Cell

A Large Intergenic Noncoding RNA Induced by p53 Mediates Global Gene Repression in the p53 Response

Maite Huarte,[1,2,*] Mitchell Guttman,[1,3] David Feldser,[3,4] Manuel Garber,[1] Magdalena J. Koziol,[1,2] Daniela Kenzelmann-Broz,[5,6] Ahmad M. Khalil,[1,2] Or Zuk,[1] Ido Amit,[1] Michal Rabani,[1] Laura D. Attardi,[5,6] Aviv Regev,[1,3] Eric S. Lander,[1,3,7] Tyler Jacks,[3,4] and John L. Rinn[1,2,*]

[1]The Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA
[2]Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA
[3]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[4]The Koch Institute for Integrative Cancer Research, Cambridge, MA 02139, USA
[5]Department of Radiation Oncology
[6]Department of and Genetics
Stanford University School of Medicine, Stanford, CA 94305, USA
[7]Department of Systems Biology, Harvard Medical School, Boston, MA 02114, USA
*Correspondence: mhuarte@broadinstitute.org (M.H.), jrinn@broadinstitute.org (J.L.R.)
DOI 10.1016/j.cell.2010.06.040

SUMMARY

Recently, more than 1000 large intergenic noncoding RNAs (lincRNAs) have been reported. These RNAs are evolutionarily conserved in mammalian genomes and thus presumably function in diverse biological processes. Here, we report the identification of lincRNAs that are regulated by p53. One of these lincRNAs (lincRNA-p21) serves as a repressor in p53-dependent transcriptional responses. Inhibition of lincRNA-p21 affects the expression of hundreds of gene targets enriched for genes normally repressed by p53. The observed transcriptional repression by lincRNA-p21 is mediated through the physical association with hnRNP-K. This interaction is required for proper genomic localization of hnRNP-K at repressed genes and regulation of p53 mediates apoptosis. We propose a model whereby transcription factors activate lincRNAs that serve as key repressors by physically associating with repressive complexes and modulate their localization to sets of previously active genes.

