RESEARCH HIGHLIGHT

PINTing for p53
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Abstract
A new study identifies the long noncoding RNA Pint as a regulator of cellular proliferation and a target of the p53 pathway.

Introduction: the new revolution in genomics
Analysis of the massive quantity of data generated by next-generation sequencing has given us a new appreciation of the complexity of the transcriptome, and has also shed light on the more than 90% of the genome that was previously thought not to have critical functions. Among these findings is the realization that a large number of noncoding RNAs map to extra- and intragenic regulatory elements known as enhancers, and that the expression of such noncoding transcripts has important functional consequences in transcriptional regulation [1-4]. However, non-enhancer classes of long intergenic non-coding RNAs (lincRNAs) may fulfill other functions in the nucleus or the cytoplasm. These transcripts are predominantly polyadenylated and processed by the splicing machinery. Importantly, many of these lincRNAs are targeted by specific transcription factors responding to critical cellular signaling pathways.

The prevailing theory regarding the mechanism of action of many nuclear lincRNAs stipulates their association with chromatin regulatory complexes, providing additional binding energy for their targeting to specific genomic loci; indeed, a number of lincRNAs have recently been shown to exhibit chromatin-binding behavior as a mechanism of gene regulation. In this issue of Genome Biology, Marín-Béjar et al. [5] identify a lincRNA termed tumor protein 53 (p53)-induced noncoding transcript (Pint) that associates with Polycomb repressive complex 2 (PRC2) and regulates transcription.

p53 targets lincRNAs
p53 has been heralded as the gatekeeper of the genome in human cancers, owing to the large number of tumors that display mutations in this transcription factor. A significant effort has therefore been directed toward understanding the direct targets of p53 transcriptional regulation and elucidating the cellular pathways that such p53 targets control. Recent studies have uncovered long noncoding RNAs as novel targets of p53 tumor suppression [6,7]. Marín-Béjar et al. used custom tiling microarrays in a mouse model system to identify lincRNAs that are regulated by p53. This analysis resulted in the identification of Pint, a lincRNA with multiple isoforms.

Subsequent experiments focused on the longest Pint isoform containing four exons, which displayed a high level of expression in most tissues examined. Analysis of the genomic locus of Pint revealed three p53 binding sites: a promoter-proximal binding site and two distal binding sites a few hundred thousand base pairs from the transcriptional start site. Functional analysis revealed that p53 binds to these regulatory sites and mediates the activation of Pint following induction of p53.

Mouse Pint promotes growth
To assess Pint functions, Marin-Bejar et al. depleted Pint levels using antisense oligos, and measured cellular growth before and after induction of DNA damage. Pint depletion led to decreased cellular proliferation, which was more prominent following DNA damage induction. Remarkably, overexpression of Pint led to an increase in cellular growth, strongly suggesting a trans-mediated mechanism of action for Pint in controlling proliferation. An analysis of the consequences of Pint depletion, on the other hand, demonstrated that lowering Pint concentration causes an increase in apoptosis and a decrease in the fraction of cells in the S-phase of the cell cycle. As expected, overexpression of Pint had opposite effects on apoptosis and cell cycle progression. Importantly, manipulation of Pint levels had similar effects in
multiple mouse cell types, suggesting a general mechanism of action for regulation of cellular growth.

To gain further insight into the mechanism by which Pint regulates proliferation, Marín-Béjar et al. depleted Pint levels following the induction of DNA damage and analyzed gene expression changes using a microarray platform. Consistent with the role of mouse Pint in the regulation of proliferation, gene expression changes in pathways regulating cellular growth and survival were uncovered, including TGF-β and MAPK pathways. Changes in gene expression were also observed in transcripts regulated by the p53 pathway. Indeed, depletion of p53 resulted in changes in gene expression that partially overlapped that of Pint depletion. Marín-Béjar et al. surmised that such gene expression changes are mediated in trans, given that the neighboring genes did not display changes in their expression following depletion of Pint, although the gene expression changes following overexpression of Pint were not analyzed.

It would be informative to know whether Pint overexpression induces opposing changes in gene expression to those seen following its depletion. Such overexpression experiments would also allow for detailed structure/function analysis of Pint with regard to gene expression and cellular proliferation.

**PINT binds PRC2 and influences its chromatin residence at a subset of genes**

To gain insight into the molecular basis of Pint transcriptional regulatory function, Marín-Béjar et al. examined its association with PRC2. The current model for the targeting of PRC2 implicates noncoding RNAs in the recruitment of this complex to its genomic sites [8-10]. Pint was found to be highly enriched in the nucleus and to associate directly with the PRC2 complex. Moreover, depletion of Pint resulted in decreased chromatin residence of PRC2 at a subset of Pint-regulated genes displaying histone H3 lysine 27 methylation, a mark of transcriptional repression.

