

P53 and the defenses against genome instability caused by transposons and repetitive elements

Arnold J. Levine^{1)2)*}, David T. Ting³⁾⁴⁾ and Benjamin D. Greenbaum⁵⁾

The recent publication by Wylie et al. is reviewed, demonstrating that the p53 protein regulates the movement of transposons. While this work presents genetic evidence for a piRNA-mediated p53 interaction with transposons in *Drosophila* and zebrafish, it is herein placed in the context of a decade or so of additional work that demonstrated a role for p53 in regulating transposons and other repetitive elements. The line of thought in those studies began with the observation that transposons damage DNA and p53 regulates DNA damage. The presence of transposon movement can increase the rate of evolution in the germ line and alter genes involved in signal transduction pathways. Transposition can also play an important role in cancers where the p53 gene function is often mutated. This is particularly interesting as recent work has shown that de-repression of repetitive elements in cancer has important consequences for the immune system and tumor microenvironment.

Keywords:

■ cancer biology; cancer immunology; endogenous elements; genome stability; innate immunity; repetitive elements; transposons

Introduction

As it became clear that genetic material was composed of DNA it was relatively simple to estimate the number of possible genes in the human genome by calculating how much DNA was present in a single cell. If the average gene encoded the information for a protein of 100 amino acids, then it contained about 300 nucleotides while the genome contained approximately three billion base pairs, or the capacity for about 10 million genes. There were many assumptions in this calculation, so it was considered too high a number. This problem came to be called the *c*-value paradox. Why was there so much DNA in each cell?

The first clue that the sequence structure of the genome was more complex came from following the rate of re-association of DNA double strands (a second-order rate equation) and the clear finding that genomes contained repetitive sequence elements found at various concentrations, totaling up to 25–65% of eukaryotic DNA [1]. It was immediately proposed that such sequences played a role in shaping gene regulation [2]. The nature of these repetitive DNA sequences became clear upon the sequencing of the human genome [3, 4]. About 50% of the human genome is composed of repetitive sequence elements whose origins derive from several diverse biological processes (Table 1).

These repeats can be classified into autonomous and non-autonomous transposition species. Autonomous species have

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¹⁾ Institute for Advanced Study, School of Natural Sciences, The Simons Center for Systems Biology, Princeton NJ, USA

²⁾ Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA

³⁾ Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown MA, USA

⁴⁾ Department of Medicine, Massachusetts General Hospital, Boston MA, USA

⁵⁾ Department of Medicine, Hematology, and Medical Oncology, and Pathology, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

*Corresponding author:

Arnold J. Levine
E-mail: alevine@ias.edu

Abbreviations:

(H)ERV, (human) endogenous retrovirus; LINE, long interspersed nuclear element; LTR, long terminal repeat; SINE, short interspersed nuclear elements.

Table 1. Classes of repetitive elements in the human genome

Repetitive element	Type	Estimate of number of copies	Percentage of genome (%)	Typical length (bp)
Satellite	Tandem	427,000	3	2–100
SINE element	Interspersed	1,800,000	15	100–300
DNA transposons	Interspersed	465,000	3	200–2,000
LTR transposons	Interspersed	718,000	9	200–6,000
LINE retrotransposon	Interspersed	1,507,000	21	500–8,000
Total		4,917,000	51	

their own active mechanism for replication and include DNA transposons and retrotransposons. DNA transposons employ DNA-dependent DNA synthesis to replicate a transposon either in the genome or excised from the genome, and transpose it to another site in the genome. By contrast the LINE and the LTR (long terminal repeat) retrotransposons employ reverse transcriptase to copy RNA into DNA, which is inserted into the genome in a quasi-random fashion. A subset of retrotransposons derive from the LTR-containing retrotransposons, which originate from exogenous retroviruses that infect germline cells and insert their DNA into the genome, thus becoming endogenous retroviruses (ERVs). These viruses can then amplify their copy number by transcribing their DNA and copying the RNA into DNA that inserts into chromosomes at diverse sites.

