

p53 cooperates with DNA  
methylation and a suicidal  
interferon response to maintain  
epigenetic silencing  
of repeats and noncoding RNAs

2013, Katerina I. Leonova et al.

Kolmogorov Mikhail

# Noncoding DNA

- Mammalian genome contain an abundance of noncoding DNA sequences
- Evolutionarily younger than structural genes, and are generally considered “genomic junk”
- DNA transposons and retrotransposons
- Short, interspersed nuclear elements (SINEs)
- Tandemly organized repeats known as “satellite DNAs”
- More than 50% of the human and mouse genomes

# Evolution history

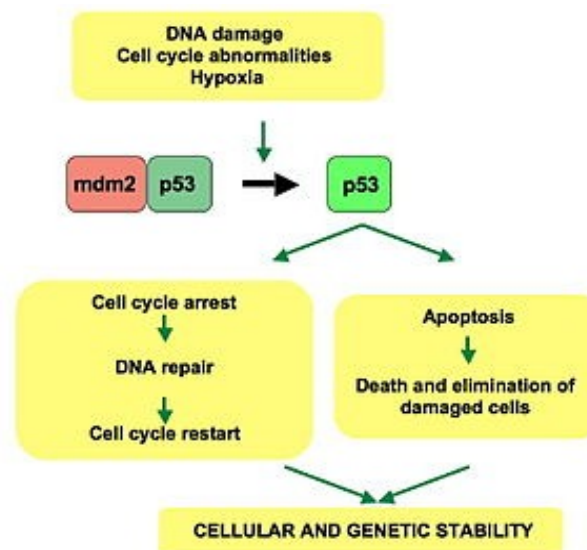
- Analysis of the phylogeny of SINEs suggests that they accumulated through multiple “explosions,” or bursts of amplification
- Started about 65 million years ago
- Located predominantly in intergenic areas, pseudogenes, and noncoding regions of genes
- Genetic catastrophes, presumably contributed to the diversity of mammalian species

# SINE

- It is logical to assume that maintenance of the genetic stability of mammalian cells and organisms depends on their ability to prevent the expression and amplification of SINEs
- Most of SINEs are located in heavily methylated regions of DNA and are believed to be epigenetically silenced by DNA methylation
- Under certain circumstances both retroelements and some classes of satellite DNA can be transcribed
- Transcription of both retroelements and satellite DNA was observed in tumors

# p53

- Tumor suppressor protein
- Plays a role in apoptosis, genomic stability, and inhibition of angiogenesis
- It can activate DNA repair proteins when DNA has sustained damage
- It can arrest growth by holding the cell cycle at the G1/S regulation point on DNA damage recognition
- It can initiate apoptosis, if DNA damage proves to be irreparable



# p53-Null and p53-WT cells

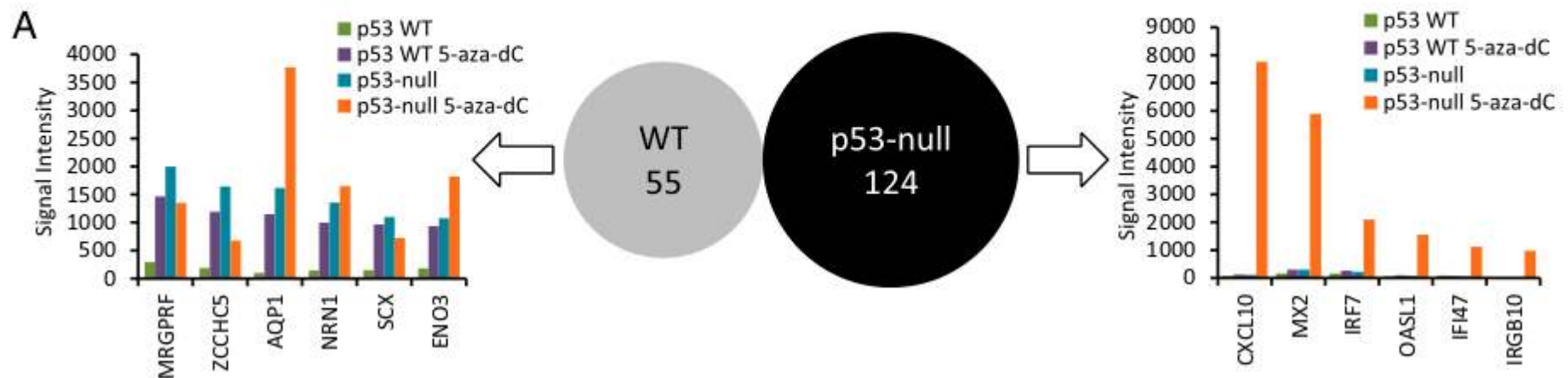
- 2004, Nieto et al.: in vitro treatment with the DNA-demethylating agent 5-aza-2'-deoxycytidine (5-aza-dC) induced apoptosis in mouse embryonic fibroblasts (MEFs) lacking p53 but not in those with WT p53
- MEF become highly sensitive to 5-aza-dC following either shRNA-mediated knockdown of p53 or ectopic expression of a dominant-negative p53 mutant
- Death of was associated with activation of caspases 3 and 7, indicating involvement of apoptosis