To mechanistically link the PRC2 complex and changes in cellular proliferation by altering Pint levels, Marín-Béjar et al. examined the consequences of Pint manipulation in 3T3 cells in which the Ezh2 subunit of PRC2 was depleted. Whereas overexpression of Pint in control cells promoted growth, increased expression of Pint in the absence of PRC2 did not significantly affect cellular proliferation. These results point to a critical role for PRC2 in mediating the growth regulatory function of Pint. It is important to note that although Pint may cooperate with PRC2 at a subset of its targets, it is likely that other chromatin regulators may also associate with Pint and its scope of interaction with chromatin regulatory complexes may be much larger than PRC2 alone.

**Human PINT displays tumor suppressive function**

Having established functional and molecular characteristics of Pint in a mouse model, Marín-Béjar et al. turned their attention to the analysis of a possible homolog of Pint in human cells (PINT), initially identified on the basis of synteny with the mouse genome. It has often been difficult to discern the evolutionary conservation of lincRNAs, given that the evolutionary pressures maintained through codon usage in protein-coding genes are not present. However, in most cases the overall genomic position and short stretches of sequence conservation are preserved among close mammalian species. Indeed, small patches of PINT 5′-end sequences have homology with the mouse Pint. Moreover, similar to mouse Pint, human PINT is regulated by p53 through proximal and distal genomic binding sites.

But the functional conservation between human PINT and mouse Pint turned out to be only at a superficial level. Surprisingly, human PINT overexpression suppressed cellular proliferation, an opposite effect to that seen with mouse Pint. Indeed, analysis of a panel of human colorectal tumors revealed a significant downregulation in PINT, consistent with a role in tumor suppression. Therefore, it is tempting to speculate that PINT may function as a critical target of p53 in cancer cells and may contribute to the tumor suppressive function of p53 in human cancers.

**Concluding remarks**

We are at the very early stages of our functional understanding of lincRNAs and noncoding RNAs in general. There is much to learn about the biogenesis pathway of lincRNAs and their mechanism of action. Clearly, the current work on Pint has begun to shed light on the biological function of lincRNAs in cellular proliferation and DNA damage response through the p53 protein, in this case, intriguingly, as a mediator of p53 autoregulation. The next steps will entail the genetic dissection of Pint in model organisms and a greater insight into the sequence or structural requirements in Pint that govern interactions with PRC2 and other chromatin regulatory complexes. These are high times for the study of long noncoding RNAs as the new players in town regulating mammalian gene expression.

**Abbreviations**

Ezh2: Enhancer of zeste homolog 2; lincRNA: Long intergenic non-coding RNA; MAPK: Mitogen-activated protein kinase; p53: Tumor protein 53; Pint: p53-induced transcript; PRC2: Polycomb repressive complex 2; TGF: Transforming growth factor.

**Competing interests**

The author declares that he has no competing interests.

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References

1. De Santa F, Barozzi I, Mietton F, Ghisletti S, Polletti S, Tusi BK, Müller H, Ragoussis J, Wei CL, Natoli G: A large fraction of extragenic RNA PolII transcription sites overlap enhancers. PLoS Biol 2010, 8:e1000384.

2. Kim TK, Hemberg M, Gray JW, Costa AM, Bear DM, Wu J, Harmin DA, Laptewicz M, Barbana-Haley K, Kuersten S, Markenscoff-Papadimitriou E, Kuhl D, Bito H, Worley PF, Kreiman G, Greenberg ME: Widespread transcription at neuronal activity-regulated enhancers. Nature 2010, 465:182–187.

3. Orom UA, Marchese FP, Athie A, Sánchez Y, González J, Segura V, Huang L, Moreno I, Navarro A, Monab M, García-Foncillas J, Rinn JL, Guo S, Huarte M: PINT lincRNA connects the p53 pathway with epigenetic silencing by the Polycomb repressive complex 2. Genome Biol 2013, 14:104.

4. Huarte M, Guttmann M, Feldser D, Garber M, Kozol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Ibarra M, Attardi LD, Regev A, Lander ES, Jacks T, Rinn JL: A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 2010, 142:409–419.

5. Liu Q, Huang J, Zhou N, Zhang Z, Zhang A, Lu Z, Wu F, Mo YY: LncRNA loc285194 is a p53-regulated suppressor. Nucleic Acids Res 2013, 41:4976–4987.

6. Rinn JL, Helms JA, Farnham PJ, Segal E, Chang HY: Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007, 129:1311–1323.

7. Kaneko S, Li G, Son J, Xu CF, Margueron R, Neubert TA, Reinberg D: Phosphorylation of the PRC2 component Ezh2 is cell cycle-regulated and up-regulates its binding to ncRNA. Genes Dev 2010, 24:2615–2620.

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