Long noncoding RNA LINP1 regulates repair of DNA double-strand breaks in triple-negative breast cancer

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Long noncoding RNAs (lncRNAs) play critical roles during tumorigenesis by functioning as scaffolds that regulate protein-protein, protein-DNA or protein-RNA interactions. Using a clinically guided genetic screening approach, we identified lncRNA in nonhomologous end joining (NHEJ) pathway 1 (LINP1), which is overexpressed in human triple-negative breast cancer. We found that LINP1 enhances repair of DNA double-strand breaks by serving as a scaffold linking Ku80 and DNA-PKcs, thereby coordinating the NHEJ pathway. Importantly, blocking LINP1, which is regulated by p53 and epidermal growth factor receptor (EGFR) signaling, increases the sensitivity of the tumor-cell response to radiotherapy in breast cancer.

Triple-negative breast cancer (TNBC), an aggressive subtype associated with poor clinical outcomes, represents approximately 10–20% of breast cancer cases1–3. A large proportion of TNBC (50–75%) exhibits the molecular subtype known as basal-like breast cancer, characterized by high expression of genes that are normally expressed in the basal epithelial layer. The absence of estrogen-receptor and progesterone-receptor expression and Her2 amplification limits the therapeutic options for this disease to surgery with adjuvant chemotherapy and radiotherapy1–3. Currently, there are no effective targeted therapies, although EGFR amplification, TP53 mutation, BRCA1 and BRCA2 loss and PI3-kinase-pathway activation have been exploited for TNBC treatment. Given the lack of recurrent, targetable genomic alterations, functional characterization of the TNBC genome is crucial to identify driver genomic events1–3. The human genome contains ~20,000 protein-coding genes (PCGs), representing less than 2% of the total genome, whereas nearly 70% of the human genome is transcribed into RNA, thus yielding thousands of noncoding RNAs4. However, because genomic studies of TNBC have mainly focused on PCGs, the functions of noncoding genes remain largely unknown.

lncRNAs are defined as RNA transcripts >200 nt that lack apparent protein-coding potential5–13. More than 15,900 lncRNA genes have recently been identified in the human genome, on the basis of GENCODE annotations4. Notably, their expression is strikingly cell-type- or tissue-restricted and, in many cases, is even primate specific. Investigations of lncRNAs have demonstrated that they can serve as scaffolds or guides regulating protein-protein or protein-DNA interactions, as decoys that bind proteins or microRNAs (miRNAs), or as enhancers of gene expression when transcribed within enhancer regions or their neighboring loci. Because of the highly dysregulated expression of lncRNAs in cancer14,15, lncRNAs have been surmised to contribute to tumorigenesis. In fact, certain lncRNAs have been shown to function as oncogenes or tumor suppressors5–13. For example, HOTAIR induces breast cancer metastasis16 by operating as a tether that links EZH2 (PRC2) and LSD1, thereby coordinating their epigenetic regulatory functions17. LINK-A promotes metabolic reprogramming toward glycolysis as well as tumorigenesis, and its expression is increased in TNBC18.

DNA repair, a collection of processes by which damaged DNA is identified and corrected in cells, is essential to genomic integrity and is involved in tumorigenesis. Although multiple proteins that mediate DNA repair have been identified, it is presently unknown whether RNA molecules are also components of the DNA-repair machinery. NHEJ is one of the major pathways for repairing damaged DNA in cancer cells19–26. In response to DNA double-strand breaks (DSBs), the Ku80–Ku70 heterodimer associates with the broken ends, forming a clamp-like complex that recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to sites of damage. Additional protein factors, including Artemis, DNA ligase IV, XRCC4, and XLF assemble with the Ku80–Ku70–DNA-PK complex and promote processing and ligation of the broken ends19–26. To identify lncRNAs that are functionally involved in tumorigenesis of TNBC, we analyzed the expression profile of lncRNAs in The Cancer Genome Atlas (TCGA) breast cancer data sets and performed a clinically guided genetic screening in TNBC cell lines.

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RESULTS
Identification of the TNBC-associated lncRNA LINP1
To identify lncRNAs associated with TNBC, we analyzed differences in lncRNA expression among the distinct pathological and molecular subtypes of breast cancers in the TCGA data set (Fig. 1a). We found 330 (fold change ≥2) and 45 (fold change ≥5) lncRNAs whose expression was significantly higher in TNBC compared with non-TNBC tumors. When the nonbasal tumors (luminal A, luminal B and Her2 enriched) were treated as a whole, a total of 402 (fold change ≥2) and 69 (fold change ≥5) lncRNAs were expressed at a significantly higher level in basal tumors. When we compared four molecular subtypes individually, we identified 164 (fold change ≥2) and 75 (fold change ≥5) lncRNAs that were specifically enriched in basal subtypes. By cross-comparing the three gene lists, we found an overlap of 154 (fold change ≥2) and 35 (fold change ≥5) lncRNAs whose expression was enriched in TNBC tumors (Fig. 1b and Supplementary Table 1), including the most recently identified TNBC-enriched lncRNA, LINK-A18. To identify the lncRNAs that are functionally involved in TNBC, we performed short interfering RNA (siRNA) screening in MDA-MB-231 cells. Of the 35 highly enriched lncRNA candidates, expression of 20 was detected in MDA-MB-231 cells. We designed 40 siRNAs targeting 20 lncRNA candidates (Supplementary Table 2) and individually transfected them into MDA-MB-231 cells, which we treated with doxorubicin (a first-line chemotherapy drug for TNBC1–3) 48 h after siRNA transfection (Supplementary Fig. 1a). We measured apoptosis via caspase3 activity 24 h after doxorubicin treatment and identified the lncRNA ENSG00000223784 (LINP1) as a strong candidate.