Non-autonomous transposition repeats include the SINE elements (short interspersed nuclear elements), which are thought to arise from RNA species in the cell that are retrotransposed by active retrotransposons, the LINE retrotransposon being the most prevalent. The RNAs copied into SINES are all produced by RNA polymerase III, and the most common SINES derive from tRNAs, 7S RNA, and 5S RNA components of the ribosomes in a cell, i.e. the most abundant RNAs. The copying of mRNA to produce DNA pseudo-genes adds another small number of repetitive elements to a genome. Tandem satellite sequences are classically thought to arise by stuttering at DNA replication forks and copying the same DNA sequences one or more times. They then can be expanded by unequal crossing over during recombination. More recent data indicates that some centromeric and pericentromeric repeats in human or mouse chromosomes (producing HSATII or GSAT RNAs, respectively) can borrow an endogenous reverse transcriptase, produce a DNA copy, and insert back into the centromeric region from where they originate [5]. Together, these repetitive elements have found similar, but distinct mechanisms of expansion in genomes, generating a vast landscape upon which evolution can act. The evolution of p53's function is deeply entangled with this landscape.

Do repetitive elements have a function selected for by a host?

Most transposable elements in the human genome were inserted into the germ line millions of years ago and as such have accumulated mutations or have been only partially

copied by reverse transcription, becoming defective movable elements. Of the approximately 1.5 million LINE elements in the human genome, it is estimated that about 80–100 elements are still fully functional and capable of transposition [6]. Of the many human ERVs (HERVs), all are likely partially defective but some of the viruses do make viral particles (HERV-L and K), possibly by employing the many DNA copies in a genome to produce some functional proteins that complement the defects in each viral genome and assemble a virus particle.

Whether these repetitive elements are selfish genetic parasites in our genome that find a way to self-replicate, or have a functional role to play in normal or pathological situations remains unclear, but this has been an intensively studied subject, particularly regarding their role in the regulation of gene expression [7]. What is quite clear is that cells go to great lengths to silence the genomic information contained in repetitive elements, at least during most normal developmental and adult phases of our lifespans. Viable or movable repetitive elements in the mouse or human genome appear to be able to be transcribed, and insert new DNA copies into the genome at each generation in the germ line during meiosis or early in development in stem cells [8–12]. This could accelerate the rates of evolution and give rise to offspring that could be selected for or against in subsequent generations.

Recently it has been shown that selected LINE1 elements can replicate and move during normal neuronal development producing somatic mosaicism in mouse and human neurons of the central nervous system [13, 14]. More recent data using single cell sequencing has revealed that LINE1 retrotransposition does occur in individual human neurons. The frequency of these events may not be as high as previously thought, though their general contribution to diversity is still being understood [15, 16]. Expression of HERVs has also been found to be potentially important in normal human development. HERV-K viral particles have been shown to be expressed at the 8-cell stage of human embryogenesis, which may lead to species- and individual-specific cellular modulation in early development [17]. Intriguingly, this work also suggested that HERV-K proteins may provide an immunoprotective effect against certain exogenous viruses. In sum, the activation of repetitive elements appears in critical stages of early human development, germ cells, and plastic adult cells providing a mechanism for driving inter- and intra-individual variation as well as potential immunomodulatory effects. Thus it is possible repeats have, in some cases, a beneficial functional effect for the normal host beyond their parasitic behavior.

The p53 protein functions to restrain the movement of repetitive elements

The p53 protein is a transcription factor whose levels and activity are induced by a wide variety of stresses such as genomic damage [18]. This protein is highly conserved with regard to its primary amino acid sequence and protein structure. Furthermore, the DNA sequence specifically bound by this protein is highly conserved among diverse species from sea anemone to humans [19, 20]. In the sea anemone, *Drosophila* and *Caenorhabditis* the p53 protein is mostly expressed in the germ line and it enforces fidelity of genome integrity by killing (using a conserved apoptotic mechanism) cells containing DNA damage and abnormal genomes. In vertebrates, the p53 protein is also expressed in somatic tissues and enforces genome stability by acting as a tumor suppressor gene. Strikingly, it is the most frequently mutated gene across human cancers.