# Hypothesis

- Hypersensitivity of p53-null cells to 5-aza-dC might be caused by the activation of transcription of some “killer” genes that normally are repressed by the combined action of a DNA methylation and the transrepressor function of p53
- Sets of transcripts activated by 5-aza-dC treatment in p53-WT and p53-null MEFs were compared using microarray

# Different genes expression

- Treatment of p53-WT and p53-null MEFs with 5-aza-dC led to activation of 55 and 124 genes, respectively.
- There were no genes shared between these two lists

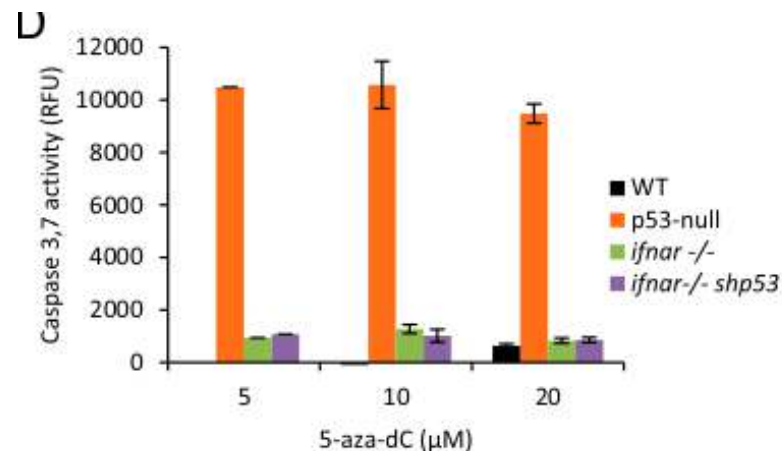


- Most of genes from p53-null cells are known transcriptional targets lying downstream of type I IFNs (IFN- $\alpha$  and IFN- $\beta$ )



# Lethal IFN Response

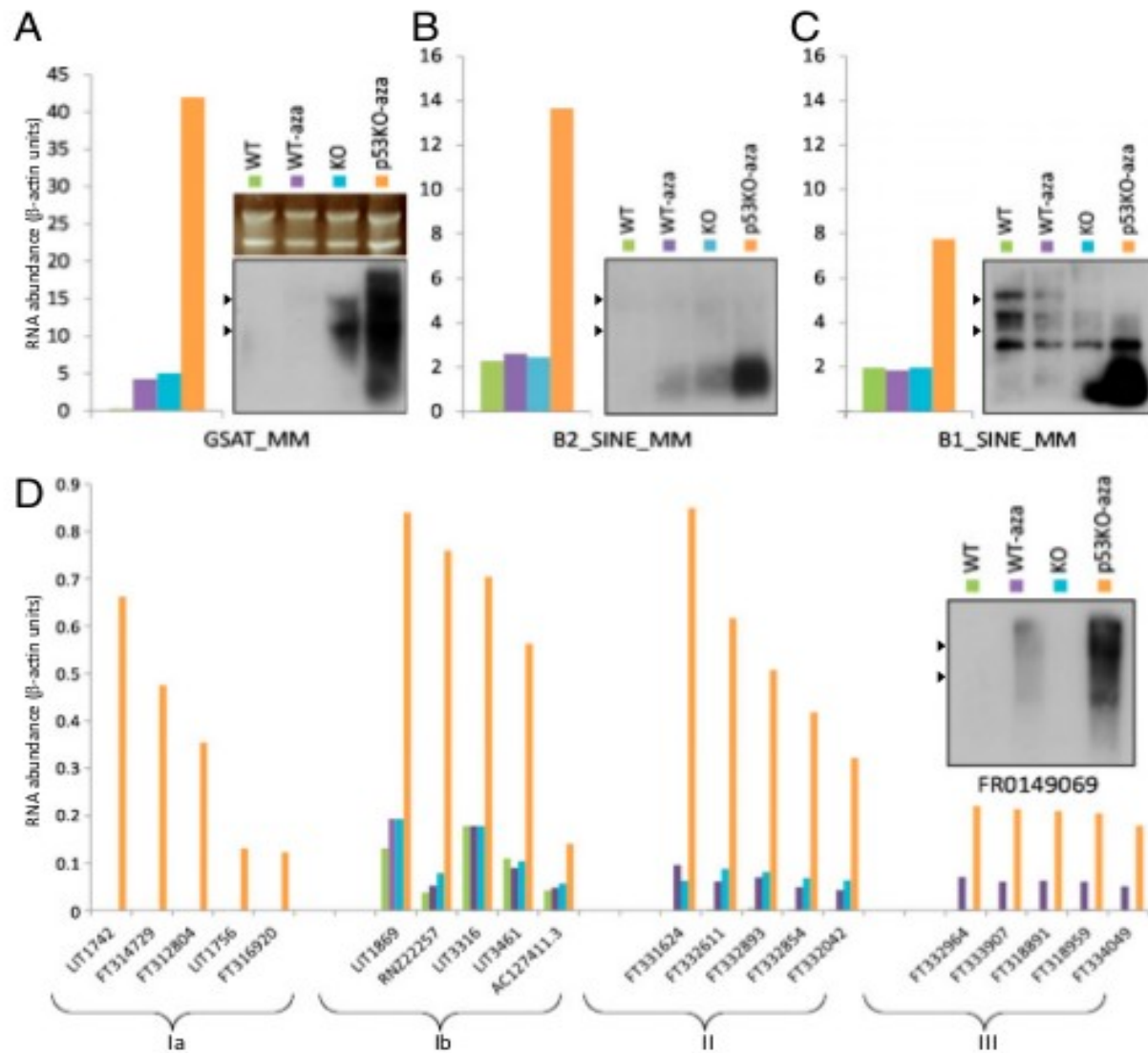
- Production of type I IFNs is a major antiviral response that limits the infectivity of a wide range of DNA and RNA viruses
- Treatment with 5-aza-dC strongly repressed virus replication only in p53-null cells
- No signs of caspase activation were detected in IFNAR<sup>-/-</sup> MEFs treated with 5-aza-dC