To corroborate the above findings, we analyzed RNA-seq data of breast cancer cell lines (n = 46) from the Cancer Cell Line Encyclopedia (CCLE) data set17. We found that LINP1 was expressed at a significantly higher level in basal lines than in nonbasal lines, in agreement with TCGA data (Fig. 1c and Supplementary Fig. 1b). We chose two TNBC lines (MDA-MB-231 and MDA-MB-468), a triple-negative immortalized breast line (MCF10A), and one estrogen-receptor-positive line (MCF7) as models for functional assays. Northern analysis confirmed the RNA-seq results, revealing that LINP1 expression was highly enriched in TNBC lines as well as MCF10A cells but was undetectable in MCF7 cells (Supplementary Fig. 1b,c). As anticipated, transduction of LINP1 siRNAs in the three
Expression and genomic alteration of LINP1 in breast cancer

To define the molecular and pathological relevance of LINP1 in TNBC, we extracted the expression and copy-number alteration data for LINP1 and key known breast cancer–associate genomic alterations, along with the clinical annotations, from TCGA (Fig. 2a–d). We observed significantly higher LINP1 expression in basal breast cancer (Fig. 2e), a result consistent with our earlier findings (Fig. 1a). Notably, the somatic copy number of LINP1 was significantly amplified in basal breast cancer (Fig. 2f), and there was a significant positive correlation between the gene copy number and RNA expression of LINP1 in the breast cancer samples (R = 0.26). These observations suggest that gains in the somatic copy number of the LINP1 gene is a mechanism by which the RNA expression level of LINP1 is increased in basal breast tumors. We further analyzed the correlation between the LINP1 expression and expression of key molecular markers for breast cancer. We found that whereas LINP1 expression was positively correlated with expression of EGFR and CDKN2A mRNAs, it was negatively correlated with RB1 expression (Fig. 2g). Intriguingly, we observed that the expression of LINP1 was significantly higher in cells expressing TP53 mutants rather than wild-type (WT) TP53 (Fig. 2h).

Finally, we found that the LINP1 RNA was present in normal breast tissues (Supplementary Fig. 2a) and was distributed in both nuclear and cytoplasmic fractions of cells (Supplementary Fig. 2b).

LINP1 associates with proteins of the NHEJ pathway

To explore the molecular mechanisms underlying the biological activity of LINP1, we used an RNA pull-down assay followed by MS to identify LINP1-associated proteins (Fig. 3a and Supplementary Table 3). Interestingly, two proteins involved in the NHEJ pathway19–26, Ku80 and DNA-PKcs, were present only in LINP1-associated samples (Supplementary Fig. 3a). To verify this result, we analyzed the lncRNA-pulldown protein samples by western blotting with antibodies specific to Ku80 and DNA-PKcs. We observed strong signals for Ku80 and DNA-PKcs in proteins pulled down with LINP1 RNA but not in proteins associated with either antisense LINP1 or beads alone (Fig. 3b), thus confirming that Ku80 and DNA-PKcs are indeed specifically enriched in the LINP1-associated protein complex. To confirm that the association between LINP1 and Ku80 DNA-PKcs was not an in vitro artifact, we tested the interaction between endogenous LINP1 and these two proteins by capture hybridization analysis of RNA targets (CHART)28 (Fig. 3c,d). LINP1 was enriched from the cross-linked chromatin extracts by C oligonucleotides (oligos) 1.2 and 1.4 but not by C oligo 1.2S and 1.4S (Fig. 3e). Furthermore, western analysis detected Ku80 and DNA-PKcs only in the complexes that were enriched by C oligos 1.2 and 1.4 (Fig. 3f). Together, these results strongly suggest that endogenous LINP1 interacts with the Ku80–Ku70 heterodimer and DNA-PKcs. To confirm the interaction of LINP1 and Ku80 DNA-PKcs, we performed RNA-immunoprecipitation assays (RNA-IP) in which RNA–protein complexes were immunoprecipitated...
with Ku80-, Ku70-, or DNA-PKcs-specific antibodies (Fig. 3g,h). Compared with the IgG-bound complexes, the complex bound by Ku80-, Ku70-, and DNA-PKcs-specific antibodies had significantly higher levels of LINP1 RNA. We observed no enrichment of the negative control GAPDH in the complexes immunoprecipitated by antibodies specific to Ku80, Ku70, or DNA-PKcs (Fig. 3i). In addition, RNA-IP experiments with UV-cross-linked RNA indicated that LINP1 was associated with the Ku80–Ku70 heterodimer and that LINP1 appeared to directly bind Ku80 but not to Ku70 (Fig. 3i). Finally, we mapped the regions of LINP1 that interact with Ku80 and DNA-PKcs by using RNA pulldown assays and identified a 300-nm region in the 5′ region of the LINP1 transcript (nts 1–300) that was essential for interaction with Ku80, and a 317-nm region within the 3′ region (nts 600–917) that was required for interaction with DNA-PKcs (Supplementary Fig. 3b). In aggregate, our findings indicate that Ku80 and DNA-PKcs are LINP1-associated proteins and that LINP1 uses distinct regions to interact with these two proteins.

**LINP1 serves as a modular scaffold in the NHEJ pathway**

Given that LINP1 RNA binds to Ku80 and DNA-PKcs, two proteins with established roles in the NHEJ pathway, we hypothesized that LINP1 might play a role in DSB repair. We therefore examined the effect of LINP1 knockdown on the repair of ionizing radiation (IR)-induced DNA damage, by using comet assays. Whereas the level of DNA damage gradually returned to the baseline in the control cells 24 h after IR treatment, it remained high in the LINP1-knockdown cells, thus suggesting that DNA repair was delayed in cells with LINP1 inhibition (Fig. 4a,b). We further confirmed this result on the basis of differences in phosphorylated histone H2AX (γ-H2AX) levels at various time points after IR treatment; the LINP1-knockdown cells, compared with control cells, had higher levels of γ-H2AX for prolonged time periods (Supplementary Fig. 4a). Whereas the level of γ-H2AX in the control cells 24 h after IR treatment was comparable to that at 0 h, γ-H2AX levels remained high in the LINP1-knockdown cells. Furthermore, we counted the number of γ-H2AX-positive foci formed in the control and LINP1-knockdown cells in response to the IR treatment. Consistently with our previous result, the number of γ-H2AX-positive foci quickly diminished in control cells but was sustained in LINP1-knockdown cells (Fig. 4c,d). Together, these observations suggest that DSB-repair activity is impaired by LINP1 knockdown. To test whether NHEJ is the pathway affected by LINP1, we used an NHEJ reporter assay. In MDA-MB-231 cells, in which LINP1 is highly expressed, we observed a decrease in NHEJ activity after LINP1 knockdown (Fig. 4e); conversely, in MCF7 cells, in which LINP1 expression is undetectable, NHEJ activity increased dramatically when the cells were transduced with LINP1 but not with control or antisense LINP1 (Fig. 4e). These results support the idea that LINP1 enhances DSB repair via the NHEJ pathway.