Recently Wylie and co-workers [21] employed both *Drosophila* and zebrafish models to demonstrate that the wild type p53 protein, genetically interacting with piwi RNA protein complexes in the female germ line, prevented the movement of repetitive mobile elements. These piwi RNA protein complexes have previously been shown to be important for repetitive element silencing across species [22–24]. Females with mutant p53 genes had increased levels of mobile element RNAs. As expected for random integration events of DNA copied from these RNAs, the eggs had reduced fertility and defective egg formation the extent of which was variable from animal to animal. It had been previously shown with *Drosophila* that double-stranded DNA break formation in meiosis, carried out by the highly conserved meiotic spo11 nuclease, increased the p53 protein levels in eggs undergoing recombination [25]. Increased p53 levels are found in all eukaryotes examined to date in response to single- or double-stranded breaks in DNA. Wylie and co-workers demonstrated that the p53/spo11- double mutants (with fewer DNA breaks in the DNA and no recombination) have much reduced levels of RNAs from mobile elements in their eggs. Thus spo11 is epistatic to p53 for derepression of mobile element transposition. Not only are p53 and spo11 in the same pathway but mutations in the proteins of the piwi RNA protein complex have also been shown to induce or increase p53 levels and activity relating p53 function to the piwi RNA protein complex activity [26]. Replacing the defective *Drosophila* p53 gene and protein with a wild-type human p53 protein-reduced retrotransposon RNA levels in eggs, while replacing the defective *Drosophila* gene with a mutant human p53 gene retained high levels of retrotransposon RNAs in eggs. In zebrafish with wild-type p53 levels, the open reading frame –1 (ORF-1) of a LINE element was silent in the embryo, but high levels of ORF-1 protein were expressed in p53–/– embryos. In p53 wild-type embryos, the integrated DNA LINE transcriptional enhancer region was shown to contain the transcriptionally repressive histone chromatin marks, H3K9me3. In zebrafish with a mutant p53 gene, these repressive marks were not found and LINE RNA was produced.

In parallel, it has been known for some time that cancers can express LINE-1 and other LTR retrotransposons (HERVs),

as well as SINES, and a many other repetitive elements [27]. Wylie and co-workers demonstrate a correlation between the expression of LINE-1 ORF-1 and the presence of mutant p53 genes in Wilms tumor and colon cancers consistent with their hypothesis that p53, along with DNA methylation, histone modifications, and the piRNA protein complexes regulate repetitive element movement. Their publication makes the compelling case that p53 plays a central role in regulating repetitive elements in germ line and somatic cells [21]. A number of previous publications are consistent with this idea, as will be discussed in the next section.

The mechanisms that permit p53 to restrain repetitive elements

The p53 gene and its protein are a compelling example of the co-evolution of genome stability and regulation of endogenous elements. Hoh and co-workers [28] developed the first algorithm to identify the genes regulated by the p53 protein employing the consensus p53 DNA sequence binding sites. It was soon found that p53 binding sites appear in the THE1-MaLR family of transposons [29]. Wang and co-workers found that p53 binding sites within ERV-derived LTRs accounted for 30% of p53 binding sites, and that p53 regulated the expression of many nearby genes [30]. When this algorithm was employed by Harris and co-workers [31] they found that LINE-1 elements contained a 15 nucleotide sequence in their promoter that binds a wild-type p53 protein. Recent work has found that the p53 response elements associated with transposons are less conserved but of higher occupancy and lower accessibility than non-transposon associated response elements [32]. Moreover, transposable elements have been further linked to non-coding RNA regulation, where p53 has emerged to have a regulatory role [33, 34].