# Massive Transcription of Repetitive Elements

- High-throughput RNA sequencing was used technique to build a picture of RNA species induced by 5-aza-dC treatment
- Three types of RNA transcripts were specifically and significantly more abundant in the RNA sample from 5-aza-dC–treated p53-null MEFs.
- Both major classes of mouse SINEs, namely B1 and B2
- Near-centromeric major ( $\gamma$ ) satellite repeats (GSAT)
- Large number of different noncoding RNA species (ncRNAs)

# Massive Transcription of Repetitive Elements



# Transcription of repeats and ncRNAs in tumors

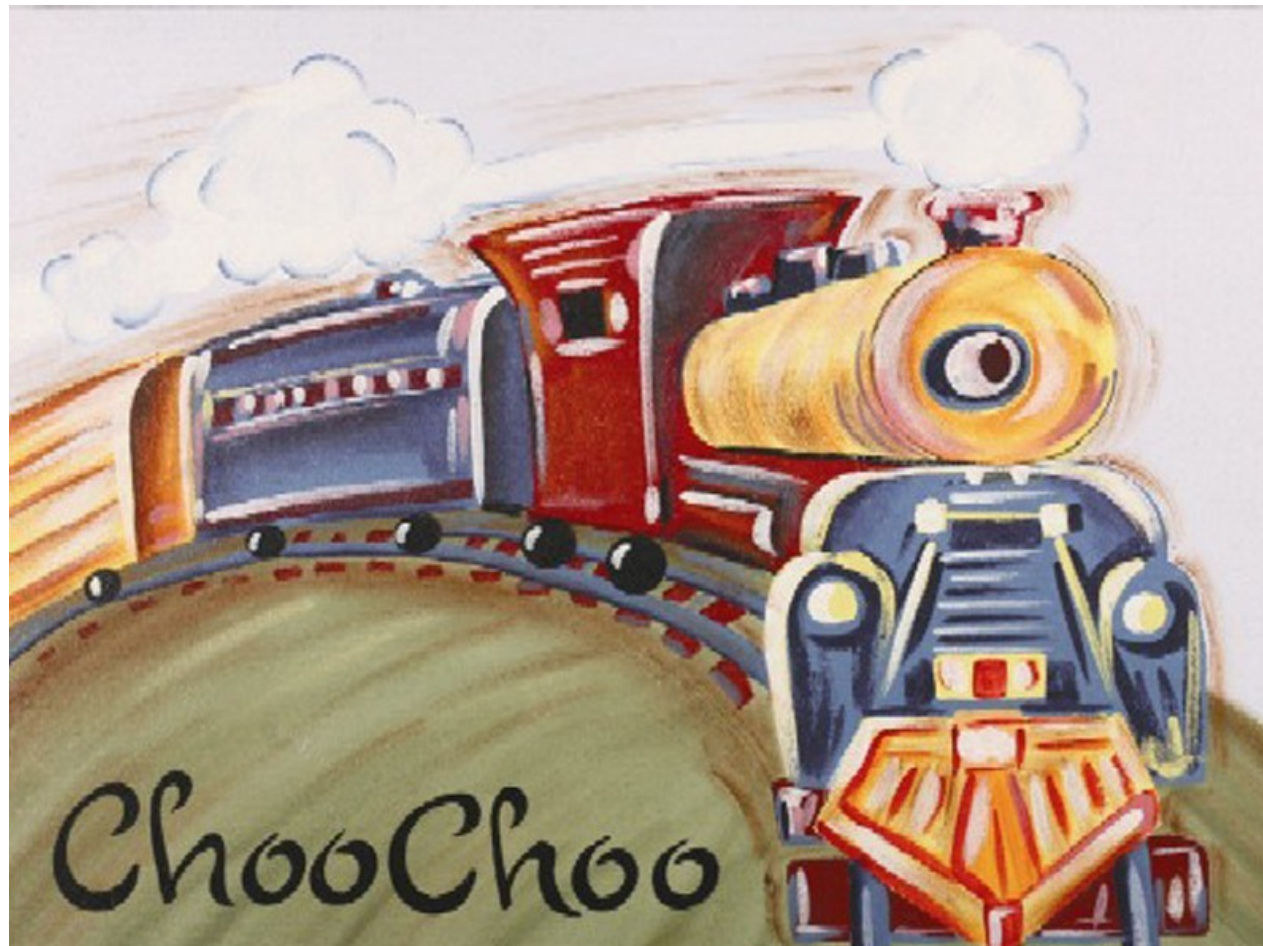
- p53 is the most frequently mutated gene in tumors (or it can be inactivated by other means)
- Three mouse tumor-derived cell lines SCC-VII, CT26, and LLC cells showed strong, intermediate, and undetectable expression of GSAT RNA respectively, upon treatment with 5-aza-dC
- Decreased genome-wide DNA methylation is another common property of tumors acquired during in vivo growth
- Transcription of repeats leading to the induction of an IFN response might occur spontaneously during tumor growth and progression in vivo