**Figure 3** LINP1 RNA associates with Ku80 and DNA-PKcs. (a) Schematic representation of the RNA pulldown assay. WB, western blotting. (b) Western analysis of Ku80 and DNA-PKcs levels in the protein complexes pulled down by LINP1, antisense LINP1, or empty beads from whole cell extracts of MDA-MB-231 cells. Input, 5% input of whole cell lysates. (c) Schematic representation of the CHART assay. (d) Schematic diagram of LINP1 RNA, the 11 C oligos (red bars) designed for the CHART assay, and the three primer sets (convergent arrows) used in the RNase H-sensitivity assay. RNase H sensitivity is indicated with blocks with different shades of red, with darker shades indicating higher sensitivity. (e) Enrichment of LINP1 with C oligos 1.2 and 1.4, as measured by qRT–PCR. GAPDH is a negative control. (f) Presence of Ku80 and DNA-PKcs in complexes enriched in C oligos 1.2 and 1.4, detected by western analysis. C oligos 1.2S and 1.4S are nonspecific controls. (g) Schematic representation of an RNA-IP assay. (h) Results from native RNA-IP (top) and cross-linking and immunoprecipitation (CLIP) (bottom) with Ku80-, Ku70-, and DNA-PKcs-specific antibodies. qRT–PCR assays showing the level of LINP1 and GAPDH assays showing the level of LINP1 and GAPDH (nonspecific control) in the coprecipitates. Ku80 (left), Ku70 (middle) and DNA-PKcs (right). Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 technical replicates. Uncropped images of gels are shown in Supplementary Data Set 2.
We next analyzed the dynamic levels of chromatin-associated LINP1, Ku80, DNA-PKcs, and γ-H2AX in response to IR treatment. We isolated chromatin-associated complexes at 10-min intervals from 0 to 60 min after irradiation. We measured the levels of Ku80, DNA-PKcs, and γ-H2AX in chromatin-associated complexes by western blot analysis and the level of LINP1 by qRT–PCR. In response to IR treatment, the levels of Ku80, DNA-PKcs, γ-H2AX, and LINP1 all increased in the chromatin-associated complex (Fig. 4f). Because the level of LINP1 remained unchanged in the whole cell lysates (Fig. 4f), this observation suggests that LINP1 was recruited to the chromatin after IR treatment. Furthermore, we found that IR treatment induced the association between LINP1 and Ku80 or DNA-PKcs, as measured by RNA-IP analysis (Fig. 4g).

To further define the mechanism responsible, we analyzed the effects of LINP1 knockdown on the levels of chromatin-associated Ku80 and DNA-PKcs after IR treatment. Whereas LINP1 knockdown significantly decreased the level of chromatin-associated DNA-PKcs, it had no effect on Ku80 (Fig. 4h). We then knocked down Ku80 or DNA-PKcs to test whether either factor affects the chromatin recruitment of LINP1. Interestingly, we found that Ku80 knockdown resulted in a significant decrease in chromatin-associated LINP1 (Fig. 4i), but DNA-PKcs knockdown resulted in no significant changes in LINP1 on chromatin (Fig. 4j). Finally, we examined the effect of LINP1 on the interaction of Ku80 and DNA-PKcs by IP followed by western blotting.

In LINP1-knockdown cells, there was less association between Ku80 and DNA-PKcs after IR treatment (Supplementary Fig. 4b). Collectively, our results suggest that LINP1 may serve as an RNA scaffold that enhances the molecular interaction between Ku80 and DNA-PKcs in the NHEJ pathway.

**LINP1 expression is activated by the EGF signaling pathway**

The observed correlation between LINP1 and EGFR expression (Fig. 2g) is intriguing because overexpressed or amplified EGFR has been reported in TNBC1. To further confirm this finding, we analyzed LINP1 and EGFR RNA expression in the CCLE data set. Expression of LINP1 positively correlated with EGFR expression in cancer cell lines (Fig. 5a), and these results were consistent with those from primary specimens (Fig. 2g), thus indicating that the EGR–LINP1 correlation is cell autonomous and has no contribution from tumor stromal RNA. We treated three breast lines with EGF and measured the expression of LINP1 and the expression of transcription factors downstream of the EGF pathway in the CCLE data set and found...
**Figure 5** LINP1 is activated by the EGF signaling pathway. (a) Correlation between LINP1 and EGF expression in CCLE. Expression levels of 935 cancer cell lines from CCLE were determined by the RPKM (reads per kilobase per million mapped reads) from RNA-seq. R value was calculated by two-sided Pearson’s test. (b) Expression of LINP1 in MDA-MB-468, MCF10A, and MCF7 cells treated with EGF or control. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 technical replicates. (c) Schematic diagram of EGFR signaling. Small-molecule inhibitors of EGFR signaling and their specific targets are indicated. (d) Expression of LINP1 in MDA-MB-468 and MCF10A cells treated with different small molecules that inhibit different parts of the EGFR pathway. RNA expression was analyzed by qRT–PCR. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 technical replicates. (e) Left, quantification of the amount of LINP1 promoter bound to c-Jun or c-Fos in MDA-MB-468, MCF10A, and MCF7 cells. The promoters were pulled down by antibodies to c-Jun or anti-c-Fos and measured by qPCR analysis. GAPDH is a negative control. Middle and right, quantification of the amount of LINP1 promoter bound to c-Jun or c-Fos in MDA-MB-468 (middle) and MCF7 (right) cells, which were treated with EGF. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 technical replicates. (f) Left, illustration of an AP1-binding site at −102 (red indicates the consensus motif) and the sequences of the AP1 mutations (blue indicates the mutant nucleotides). Middle, luciferase reporter assay of the promoter activities of the LINP1 core promoter construct and its AP1-mutant counterpart in MDA-MB-468 cells treated with EGF. Right, luciferase (Luc) reporter assay assessing the promoter activities of the LINP1 core promoter construct in MDA-MB-468 cells with different small molecules that inhibit different parts of the EGFR pathway. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 technical replicates.