Most of the p53 protein binding sites in defective LINE-1 elements are negative regulators of transcription. This negative regulation was also shown to be the case for p53 DNA binding sites found in *cis*-acting regulators of HERV-1-LTRs [35]. However, a p53 LINE-1 retrotransposon DNA binding site that is a recent addition to the primate germ line (20 million years ago) can increase the LINE-1 RNA transcription (in the absence of CpG methylation, chromatin repression, and piRNA clustering). Based upon these observations, most repetitive DNA sequences are negatively regulated by genome CpG methylation, heterochromatin repression, and piwi RNA protein complexes. When these restraints upon expression are lost the wild type p53 protein can promote transcription of some LINE-1 genomes, this results in an RNA to DNA transposition and the insertion of this DNA is always mediated by double-stranded breaks [36]. The persistence of double-stranded breaks, which are now amplified by increased transposition, activates p53 resulting in cellular apoptosis and the enforcement of genomic stability in the organism by the killing of those cells with transpositions, reinforcing the regulatory role. In *Drosophila*, DNA damage by gamma radiation results in germ line p53 activation and the transcription of several genes (reaper and

sickle), which initiate apoptosis. Thus, germ line and somatic fidelity are enforced by death.

In the germ line of female mice (but not male mice) with a p53 mutation, litter sizes are smaller, as was the case with *Drosophila* females with p53 mutants [37, 26]. In addition, about one third of the female offspring produced from p53^{-/-} × p53^{-/-} crosses had birth defects in the C57Bl/6 genetic background [37]. When the insulin-like growth factor-2 (IGF-2) gene CpG methylation pattern was examined in these p53^{-/-} offspring many of these defective mice had an altered epigenetic pattern [38]. These data suggest that the absence of the p53 protein could impact the fidelity of CpG methylation of epigenetic marks. Several observations support this hypothesis. Jackson-Grusby and co-workers [39] knocked out the DNA methyltransferase-1 gene (Dnmt1) in mouse cells. After two cell divisions in the absence of this methyl-CpG copy transferase, the cells underwent a p53-mediated apoptosis indicating that the p53 protein senses and ensures the fidelity of this methylation pattern. Mutant p53 fails to do this, and enhances epigenetic plasticity. Takahashi and Yamanaka [40] had first demonstrated that the addition of four transcription factors (c-myc, KLF-4, Oct-4, and Sox-2) to fibroblasts in cell culture results in the epigenetic reprogramming of these cells and the induction of pluripotent stem cells (iPS cells). However, if this is done in the absence of wild-type p53 the efficiency of the formation of iPS cells can increase from 10- to 100-fold, the time it takes to produce iPS cells is shorter by 5- to 10-fold and one can eliminate the need for c-myc and KLF-4 in producing iPS cells [41, 38]. Clearly there is a relationship between p53 surveillance of epigenetic marks and altered marks activating wild-type p53 [42].

Indeed the first suggestion that the p53 protein directly regulates the expression of repetitive DNA elements in cancerous cells, was demonstrated by Haoudi et al. [43]; Noutsopoulos et al. [44]; and by Leonova and co-workers, who showed that p53 mutations were required to observe the epigenetic changes and the expression of SINE repeats, satellite repeats, and other lncRNAs [45]. The treatment of mouse cancerous cells containing a mutant p53, but not wild-type p53, with 5'-aza-2'-deoxycytidine (which prevents CpG methylation in DNA) resulted in large increases in the transcription of these repetitive elements in these cells [45]. The presence of double-stranded repetitive RNAs and DNA:RNA complexes induced the innate immune system in these cells to produce interferon, which led to cell death [45]. A wide variety of human and mouse carcinomas were found to express abundant satellite and LINE repeats compared to normal tissues [46]. The highest levels of cancer-specific DNA repeats observed were the HSATII and GSAT satellites in human and mouse tumors, respectively (Fig. 1). Interestingly, these same HSATII and GSAT satellites were identified by computational sequence analysis for their unusual sequence motifs (high-CpG content) compared to other RNA transcripts, and that these motifs could trigger the innate immune system to produce cytokines [47]. Similarly, results indicating that HERV expression, can initiate the innate immune response in a variety of malignancies leading to alterations in the local tumor microenvironment that can affect the response to immunotherapies [48–50]. This parallels the suggestion that HERV-K expression in human

