then recruits additional NHEJ proteins to the DSBs, which process and subsequently ligate the broken DNA ends⁶. One well-characterized Ku-interacting protein is the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a serine/threonine protein kinase that is essential for NHEJ in human cells⁷. Zhang *et al.*² have demonstrated that LINP1 interacts with and enhances formation of the Ku–DNA–PKcs complex. Moreover, depletion of LINP1 attenuates DSB repair, induces radiation sensitivity and, in mouse xenografts, delays tumor growth after exposure to IR. Together, their data reveal that the interaction of LINP1 with the Ku–DNA–PKcs complex enhances NHEJ-mediated DSB repair and that depleting LINP1 improves the response to IR *in vitro* and *in vivo*.

Zhang *et al.*² have also identified new links among epidermal growth factor signaling, p53 status and LINP1 that enhance the implications of these results for potential cancer therapy. Specifically, they report that epidermal growth factor receptor (EGFR), which is frequently amplified in TNBC, increases LINP1 expression through the Ras–MAP kinase pathway, whereas wild-type p53 decreases LINP1 expression through a miR-29-dependent pathway. Because p53 is frequently mutated in TNBC, dual regulation of LINP1 by overactivation of EGFR signaling combined with a loss of downregulation by p53 may enhance NHEJ and promote resistance to IR and chemotherapy². Furthermore, because LINP1 links Ku and DNA-PKcs, which jointly initiate NHEJ, modulating LINP1 may provide an additional mechanism to control pathway choice in DSB repair. Inhibition of the MRE11 endonuclease, which licenses repair via homologous recombination, promotes a switch to NHEJ repair, thereby eliminating repair defects from BRCA2 mutations⁸. Targeting LINP1 expression may similarly enable a new therapeutic strategy in TNBC that can overcome the effects of defects in p53 and EGFR and the resultant LINP1 dysregulation.

The study by Zhang *et al.*² additionally raises interesting biological questions regarding the mechanisms of DSB repair. Whereas NHEJ is largely defined by its ability to detect and repair DSBs, Ku also interacts with RNA, specifically the telomerase-associated RNA component hTR^{9,10}, and this interaction may facilitate regulation of proteins involved in telomere-length maintenance¹¹. Like LINP1, large segments of hTR are predicted to form stem-loops and double-stranded RNA elements, thus raising the possibility that Ku may interact directly with RNA secondary structures. The core DNA-binding region of Ku70–Ku80 contains a preformed ring domain that binds to ends of double-stranded DNA with high affinity and low sequence specificity¹². However, Ku can also interact with circular single-stranded DNA^{13,14}, most probably through stem-loop structures. As mentioned above, the interaction of Ku with DNA promotes the recruitment of additional NHEJ proteins, including DNA-PKcs, which is flexibly linked to Ku80 via Ku80's C-terminal

domain¹⁵. How LINP1 provides a scaffold to stabilize this interaction, and how this enhanced stability promotes NHEJ, remain to be determined. Other factors known to enhance NHEJ include aprataxin and polynucleotide kinase phosphatase like protein (APLF), which interacts with Ku80 and XRCC4 (ref. 16), thus suggesting that perhaps, like LINP1, APLF may also have a scaffolding role. Indeed, emerging data underscore the importance of scaffolding molecules in both homologous recombination and NHEJ. Consequently, both DSB-repair pathways are increasingly being viewed as dynamic interactive assemblies of multiprotein complexes rather than as linear pathways in which each protein leaves, and the next one takes its place^{6,17}. It will be exciting to elucidate how LINP1 and other potential DSB-repair scaffolds facilitate interactions required for regulation, end processing and ligation while helping to tether DNA ends. In addition, it will be interesting to determine whether LINP1 and NHEJ are involved in the translocations and deletion breakpoints associated with non-B-DNA-forming sequences in cancer genomes¹⁸.

The findings of Zhang *et al.*² may also be more broadly relevant to DNA-damage-response mechanisms, because recent screens have revealed that over 50% of genes that induce endogenous DNA damage when suppressed are involved in RNA metabolism^{19,20}. Although recent research has focused largely on the roles of R loops in the DNA-damage response²¹, the present study² suggests that lncRNAs and other RNA molecules may be directly involved in regulating DNA repair, and their potential multifaceted effects in cancer and DNA-repair biology may open new avenues for research and possible therapeutic strategies.

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Figure 1.

Regulation of NHEJ by LINP1 in TNBC. IR and/or chemotherapeutic agents induce DSBs that are detected by the Ku70–Ku80 heterodimer (orange). The C-terminal region of Ku80 forms a flexible arm that interacts with DNA-PKcs (blue). Recruitment of the XLF–XRCC4–DNA ligase IV–APLF–PNKP complex results in ligation of the DSB. Zhang *et al.*² have shown that LINP1 interacts with the Ku–DNA–PKcs complex and promotes NHEJ, possibly by acting as a flexible scaffold that enhances the interaction of DNA-PKcs with Ku–DNA. LINP1 expression is itself enhanced by EGFR amplification and is negatively

regulated by wild-type p53. MAPK, MAP kinase; XLF, XRCC4-like factor; LIG4, DNA ligase IV; PNKP, polynucleotide kinase–phosphatase; slashes denote ‘and’. Targeting LINP1 may provide a new strategy for regulating NHEJ, thereby increasing the sensitivity of TNBC cells to radiation and chemotherapy. The scaffolding function of LINP1 in linking Ku and DNA-PKcs supports the concept of a dynamic multiprotein machine ‘core’ that mediates concerted NHEJ functions.

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