

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, Marín-Béjar *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

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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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