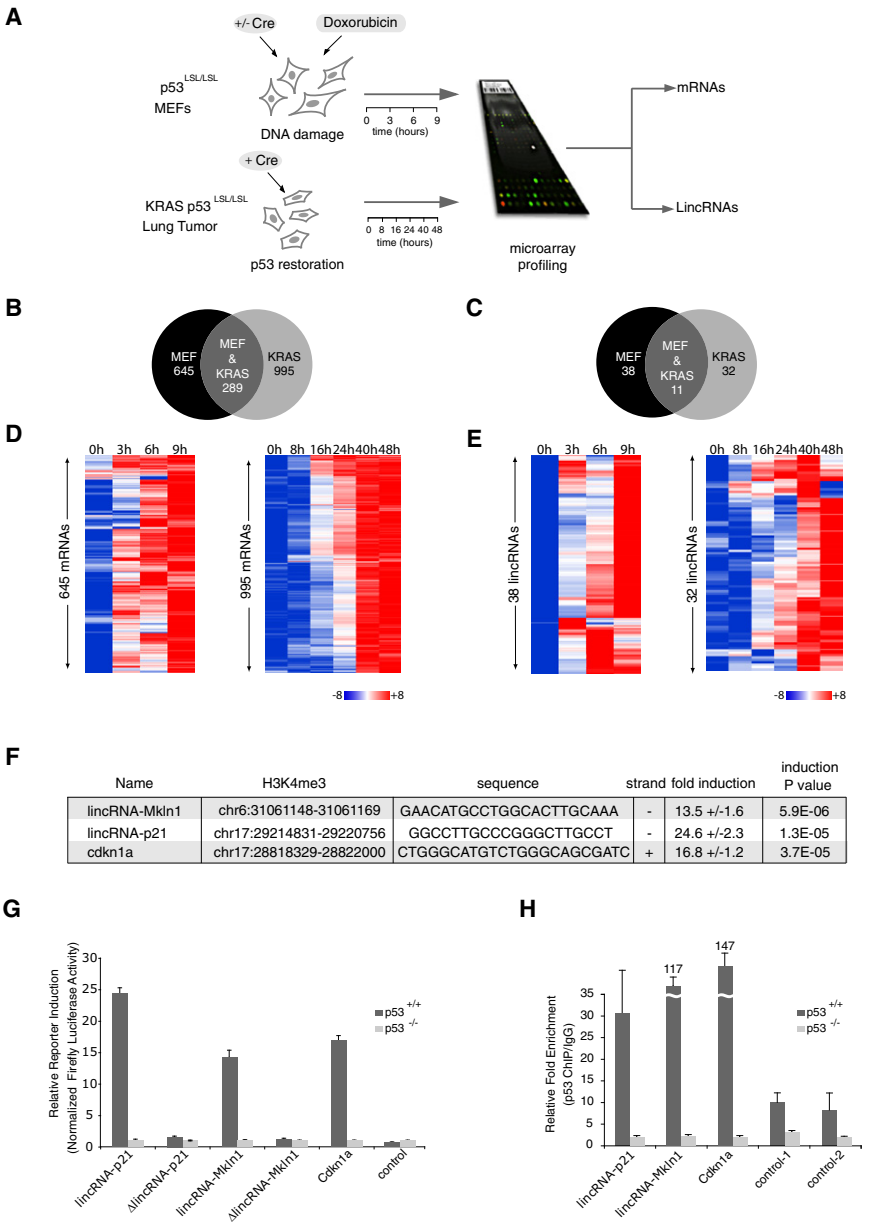
INTRODUCTION

It has become increasingly clear that mammalian genomes encode numerous large noncoding RNAs (Mercer et al., 2009; Ponting et al., 2009; Mattick, 2009; Ponjavic et al., 2007). It has been recently reported the identification of more than 1000 large intergenic noncoding RNAs (lincRNAs) in the mouse genome (Carninci, 2008; Guttman et al., 2009). The approach to identify lincRNAs was by searching for a chromatin signature of actively transcribed genes, consisting of a histone 3-lysine 4 trimethylated (H3K4me3) promoter region and histone 3-lysine 36 trimethylation (H3K36me3) corresponding to the elongated transcript (Guttman et al., 2009). These lincRNAs show clear evolutionary conservation, implying that they are functional (Guttman et al., 2009; Ponjavic et al., 2007).

In an attempt to understand the potential biological roles of lincRNAs, a method to infer putative function based on correlation in expression between lincRNAs and protein-coding genes was developed. These studies led to preliminary hypotheses about the involvement of lincRNAs in diverse biological processes, from stem cell pluripotency to cell-cycle regulation (Guttman et al., 2009). In particular, we observed a group of lincRNAs that are strongly associated with the p53 transcriptional pathway. p53 is an important tumor suppressor gene involved in maintaining genomic integrity (Vazquez et al., 2008). In response to DNA damage, p53 becomes stabilized and triggers a transcriptional response that causes either cell arrest or apoptosis (Riley et al., 2008).

The p53 transcriptional response involves both activation and repression of numerous genes. While p53 is known to transcriptionally activate numerous genes, the mechanisms by which p53 leads to gene repression have remained elusive. We recently reported evidence that many lincRNAs are physically associated with repressive chromatin modifying complexes and suggested that they may serve as repressors in transcriptional regulatory networks (Khalil et al., 2009). We therefore hypothesized that p53 may repress genes in part by directly activating lincRNAs, which in turn regulate downstream transcriptional repression.

Here, we show that lincRNAs play a key regulatory role in the p53 transcriptional response. By exploiting multiple independent cell-based systems, we identify lincRNAs that are transcriptional targets of p53. Moreover, we find that one of these p53-activated lincRNAs—termed lincRNA-p21—serves as a transcriptional repressor in the p53 pathway and plays a role in triggering apoptosis. We further demonstrate that lincRNA-p21 binds to