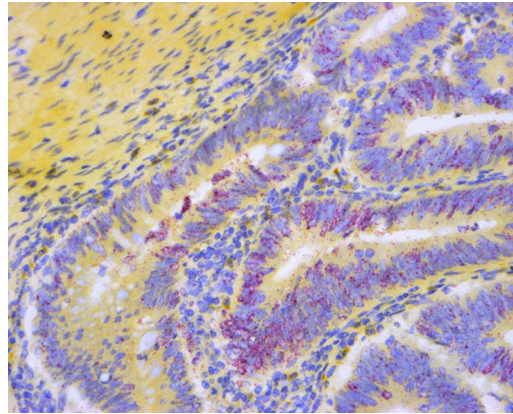


Figure 1. Primary colon adenocarcinoma stained with RNA in situ hybridization assay for HSATII non-coding RNA (red dots) and counter stained with hematoxylin. The magnification is 200×. Colon cancers are often p53 deficient. Detection shows abundant HSATII expression in tumors.

embryos have immunoprotective effects [17]. Altogether, the suppression of these repeats by p53 appears to be important in modulating the tumor cell innate immune response across many malignancies. However, the relative contribution of these different repeats to the shaping of the immune microenvironment remains to be determined. Thus there is a growing literature that demonstrates a functional consequence of expression of repetitive elements in a cell resulting in its recognition by the immune system.

In addition to these effects on the innate immune system, the expression of repetitive elements in cancer can drive genomic instability through retrotransposon insertional mutagenesis [51–53] as well as chromosomal instability driven by satellite repeat expression [54, 55]. The mutations that drive tumors in p53^{-/-} germline knockout mice result from genomic instability and are most commonly deletions, gene amplifications and rearrangements on a background of aneuploidy and chromotrypsis [56]. Based upon all of these observations it is not surprising that the loss of p53 gene functions through mutation is the single most common gene mutation observed in human cancers.

Conclusions

The first indication of a conserved p53 gene in evolution is found in modern choanoflagellates and sea anemones (early multicellular organisms) which can be traced back to a common ancestor with humans of about 800 million to a billion years ago. Not only is the amino acid sequence and protein structure of p53 conserved but the specific DNA binding site sequences employed by p53 are also conserved over this time frame. In those organisms, p53 monitors the fidelity of the germ line by killing cells with DNA damage. The repetitive DNA elements of transposons initiate double-stranded DNA breaks during movements in the genome activating the p53 protein and killing cells that undergo transposition. As such the p53 protein and the regulation of genomic instability by transposition have co-evolved in

organisms, eventually moving the p53 gene functions from the germ line into the somatic tissues of vertebrates where it becomes a tumor suppressor gene as well as a germ line fidelity factor. During this evolutionary process some transposable elements have acquired p53 DNA binding sites. After a double-stranded break, p53 is activated for transcription and some of the p53 DNA binding sites in the LINE elements attract the p53 protein and start a new round of mRNA synthesis and retrotransposition, setting up a positive feedback loop and resulting in p53-mediated cell death. When a germ line cell escapes death the newly inserted retrotransposon DNA may now activate adjacent genes for p53-regulated transcription, impacting the p53 signal transduction pathway which appears to have evolved in this manner.

Three biological processes play a role in regulating the expression and movement of transposons in the genome. The first is the methylation of cytosine residues in DNA at CpG sites and the associated chromatin modifications that produce condensed heterochromatin preventing access to enhancer/promotor elements required for transcription. The second process employs piwi proteins and their associated piwi RNA complexes, which prevent transcription of repetitive elements and can even degrade retrotransposon RNAs after their transcription. The third process that triggers a response to double stranded breaks in the genomic DNA for recombination and the insertion of retrotransposons is the p53 stress response which can initiate a program of transcription resulting in cell cycle arrest, senescence, or apoptosis eventually repairing the DNA damage or killing the cell. The loss of any one of these three processes can lead to movement of transposons and even genomic instability. Indeed these three processes communicate with each other and are interactive. Mutation of piwi proteins increases and activates the p53 transcription factor [21]. The alteration or failure of copying methylated CpG residues by DNA methyltransferase results in the activation of p53 transcription and the killing of the cell with an altered pattern of methylation [39]. A mutant p53 protein alters the probability of faithfully copying methylated cytosine residues in genomic DNA and also permits the removal of cytosine methylation from CpG residues during the reprogramming of cells to produce stem cells [38]. Clearly these three biological processes are cooperative and interactive with loss of CpG methylation or piwi protein mutations activating the p53 transcription factor to kill the cell. Thus the p53 protein acts as a suppressor of changes in CpG methylation and piwi mutational alterations.