**Figure 6** LINP1 is repressed by the p53 signaling pathway. (a) LINP1 expression in the CCLE breast cancer cell lines in which the TP53 mutation status is known. P < 0.01 by two-tailed Student’s t test; n, number of breast cancer cell lines used for analysis. (b) LINP1 expression in cells of different TP53 status, in response to nutlin-3a treatment. MCF10A and HCT116, TP53 WT; MDA-MB-231, TP53 mutant; HCT116 with TP53 deletion, TP53 null. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 technical replicates. NS, not significant. (c) Luciferase assay measuring the transcription activity of LINP1 and construct containing a known p53 binding site in cells expressing control vector or WT TP53. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 independent cell cultures. (d) Left, sequence alignment showing the complementarity between LINP1 exon 2 and miR-29. Red, seed sequence of miR-29. Middle, LINP1 expression in cells with different TP53 status after treatment with vehicle or miR-29. Right, luciferase assay measuring the activity of WT or mutant LINP1-luciferase fusion reporter constructs in response to treatment with miR-29 mimic. Error bars, s.d. *P < 0.05 by two-tailed Student’s t test; n = 3 independent cell cultures.
EGFR inhibitor (Gefitinib), the MEK inhibitor (AZD6244), and the JNK inhibitor (SP600125) all significantly reduced the expression of LINP1 in triple-negative lines, whereas the PI3K inhibitor (LY294002) had little effect on the expression of LINP1 (Fig. 5d). By analyzing the binding of c-Jun or c-Fos from the chromatin immunoprecipitation–sequencing (ChIP–seq) data from ENCODE, we observed a strong enrichment of c-Jun or c-Fos to the promoter region of LINP1 in MDA-MB-231, MDA-MB-468, and MCF10A cells but not in MCF7 cells (Supplementary Fig. 5). Importantly, we found that EGF treatment further increased binding between c-Jun or c-Fos and the LINP1 promoter in MDA-MB-468 cells but had no effect in MCF7 cells (Fig. 5e). We found a consensus AP1-binding site at −102 bp from the transcription start site of LINP1 (Fig. 5f). EGF treatment significantly increased the promoter luciferase activity of WT but not that of AP1-mutated LINP1 (Fig. 5f). Consistently with the effects of EGF treatment, the EGFR inhibitor (gatifinib), the MEK inhibitor (AZD6244) and the JNK inhibitor (SP600125) all significantly reduced the activities of the reporter, whereas the PI3K inhibitor (LY294002) had little effect on activity (Fig. 5f). Together, these findings demonstrate that EGF signaling, specifically its activation of the RAS–MEK–JNK pathway, is involved in regulating LINP1 expression in TNBC.

LINP1 expression is repressed by the p53 signaling pathway

TNBC shows a high frequency of TP53 mutations. The differential expression of LINP1 in breast cancer specimens with different TP53 mutation statuses (Fig. 2h) suggests that the p53 pathway might play a role in regulating the expression of LINP1. To delineate the possible link to p53, we looked for a correlation between LINP1 expression and TP53 mutation status in breast cancer cell lines from CCLE. Consistently with our findings in primary tumors, the breast cancer cells with mutant TP53 had a significantly higher expression of LINP1 (Fig. 6a). Next, we found that nutlin-3a treatment significantly decreased the expression of LINP1 in MCF10A and HCT116 cells, which express WT p53, but not in MDA-MB-231 cells with mutated TP53 or HCT116 cells with homozygous TP53 deletion (Fig. 6b), thus suggesting that LINP1 expression is negatively regulated by the WT p53 pathway. We used the LINP1 promoter luciferase reporter.
in cells transduced with WT TP53 cDNA and used a luciferase construct containing a known p53-binding site as the positive control. Whereas the activity of a construct containing a known p53 binding site dramatically increased after TP53 expression, the luciferase activity of the LINP1 construct remained unchanged (Fig. 6c), thus suggesting that p53 may not directly regulate the transcription of LINP1. Consistently with this observation, ChIP-seq data from HCT116 cells also revealed a clear p53 binding signal in the CDKN1A (p21) promoter in TP53 WT cells but not in the LINP1 promoters in any of the cell lines studied. Together, these results suggest that p53 may regulate LINP1 expression via an indirect pathway.

Next, we observed two regions in LINP1 exon 2 (Fig. 6d) that are fully complementary to the seed sequence of miR-29, which is positively and directly regulated by p53 (ref. 30). To determine whether p53 regulates LINP1 expression via miR-29, we treated the cells with nutilin-3a and confirmed an increase in miR-29 expression in TP53 WT but not mutant or null cells (Supplementary Fig. 6). Then we transduced a miR-29 mimic into MCF10A and HCT116 cells, as well as into HCT116 cells bearing a homozygous TP53 deletion, and measured LINP1 levels. In all three lines, expression of the miR-29 mimic decreased expression of LINP1 (Fig. 6f). Finally, we generated reporter constructs in which WT LINP1 or LINP1 with an miR-29 seed-sequence mutation was inserted into the 3′ untranslated region of a luciferase reporter gene. After cotransfection the reporter construct with miR-29 mimics into cells, we found that the expression of the WT construct was significantly reduced in cells cotransfected with the miR-29 mimic. Importantly, unlike the WT LINP1 control construct, the mutant LINP1 construct retained a high level of expression despite the expression of miR-29 mimic (Fig. 6d). Together, these results confirm a role for the p53 pathway in repressing LINP1 expression and indicate that miR-29 is a mediator of p53-regulated LINP1 expression.

Alteration of LINP1 modulates radiation sensitivity

Radiation treatment is currently one of the standard therapies for patients with TNBC1–3, and previous studies have demonstrated that NHEJ is a key determinant of IR resistance in cancer cells19–26. We hypothesized that LINP1 may regulate the IR response by increasing NHEJ activity. First, we assessed the effect of LINP1 knockdown on the IR sensitivities of three cancer cell lines: MDA-MB-231 and MDA-MB-468, which express high levels of LINP1, and MCF7, which has undetectable levels of LINP1 expression. We introduced LINP1-specific short hairpin RNAs (shRNAs) into these cells, which we then treated with different doses of IR. We then assessed cell survival one week after the IR treatments. LINP1 shRNA expression significantly decreased survival after IR treatment in MDA-MB-231 and MDA-MB-468 but not in MCF7 cells (Fig. 7a). In contrast, expression of LINP1 RNA in MCF7 cells rendered the cells more resistant to IR (Fig. 7b). Next, we established control or LINP1-shRNA-expressing MDA-MB-231-derived xenografts in nude mice. We administered a single dose of 8 Gy IR to each tumor when the tumors reached 50 mm³ and monitored the mice until the tumors reached 900 mm³ in size. In the untreated groups, we observed a slight delay of growth in the LINP1-knockdown tumors compared with the controls (Fig. 7c). In the IR-treated groups, however, the regrowth of LINP1-knockdown tumors was significantly attenuated. At the time point at which all IR-treated control tumors reached 900 mm³, the LINP1-knockdown tumors had just started to regrow. In fact, two of the seven LINP1-knockdown tumors became undetectable after IR treatment and never reemerged during the 64-day observation period after IR (Fig. 7c). qRT–PCR analysis demonstrated that the tumors expressing LINP1 shRNAs had lower endogenous LINP1 expression than controls (Fig. 7d). We next implanted MDA-MB-231-derived tumors as described above and administered a single dose of 8 Gy IR when the tumors reached 100 mm³. We harvested the tumor tissues either 0.5 h or 24 h after the IR treatment and used γ-H2AX staining to assess the level of DSBs over time. At 0.5 h after IR treatment, a substantial amount of γ-H2AX was present in both control and LINP1-knockdown tumors. At 24 h, the level of γ-H2AX in the control tumors was significantly decreased, but the level in LINP1-knockdown cells remained high (Fig. 7e,f). These observations indicated that in LINP1-knockdown tumors, compared with control tumors, the ability to repair DSBs was reduced. Collectively, our results suggest that suppression of LINP1 expression impairs DNA-repair activity in vivo, thereby sensitizing tumors to IR treatment.