# Conclusions

- The study reveals a function of p53 that, in cooperation with DNA methylation, keeps large families of interspersed and tandem repeats transcriptionally dormant
- Transcriptional derepression of repeats resulting from a combined lack of p53 function and DNA methylation was accompanied by induction of the classical type I IFN signaling pathway, which leads to apoptotic cell death

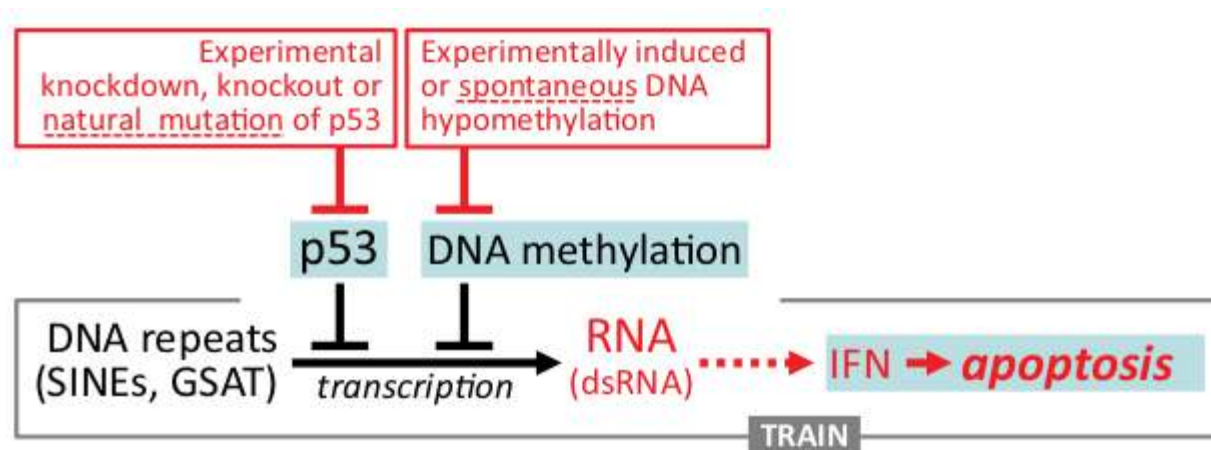
# TRAIN

- Transcription of repeats activates interferon



# Model

- Epigenetic silencing of repeats (an essential condition for genomic stability and viability of currently existing species) is controlled by three factors:
- p53-mediated transcriptional silencing
- DNA methylation-mediated suppression of transcription
- Suicidal IFN response which eliminates cells that escape the first two lines of control.



Thanks for attention