The detailed mechanisms that mediate this communication between these three processes remain unclear. The epigenetic reprogramming of cells play an important role in the origins and evolution of cancers as do mutations in the p53 gene, which is the single most common mutation observed in human cancers. Both of these changes in cells can lead to trans-determination of cell types and an epithelial-mesenchymal transition observed in cancers. There is not a great deal of information about a role for the piwi protein-RNA complexes in cancers but it seems a natural direction to explore. The movement of retrotransposons in cancers results in insertions, double-stranded breaks in the DNA and new transcriptional programs all of which increase the rates

of cellular evolution and possibly drug resistance. The transposition of a DNA element in a single cell now identifies that cell as unique (in its genome and possibly its gene expression) in an organ. The expression of retrotransposon RNA, DNA, and proteins should have an impact upon the responses of the immune system in recognizing cancers [6, 47, 57] or alerting the immune response. These ideas bring up many new avenues to explore in the future.

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References

1. Britten RJ, Kohne DE. 1968. Repeated sequences in DNA. *Science* **161**: 529–40.
2. Britten RJ, Davidson EH. 1969. Gene regulation for higher cells: a theory. *Science* **165**: 349–57.
3. Lander ES, Linton LM, Birren B, Nusbaum C, et al. 2001. Initial sequencing and analysis of the human genome. *Nature* **409**: 860–921.
4. Venter JC, Adams MD, Myers EW, Li PW, et al. 2001. The sequence of the human genome. *Science* **291**: 1304–51.
5. Bersani F, Lee E, Kharchenko PV, Xu AW, et al. 2015. Pericentromeric satellite repeat expansions through RNA-derived DNA intermediates in cancer. *Proc Natl Acad Sci* **112**: 15148–53.
6. Brouha B, Schustak J, Badge RM, Lutz-Prigge S, et al. 2003. Hot L1s account for the bulk of retrotransposition in the human population. *Proc Natl Acad Sci* **100**: 5280–5.
7. Medstrand P, Van de Lagemaat LN, Dunn CA, Landry JR, et al. 2005. Impact of transposable elements on the evolution of mammalian genome regulation. *Cytogen Genome Res* **110**: 342–52.
8. Babushok DV, Kazazian HH. 2007. Progress in understanding the biology of the human mutagen LINE-1. *Hum Mutat* **28**: 527–39.
9. Ewing AD, Kazazian HH. 2010. High-throughput sequencing reveals extensive variation in human-specific L1 content in individual human genomes. *Genome Res* **20**: 1262–70.
10. Beck CR, Collier P, Macfarlane C, Malig M, et al. 2010. LINE-1 retrotransposition activity in human genomes. *Cell* **141**: 1159–70.
11. Huang CRL, Schneider AM, Lu Y, Niranjan T, et al. 2010. Mobile interspersed repeats are major structural variants in the human genome. *Cell* **141**: 1171–82.
12. Iskow RC, McCabe MT, Mills RE, Torene S, et al. 2010. Natural mutagenesis of human genomes by endogenous retrotransposons. *Cell* **141**: 1253–61.
13. Baillie JK, Barnett MW, Upton KR, Gerhardt DJ, et al. 2011. Somatic retrotransposition alters the genetic landscape of the human brain. *Nature* **479**: 534–7.
14. Singer T, McConnell MJ, Marchetto MC, Coufal NG, et al. 2010. LINE-1 retrotransposons: mediators of somatic variation in neuronal genomes? *Trends Neuro* **33**: 345–54.
15. Evrony GD, Cai X, Lee E, Hills LB, et al. 2012. Single-neuron sequencing analysis of L1 retrotransposition and somatic mutation in the human brain. *Cell* **151**: 483–96.
16. Denli AM, Naravaiza I, Kerman BE, Pena M, et al. 2015. Primate-specific ORF0 contributes to retrotransposon-mediated diversity. *Cell* **163**: 583–93.
17. Grow EJ, Flynn RA, Chavez SL, Bayless NL, et al. 2015. Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells. *Nature* **522**: 221–5.
18. Levine AJ, Lane DJ, eds. 2010. *Cold Spring Harbor Perspectives in Biology: The p53 Family*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