DISCUSSION

Triple-negative breast cancer is a clinically challenging disease involving multistep changes in the genome1–3. To date, changes in PCGs in TNBC genomes have been the major focus5. By sifting through genomic alterations and distinguishing ‘driver’ from ‘passenger’ alterations, a number of key PCG hubs have been uncovered, including gain of EGFR signaling or loss of TP53. However, despite these pivotal findings, resistance of TNBC to standard therapy, particularly radiation and chemotherapy, has remained poorly understood at a mechanistic level. Because PCGs constitute only 2% of the human genome, it is likely that noncoding RNAs play as-yet-undefined roles in the TNBC ‘phenome’ of therapeutic resistance. In this regard, we devised a clinically guided genetic screening approach to identify functional IncRNAs in TNBC. Using the IncRNA expression profile as an initial clinical filter, we generated a relatively short list of IncRNA candidates for more extensive testing in siRNA-based functional genetic screening. On the basis of screening for apoptosis induced by the chemotherapy drug doxorubicin, we identified LINP1 as a potential IncRNA candidate that may be involved in cell death and the DNA-damage response in TNBC. Importantly, LINP1 enhances NHEJ activity by providing a scaffold for Ku80 and DNA-PKcs. Once a DSB occurs, the Ku80–Ku70 heterodimer recruits LINP1 to the damaged DNA;
LINP1 then stabilizes the Ku80–DNA-PKcs complex, thereby increasing NHEJ-mediated DNA-repair activity (Fig. 8). Because cells lacking LINP1 expression (for example, MCF7) are still competent for NHEJ-mediated repair, LINP1 does not appear to be a prerequisite for the NHEJ process. However, expression of LINP1 in non-LINP1-expressing cells enhances NHEJ repair activity. Interestingly, Ting et al. have shown that the Ku80–Ku70 complex also interacts with hTERT (TERC), a lncRNA component of telomerase, in human cells.31

We also uncovered functional links between the noncoding (LINP1 lncRNA) and protein-coding (EGFR and TP53) genomic hubs. EGFR has been reported to be highly amplified in TNBC and to serve as a potential target for treatment1–3. Notably, the EGFR pathway is known to enhance NHEJ-mediated DNA repair, and high EGFR activity is associated with radiation resistance32,33. Here, we uncovered an additional mechanism for EGFR-induced radiation resistance, whereby EGFR activation results in the upregulation LINP1 transcription via the activation of the RAS–MEK–ERK pathway and AP1 transcription factors. Thus, in cells with EGFR activation, increased levels of LINP1 stabilize the interaction between Ku80 and DNA-PKcs and enhance NHEJ-mediated DNA-repair activity. Our study also revealed that p53 activation downregulates LINP1 expression via induction of miR-29, which targets LINP1 RNA. Because LINP1’s enhancement of NHEJ activity takes place immediately after DNA damage, whereas the miR-29-mediated LINP1 downregulation occurs at a much later time point, we speculate that the p53- and miR29-mediated LINP1 regulation may serve as a negative feedback mechanism restricting the level of NHEJ-mediated DNA-repair activity in cells long after damage. High frequencies of EGFR amplification and TP53 mutations in TNBC may increase LINP1 expression at the transcriptional and post-transcriptional levels, respectively. Moreover, copy-number amplification of the LINP1 gene itself may further enhance the response to increased EGFR activity and loss of TP53 repression in TNBC.

Because of limited therapeutic targets, TNBC is typically treated with surgery and a combination of radiation and chemotherapy, which induce various types of DNA damage1–3. The NHEJ pathway, which repairs DSBs in DNA, is one of the major pathways in tumor cells that respond to radiation treatment and chemotherapeutic agents19–26. Inhibition of the NHEJ pathway has been proposed to synergize with DNA-damaging therapies for TNBC1–3. In addition, the NHEJ pathway may also be a key feature of genomic rearrangement and instability19–26, which are fundamental features of TNBC1–3. We believe that a better understanding of the role of lncRNA in the NHEJ pathway will not only provide a deeper understanding TNBC development but also help to refine the classification and treatment of this disease.

METHODS

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.Z., C.V.D. and L.Z. conceptualized and designed the experiments. Y.Z., Q. He, L.F., W.S., and C.L. performed the molecular and cellular biology experiments. Z.H., Y. Fan, Z.T. and Y.J. performed bioinformatics analysis. Y.Z., Z.H., L.Y., X.H., J.L.T., Y.F., Q. Huang, and K.M. analyzed and interpreted data. Y.Z., Y. Feng, C.V.D., and L.Z. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