F

Name	H3K4me3	sequence	strand	fold induction	induction P value
lincRNA-Mkln1	chr6:31061148-31061169	GAACATGCCTGGCACTTGCAAA	-	13.5 +/-1.6	5.9E-06
lincRNA-p21	chr17:29214831-29220756	GGCCTTGCCCGGGCTTGCCT	-	24.6 +/-2.3	1.3E-05
cdkn1a	chr17:28818329-28822000	CTGGGCATGTCTGGGCAGCGATC	+	16.8 +/-1.2	3.7E-05

Figure 1. Several LincRNAs Are p53 Transcriptional Targets

(A) Experiment layout to monitor p53-dependent transcription. p53-restored (+Cre) or not restored (−Cre) p53$^{LSL/LSL}$ MEFs were treated with 500 nM dox for 0, 3, 6, and 9 hr (upper left). KRAS (p53$^{LSL/LSL}$) tumor cells were treated with hydroxytamoxifen for p53 restoration for 0, 8, 16, 24, 40, or 48 hr (lower left). RNA was subjected to microarray analysis of mRNAs and lincRNAs.

(B and C) Venn diagrams showing the number of shared and distinct mRNAs (B) or lincRNAs (C) induced in a p53-dependent manner in the MEF or KRAS systems.

(D and E) mRNAs (D) and lincRNAs (E) activated by p53 induction (FDR < 0.05) in MEF or KRAS system. Colors represent transcripts above (red) or below (blue) the global median scaled to 8-fold activation or repression, respectively.

(F) Promoter region, conserved p53 binding motif, promoter orientation, and p53-dependent fold induction in reporter assays of lincRNA promoters induced in a p53-dependent manner (values are average of at least three biological replicates [±STD]; p values are determined by t test).

(G) p53-dependent induction of lincRNA promoters requires the consensus p53 binding elements. Relative firefly luciferase expression driven by promoters with p53 consensus motif (lincRNA-p21, lincRNA-Mkln1) or with deleted motif (ΔlincRNA-p21 and ΔlincRNA-Mkln1) in p53-restored p53$^{LSL/LSL}$ (p53$^{+/+}$) or p53$^{LSL/LSL}$ (p53$^{-/-}$) cells. Values are relative to p53$^{-/-}$ and normalized by renilla levels (average of three replicates ±STD).

(H) p53 specifically binds to p53 motifs in lincRNA promoters. p53 ChIP enrichment in p53$^{+/+}$ and p53$^{-/-}$ MEFs on regions with p53 motifs (lincRNA-p21, lincRNA-Mkln1, Cdkn1a) or two irrelevant regions (controls). Enrichment values are relative to IgG and average of 3 replicates (±STD).

See also Figure S1 and Table S1.

hnRNP-K. This interaction is required for proper localization of hnRNP-K and transcriptional repression of p53-regulated genes. Together, these results reveal insights into the p53 transcriptional response and lead us to propose that lincRNAs may serve as key regulatory hubs in transcriptional pathways.

RESULTS

Numerous LincRNAs Are Activated in a p53-Dependent Manner

As a first attempt to dissect the functional mechanisms of lincRNAs, we focused on a strong association in the expression patterns of certain lincRNAs and genes in the p53 pathway (Guttman et al., 2009). In order to determine whether these lincRNAs are regulated by p53, we employed two independent experimental systems that allow us to monitor gene expression changes at different times after p53 induction (Ventura et al., 2007).

The first system uses mouse embryonic fibroblasts (MEFs) derived from mice where the endogenous p53 locus is inactivated by insertion of a transcriptional termination site flanked by loxP sites (LSL) in the first intron. This endogenous *p53* locus (p53 $^{LSL/LSL}$) is restorable by removal of the stop element by Cre recombination (Ventura et al., 2007). The p53 $^{LSL/LSL}$ MEFs were treated with AdenoCre virus expressing the Cre recombinase to reconstitute the normal p53 allele or AdenoGFP control virus to maintain the inactive p53 $^{LSL/LSL}$ allele. Then we compared the transcriptional response between the p53-reconstituted and p53 $^{LSL/LSL}$ MEFs after 0, 3, 6, and 9 hr of DNA damage treatment with doxorubicin (we will refer to this system as "MEFs") (Figure 1A). The second system uses a lung tumor cell line