19. Belyi VA, Levine AJ. 2009. One billion years of p53/p63/p73 evolution. *Proc Natl Acad Sci* **106**: 17609–10.
20. Belyi VA, Ak P, Markert E, Wang H, et al. 2010. The origins and evolution of the p53 family of genes. *Cold Spring Harb Perspect Biol* **2**: a001198.
21. Wylie A, Jones AE, D'Brot A, Lu WJ, et al. 2016. P53 genes function to restrain mobile elements. *Genes Dev* **30**: 64–77.
22. Saito K, Nishida KM, Mori T, Kawamura Y, et al. 2006. Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the *Drosophila* genome. *Genes Dev* **20**: 2214–22.
23. Vagin VV, Sigova A, Li C, Seitz H, et al. 2006. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* **313**: 320–4.
24. Aravin AA, Hannon GJ, Brennecke J. 2007. The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. *Science* **318**: 761–4.
25. Lu WJ, Chappo J, Roig I, Abrams JM. 2010. Meiotic recombination provokes functional activation of the p53 regulatory network. *Science* **328**: 1278–81.
26. Wylie A, Lu WJ, D'Brot A, Buszczak M, et al. 2014. p53 activity is selectively licensed in the *Drosophila* stem cell compartment. *eLife* **3**: e01530.
27. Harris CR, Normart R, Yang Q, Stevenson E, et al. 2010. Association of nuclear localization of a long interspersed nuclear element-1 protein in breast tumors with poor prognostic outcomes. *Genes Cancer* **1**: 115–24.
28. Hoh J, Jin S, Parrado T, Edington J, et al. 2002. The p53MH algorithm and its application in detecting p53-responsive genes. *Proc Natl Acad Sci* **99**: 8467–72.
29. Zhurkin VB, Sirotni M, Robles A, Rauf K, et al. 2003. Distribution of p53 binding sites in the human genome reflects the diversity of p53 binding and its tumor suppressor function. *J Biomol Struct Dynam* **20**: 858–9.
30. Wang T, Zeng J, Lowe CB, Sellars RG, et al. 2007. Species-specific endogenous retroviruses shape the transcriptional network of the human tumor suppressor protein p53. *Proc Natl Acad Sci* **104**: 18613–8.
31. Harris CR, Dewan A, Zupnick A, Normart R, et al. 2009. P53 responsive elements in human retrotransposons. *Oncogene* **28**: 3857–65.
32. Su D, Wang X, Campbell MR, Song L, et al. 2015. Interactions of chromatin context, binding site sequence content, and sequence evolution in stress-induced p53 occupancy and transactivation. *PLoS Genet* **11**: e1004885.
33. Kelley D, Rinn J. 2012. Transposable elements reveal a stem cell-specific class of long noncoding RNAs. *Genome Biol* **13**: R107.
34. Younger ST, Kenzelmann-Broz D, Jung H, Attardi LD, et al. 2015. Integrative genomic analysis reveals widespread enhancer regulation by p53 in response to DNA damage. *Nucleic Acids Res* **43**: 4447–62.
35. Chang NT, Yang WK, Huang HC, Yeh KW, et al. 2007. The transcriptional activity of HERV-I LTR is negatively regulated by its cis-elements and wild type p53 tumor suppressor protein. *J Biomed Sci* **14**: 211–22.
36. Gasior SL, Wakeman TP, Xu B, Deininger PL. 2006. The human LINE-1 retrotransposon creates DNA double-strand breaks. *J Mol Biol* **357**: 1383–93.
37. Hu W, Feng Z, Teresky AK, Levine AJ. 2007. P53 regulates maternal reproduction through LIF. *Nature* **450**: 721–4.