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1. Fouliks, W.D., Smith, I.E. & Reis-Filho, J.S. Triple-negative breast cancer. N. Engl. J. Med. 363, 1938–1948 (2010).
2. Carey, L., Winer, E., Viale, G., Cameron, D. & Gianni, L. Triple-negative breast cancer: disease entity or title of convenience? Nat. Rev. Clin. Oncol. 7, 683–692 (2010).
3. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 490, 61–70 (2012).
4. Derrien, T. et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 22, 1775–1789 (2012).
5. Mercer, T.R., Dinger, M.E. & Mattick, J.S. Long non-coding RNAs: insights into functions. Nat. Rev. Genet. 10, 159–199 (2009).
6. Rinn, J.L. & Chang, H.Y. Genome regulation by long non-coding RNAs. Annu. Rev. Biochem. 81, 145–166 (2012).
7. Kutman, M. & Rinn, J.L. Modular regulatory principles of large non-coding RNAs. Nature 482, 339–346 (2012).
8. Balatska, P.I. & Chang, H.Y. Long non-coding RNAs: cellular address codes in development and disease. Cell 152, 1298–1307 (2013).
9. Ørom, U.A. & Shiekhattar, R. Long non-coding RNAs usher in a new era in the biology of enhancers. Cell 154, 1190–1193 (2013).
10. Listinsky, I.S., Bartel, D.P. lincRNAs: genomics, evolution, and mechanisms. Cell 154, 26–46 (2013).
11. Tay, Y., Rinn, J. & Pandolfi, P.P. The multilayered complexity of ceRNA crosstalk and competition. Nature 505, 344–352 (2014).
12. Sahu, A., Singhal, U. & Chinnaiyan, A.M. Long non-coding RNAs in cancer: from function to translation. Trends Cancer 1, 93–109 (2015).
13. Ling, H. et al. Junk DNA and the long non-coding RNA twist in cancer genetics. Oncogene 34, 5003–5011 (2015).
14. Jey, M.K. et al. The landscape of long non-coding RNAs in the human transcriptome. Nat. Genet. 47, 199–208 (2015).
15. Yan, X. et al. Comprehensive genomics characterization of long non-coding RNAs across human cancers. Cancer Cell 28, 529–540 (2015).
16. Gupta, R.A. et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464, 1071–1076 (2010).
17. Tsai, M.C. et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science 329, 689–693 (2010).
18. Sahu, A., Singhal, U. & Chinnaiyan, A.M. Long non-coding RNAs in cancer: from function to translation. Trends Cancer 1, 93–109 (2015).
19. Lord, C.J. & Ashworth, A. The DNA damage response and cancer therapy. Nature 491, 617–625 (2012).
20. Lindgren, D. & Bartek, J. DNA-damage response in human biology and disease. Nature 461, 1071–1078 (2009).
21. Lieber, M.R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu. Rev. Biochem. 79, 181–211 (2010).
22. Leng, C.J. & Ashworth, A. The DNA damage response and cancer therapy. Nature 481, 287–294 (2012).
23. Chiruvella, K.K., Liang, Z. & Wilson, T.E. Repair of double-strand breaks by end joining. Cold Spring Harb. Perspect. Biol. 5, a012757 (2013).
24. Deboris, L. & Roth, D.B. Modernizing the nonhomologous end-joining repertoire: alternative and classical NHEJ share the stage. Annu. Rev. Genet. 47, 433–455 (2013).
25. Mehta, A. & Haber, J.E. Sources of DNA double-strand breaks and models of recombination-mediated DNA repair. Cold Spring Harb. Perspect. Biol. 6, a016248 (2014).
26. Goldstein, M. & Kastan, M.B. The DNA damage response: implications for tumor responses to radiation and chemotherapy. Annu. Rev. Med. 66, 129–143 (2015).
27. Barretina, J. et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 483, 603–607 (2012).
28. Simon, M.D. et al. The genomic binding sites of a noncoding RNA. Proc. Natl. Acad. Sci. USA 108, 20497–20502 (2011).
29. Seluanov, A., Mittelman, D., Pereira-Smith, O.M., Wilson, J.H. & Gorbunova, V. DNA end joining becomes less efficient and more error-prone during cellular senescence. Proc. Natl. Acad. Sci. USA 101, 7624–7629 (2004).
30. Ulidal, A.P. et al. Aging and chronic DNA damage response activate a regulatory pathway involving miR-29 and p53. EMBO J. 30, 2219–2232 (2011).
31. Ting, N.S., Yu, Y., Pohorelic, B., Lees-Miller, S.P. & Beattie, T.L. Human Ku70/80 interacts directly with hTERT, the RNA component of human telomerase. Nucleic Acids Res. 33, 2090–2098 (2005).
32. Nyati, M.K., Morgan, M.A., Feng, F.Y. & Lawrence, T.S. Integration of EGFR inhibitors with radiochemotherapy. Nat. Rev. Cancer 6, 876–885 (2006).
33. Chen, D.J. & Nirdoul, C.S. The epidermal growth factor receptor: a role in repair of radiation-induced DNA damage. Clin. Cancer Res. 13, 6555–6560 (2007).
ONLINE METHODS
RNA-seq data processing. The poly(A)+ RNA-seq (Illumina) data, in BAM format, for human breast tumor specimens were generated and processed by the University of North Carolina (UNC) as part of the TCGA project. The poly(A)+ RNA-seq (Illumina HiSeq) data, in BAM format, for 933 human cancer cell lines across 21 cancer types was generated and processed by the Cancer Cell Line Encyclopedia (CCLE) project, a collaboration between the Broad Institute, the Novartis Institutes for Biomedical Research, and the Genomics Institute of the Novartis Research Foundation. RNA-seq files were downloaded from the Cancer Genomics Hub (http://cghub.ucsc.edu/). We imported the aligned reads of each BAM file to the Partek Genomic Suite (http://www.partek.com/) to obtain the expression levels for genes by summarizing the reads per kilobase per million mapped reads (RPKM) values. GENCODE annotations (version 18; http://www.gencodegenes.org/releases/18.html/) were used to define IncRNAs and PCGs. The log-transformed RPKM values of genes were further analyzed with Partek Genomic Suite and BRB-ArrayTools (http://linus.nci.nih.gov/BRB-ArrayTools.html).

SNP array data processing and copy-number analysis. The TCGA SNP array (Affymetrix genome-Wide Human SNP Array 6.0) data in CEL format of patients’ paired breast tumor and germline-derived DNA specimens was downloaded from the TCGA Data Portal (https://tcga-data.nci.nih.gov/tcga/). The CEL files were imported into the Partek Genomic Suite for subsequent segmentation and calculation of the predicted copy number for each given gene. A predicted copy number larger than 2.3 or smaller than 1.7 was considered to be a copy-number gain or loss for each gene, respectively. Amplified segments and GISTIC scores were visualized with IGV (http://www.broadinstitute.org/ igv/).

Cell culture. Cancer cell lines were purchased from the ATCC without further authentication. HCT116 WT and HCT116 TP53 cell lines were from B. Vogelstein (Johns Hopkins University) without further authentication. MDA-MB-231, MDA-MB-468, MCF7, HCT116 WT and HCT116 TP53 were cultured in RPMI1640 medium (Invitrogen) supplemented with 10% FBS, (FBS, Invitrogen). MCF10A was cultured in DMEM/F12 medium (Invitrogen) containing 5% horse serum, 20 ng/ml EGF, 0.5 mg/ml hydrocortisone, 100 ng/ml cholera toxin, and 1 µg/ml insulin. Cells were routinely tested for mycoplasma contamination with a Mycoplasma Plus PCR Primer Set (Agilent) and were found to be negative.

siRNA screening in the MDA-MB-231 cell line. A total of 20 IncRNAs were included in our initial screening in MDA-MB-231 cells (Supplementary Table 2). To reduce the off-target effect of the siRNAs, we designed two independent siRNA sequences targeting each IncRNA gene candidate. qRT–PCR was used to monitor the siRNAs’ knockdown efficiency. We found that 11 of 20 (55%) IncRNAs were efficiently knocked down by siRNAs. LINP1 and ENSG00000227036 were not target any human or mouse genes) were used as negative controls in the siRNA screening in the MDA-MB-231 cell line.

To reduce the off-target effect of the siRNAs, we designed two independent siRNA sequences (psiCheck-LINP1: TGCTGGGCAAGTGAATGAGTCTTCTTTTCTAGCCGGGTGTTTTCACCC GTGCCGG) or two mutant target sequences (psiCheck-LINP1 mutant: TCGAGTGGTGCTCCAGGATGGTGCTGAGATCTTAGCCGGGTTTTACGG TGGTGCC) to ensure specificity and to prevent off-target effects.

RNA isolation and qRT–PCR. Total RNA was extracted with Trizol Reagent (Invitrogen) and reverse transcribed with a High Capacity RNA-to-cDNA Kit (Applied Biosystems). cDNA was quantified with an ABI Viia 7 System (Applied Biosystems).

Protein isolation and western blotting. Western blotting was performed with the following primary antibodies: anti-Ku80 (cat. no. MA5-12933, clone no. 111, Thermo); anti-Ku70 (cat. no. MA5-13110, clone no. N3H10, Thermo); anti-DNA-PKcs (cat. no. MA5-13404, Thermo); anti-phospho-H2AX (cat. no. MA5-13109, clone no. 05-636, clone no. BW301, Millipore); anti-PARP (cat. no. 9542, CST); anti-β-tubulin (cat. no. 2128, clone no. 9F3, CST); anti-Lamin B (cat. no. ab9892, clone no. 119DS-F1, Abcam) were used; this was followed by incubation with secondary antibodies conjugated with horseradish peroxidase (anti-rabbit IgG HRP-linked antibody (cat. no. 7074S, CST) and anti-mouse IgG HRP-linked antibody (cat. no. NA931V, GE Healthcare Life Sciences)). Immunoreactive proteins were visualized with the LumiGLO chemiluminescent substrate (Cell Signaling). The antibody and validation information is provided in Supplementary Data Set 4.

Northern blot. 561-bp LINP1 cDNA fragment (266–826 bp) was cloned into pBluescript II SK (+). DIG-labeled RNA probe was transcribed in vitro and purified. 20 µg of the total RNA was fractionated on a 2% agarose gel containing 1x Denaturing Gel Buffer (Invitrogen). After visualization of 28S and 18S rRNAs, the RNA was blotted onto a nylon membrane (Whatman). After UV cross-linking, membranes were placed into a hybridization bag containing prewarmed DIG Easy Hybrid Membrane (Roche) and incubated for 30 min at 68 °C; this was followed by incubation with DIG-labeled LINP1 RNA probe (final concentration 50 ng/ml) for 14 h at 68 °C. The membranes were washed in 2x SSC and 0.1% SDS for 10 min twice at 68 °C, and detection was performed with ready-to-use CDP-Star Membrane Kit (Roche).

RNA pulldown assay. The cDNA sequence of LINP1 was cloned into pBluescript II SK (+). Biotin-labeled RNAs were transcribed in vitro and purified. 3 µg of biotinylated RNA was mixed with precleared human MDA-MB-231 whole cell lysate (containing 1 mg proteins) in 500 µl RIP buffer and then mixed with 50 µl washed streptavidin agarose beads at RT for 1 h. Beads were washed briefly with RIP buffer five times and boiled in SDS buffer. Then the retrieved proteins were detected by western blotting or by MS identification.

RNA immunoprecipitation (RNA-IP). For native RNA-IP, MDA-MB-231 extract was incubated with 10 µg of anti-Ku70 (cat. no. MA5-13110, clone no. N3H10, Thermo), anti-Ku80 (cat. no. MA5-12933, clone no. 111, Thermo), anti-DNA-PKcs (cat. no. MA5-13404, Thermo) antibody or control IgG (cat. no. 5415S, CST) and then with Protein A-Sepharose beads. After a total of three washes in RNA-IP buffer, beads were boiled in SDS buffer for western blotting or were resuspended in TRIZol reagent for real-time RT-PCR. UV-cross-linking RNA-IP (CLIP) was performed as previously described. Briefly, UV-irradiated
MDA-MB-231 cells were lysed in RSBI-Triton buffer, incubated with anti-Ku70 (cat. no. MA5-13110, clone no. N3H10, Thermo), anti-Ku80 (cat. no. MA5-12933, clone no. 111, Thermo), anti-DNA-PKcs (cat. no. MA5-13404, Thermo) antibody or control IgG (cat. no. 5415S, CST) and then precipitated with Protein A-Sepharose beads. Beads were then extracted for western blotting or real-time RT-PCR.

**Capture hybridization analysis of RNA targets (CHART).** Experiments were performed as previously described[28]. Briefly, cells were washed with PBS, and nuclei were disrupted by scraping cells with a Dounce homogenizer in sucrose buffer, diluted with an equal volume of glycogen buffer, and layered on top of glycogen buffer (4 mL). The cross-linked nuclei were collected by centrifugation and further cross-linked in 3% formaldehyde diluted in PBST for 30 min. Cross-linked nuclei were washed in PBST and resuspended in sonication buffer and then sheared with a Misonix sonicator 3000. CHART nuclear extracts were diluted 1:4 in NRB buffer. RNase H–mapping reactions were performed and analyzed as previously described[28]. RNase H–mapping oligonucleotides and sequences of qPCR primers are listed in Supplementary Data Set 3. Capture oligonucleotides were synthesized (Integrated DNA Technologies) to incorporate an internal hexaethylene glycol spacer (sp18) and a 3' biotin label with an extended spacer arm (3Bio-TEG); sequences are listed in Supplementary Data Set 3.