38. Yi L, Lu C, Hu W, Sun Y, et al. 2012. Multiple roles of p53-related pathways in somatic cell reprogramming and stem cell differentiation. *Cancer Res* **72**: 5635–45.
39. Jackson-Grusby L, Beard C, Possemato R, Tudor M, et al. 2001. Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. *Nature Genet* **27**: 31–9.
40. Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**: 663–76.
41. Puzio-Kuter AM, Levine AJ. 2009. Stem cell biology meets p53. *Nat Biotech* **27**: 914–5.
42. Levine AJ, Greenbaum B. 2012. The maintenance of epigenetic states by p53: the guardian of the epigenome. *Oncotarget* **3**: 1503.
43. Haoudi A, Semmes OJ, Mason JM, Cannon RE. 2004. Retrotransposition-competent human LINE-1 induces apoptosis in cancer cells with intact p53. *BioMed Res Int* **4**: 185–94.
44. Noutsopoulos D, Markopoulos G, Vartholomatos G, Kolettas E, et al. 2010. VL30 retrotransposition signals activation of a caspase-independent and p53-dependent death pathway associated with mitochondrial and lysosomal damage. *Cell Res* **20**: 553–62.
45. Leonova KI, Brodsky L, Lipchick B, Pal M, et al. 2013. P53 cooperates with DNA methylation and a suicidal interferon response to maintain epigenetic silencing of repeats and noncoding RNAs. *Proc Natl Acad Sci* **110**: E89–98.
46. Ting DT, Lipson D, Paul S, Brannigan BW, et al. 2011. Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers. *Science* **331**: 593–6.
47. Tanne A, Muniz LR, Puzio-Kuter A, Leonova, KI, et al. 2015. Distinguishing the immunostimulatory properties of noncoding RNAs expressed in cancer cells. *Proc Natl Acad Sci* **112**: 15154–9.
48. Rooney MS, Shukla SA, Wu CJ, Getz G, et al. 2015. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* **160**: 48–61.
49. Roulois D, Yau HL, Singhania R, Wang Y, et al. 2015. DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. *Cell* **162**: 961–73.
50. Chiappinelli KB, Strissel PL, Desrichard A, Li H, et al. 2015. Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. *Cell* **162**: 974–86.
51. Lee E, Iskow R, Yang L, Gokcumen O, et al. 2012. Landscape of somatic retrotransposition in human cancers. *Science* **337**: 967–71.
52. Tubio JM, Li Y, Ju YS, Martincorena I, et al. 2014. Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes. *Science* **345**: 1251343.
53. Rodić N, Steranka JP, Makohon-Moore A, Moyer A, et al. 2015. Retrotransposon insertions in the clonal evolution of pancreatic ductal adenocarcinoma. *Nat Med* **21**: 1060–4.
54. Bouzinba-Segard H, Guais A, Francastel C. 2006. Accumulation of small murine minor satellite transcripts leads to impaired centromeric architecture and function. *Proc Natl Acad Sci* **103**: 8709–14.
55. Zhu Q, Pao GM, Huynh AM, Suh H, et al. 2011. BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. *Nature* **477**: 179–84.
56. Dudgeon C, Chan C, Kang W, Sun Y, et al. 2014. The evolution of thymic lymphomas in p53 knockout mice. *Genes Dev* **28**: 2613–20.
57. Younger ST, Rinn JL. 2015. Silent pericentromeric repeats speak out. *Proc Natl Acad Sci* **112**: 15008–9.