For each CHART reaction, 100 pmol of capture oligonucleotides was added to the extract from 10^6 cells and hybridized overnight at room temperature with gentle shaking. Hybridized material was captured with 60 µl streptavidin resin (Invitrogen) 8 h at room temperature. Bound material was washed five times with WB520 buffer. Streptavidin resin was boiled in SDS buffer for western blotting or was resuspended in TRIzol reagent for real-time RT-PCR.

**Chromatin fractionation.** MDA-MB-231 cells were fractionated as previously described[37] with modification. Briefly, cells were resuspended in cytoplasmic extract (CE) buffer and incubated on ice for 5 min. Cell lysates were centrifuged at 300g for 2 min, and the supernatant (cytoplasm fraction) was removed. The remaining pellet (enriched with nuclei) was washed with CE buffer once and then lysed in buffer B on ice for 5 min. The nuclei lysate was then centrifuged at 1700g for 4 min, and the supernatant (soluble nuclear fraction) was removed. The final pellet is the chromatin fraction.

**Comet assays.** MDA-MB-231 cells were transfected with anti-LINP1 siRNAs or control siRNA 48 h before irradiation. Cells were treated with 10 µg of IR, and harvested at 0 h (before radiation), 0.5 h, 4 h, or 24 h after IR. Neutral comet assays with SYBR Gold staining (Invitrogen) were performed. The quantification of tail DNA was performed with CASP software.

**Immunofluorescence.** siRNA-treated cells were seeded on coverslips, treated with 10 µg of IR the next day, and then harvested at 0 h (before radiation), 0.5 h, 4 h, or 24 h for immunofluorescence. Cells were fixed in solution containing 3% parafomaldehyde, 2% sucrose for 10 min at room temperature. Cells were subsequently permeabilized with 0.5% Triton solution for 5 min at 4 °C and then incubated with anti-γH2AX antibody (cat. no. ab81299, Abcam; cat. no. 05-636, clone no. JBW301, Millipore) at a dilution of 1:1,000 in PBST buffer (PBS plus 0.1% Tween-20, and 0.02% NaN3) overnight at 4 °C. Cells were then washed three times with PBST and then incubated with secondary antibody for 1 h at room temperature. After being washed four times with PBST, coverslips were mounted on glass slides with Vectashield mounting medium containing DAPI (Vector Laboratories) and visualized with an Axiovert 200M inverted microscope (Zeiss).

**Chromatin immunoprecipitation (ChIP).** ChIP was performed as previously described[38] with the following modifications. 3 × 10^6 of MDA-MB-231, MDA-MB-468, MCF10A, or MCF7 cells treated with or without 200 ng/ml of EGF were harvested for ChIP experiments. Cells were cross-linked with 1% formaldehyde at room temperature for 10 min and then neutralized with 125 mM glycine for 5 min. Cells were rinsed with ice-cold PBS twice and scraped into 1 ml of ice-cold PBS. Cells were resuspended in 0.3 ml of lysis buffer and sonicated. After centrifugation, supernatants were collected and diluted in IP dilution buffer, and this was followed by immunoprecipitation with protein A-Sepharose for 2 h at 4 °C. 5 µg anti-c-Jun (cat. no. 9165S, clone no. 60A8, CST) or anti-c-Fos (cat. no. 2250S, CST) antibody (Cell Signaling Technology) or control IgG (cat. no. 27295, CST) was used for immunoprecipitation. After immunoprecipitation, 45 µl protein A-Sepharose was added and incubated for another 1 h. Precipitates were washed, and DNA was purified after removal of cross-links for real-time PCR. Primers are listed in Supplementary Data Set 3.

**In vivo tumor experiments.** MDA-MB-231 cells were transduced with lentiviruses expressing anti-LINP1 or control shRNA, and selected in puromycin on 7 days. Three million tumor cells were injected subcutaneously into 6-week-old athymic female nu/nu mice (stock no. 002019, Jackson Labs). An 8-µg single dose was precisely delivered to the tumors of anesthetized mice with a small animal radiation research platform (SARRP) after tumors had grown to approximately 50 mm³. Tumor growth was monitored every other day with a digital vernier caliper, and tumor volumes were calculated according to the formula: tumor volume (mm³) = (1/2) × π × (tumor length) × (tumor width)². For xenograft immunofluorescence, an 8-µg single dose was delivered after tumors had grown to 100 mm³. 0.5 h or 24 h after irradiation, mice were sacrificed, and tumors were harvested for immunofluorescence of γ-H2AX. The intensity of fluorescence was quantified with ImageJ. Statistical significance of the differences was evaluated with two-tailed Student’s t tests. For all the xenograft studies, the sample size of each group is indicated in the figures. We performed pilot experiments with a few mice per group and then performed larger studies if needed to reach statistical
significance; we repeated experiments to ensure reproducibility. Owing to the nature of the performed experiments, no randomization and no blinding were used because they were deemed unfeasible. However, the resulting tumors were analyzed in a blinded manner. We treated a $P$ value of less than 0.05 as a significant difference. All experiments were performed at least twice. All animal procedures were in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

**Statistical analysis.** Statistical analysis was performed with SPSS and SAS software. All results were expressed as mean ± s.d., and $P < 0.05$ indicated significance.

34. Ule, J., Jensen, K., Mele, A. & Darnell, R.B. CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods* **37**, 376–386 (2005).
35. Zhao, J. et al. Genome-wide identification of polycomb-associated RNAs by RIP-seq. *Mol. Cell* **40**, 939–953 (2010).
36. Zhang, C. & Darnell, R.B. Mapping in vivo protein-RNA interactions at single-nucleotide resolution from HITS-CLIP data. *Nat. Biotechnol.* **29**, 607–614 (2011).
37. Méndez, J. & Stillman, B. Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. *Mol. Cell. Biol.* **20**, 8602–8612 (2000).
38. Shang, Y., Hu, X., DiRienzo, J., Lazar, M.A. & Brown, M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* **103**, 843–852 (2000).