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Type	Poster
Session	Gene regulation, transcriptomics and epigenomics
Title	Transposable elements activation following global DNA demethylation: exploration of transcriptional responses.
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Motivation

Transposable elements (TEs) are genomic repetitive DNA fragments that are able to move in a new position of the genome[1]. Their activity can cause germline and somatic genomic variations and even disrupt genes, acquiring a clear role in diseases like cancer and brain disorders[2]. For these reasons, the cells have developed mechanisms to repress and/or modulate the activity of TEs. However, during evolution, TEs activity was also co-opted several times, for example to exploit their involvement in regulatory networks[3].

One of the key players involved into the modulation of TEs transcription is DNA methylation. It is an epigenetic modification, in the eukaryotic cells, occurring mostly at CG dinucleotides (CpG) and is mediated by the activity of DNA methyltransferases (DNMTs). DNMT1 is known as the enzyme involved in the maintenance of DNA methylation during the replication while, DNMT3A and DNMT3B are considered de-novo methyltransferases since they establish specific methyl-CpG during development. The general effect of CpG methylation in TEs loci is transcriptional silencing[4]. DNMTs mutations in human diseases are often heterozygous, this feature being strictly dependent upon the severity of the phenotype. Nevertheless, CRISPR-Cas9 technology has recently allowed the study of the first model of human neural progenitor cells (hNPCs) with a complete disruption of DNMT1 activity. In this model, despite the lower proliferative rate, the cells survive allowing to observe the connection between a dramatic loss of CpG-methylation and the transcriptional activation of evolutionarily young LINE L1 retrotransposons[5]. Taking advantages of the transcriptomic data generated from this model, the aim of our study is to investigate regulatory and interaction dynamics between TEs and protein coding genes.

Methods

The dataset is composed by public RNA-seq data from 3 DNMT1-/- and 3 Control hNPCs cultures published by Jonsson et al[5]. Reads were aligned to the reference human genome using STAR[6]. Expression levels quantification was achieved with htseq-count[7] while locus-specific quantification of TEs was performed with SQuiRE[8]. DESeq2[9] was used for differential expression analysis and genes/TEs showing p-adjusted<0.05 and fold change>|1.5| were classified as differentially expressed. Bedtools[10] was employed to intersect TEs and genic coordinates. Enrichment analysis were made with gProfiler2[11] setting as background the genes with at least one read in at least one sample. Enrichment with at least 5 intersected genes and an FDR<0.1 were considered significant.

Results

The complete disruption of DNMT1 activity leads to dramatic changes in the regulation of gene expression. A consequence of the global demethylation is reflected in the higher amount of genes upregulated (2188) with respect to downregulated (627). Functional enrichment associated to upregulated genes highlights the activation of genes involved in the piRNA pathway and in the pro-apoptotic transcriptional network regulated by p53. These results suggest that, by activating the piRNAs and apoptosis pathways, cells are responding to the transcriptional activation of TEs[12] and to genotoxic stimuli[13]. These results are in agreement with the lower proliferative rate of cells observed by the authors and reinforced by the enrichment of the downregulated genes which shows a significant association to the positive regulation of cell proliferation.

TEs expression analysis show that the observed global DNA demethylation is indeed associated to the transcriptional activation of TEs: 3663 TEs result upregulated while 413 are downregulated. TEs/genes overlap analysis identified 80 upregulated genes that are enriched to contain at least one concordant upregulated TE in their 3'UTR. The TEs inside 3'UTRs suggest that, those TEs might lie in a strategic position to provide sources for miRNA binding sites[14]. Enrichment analysis of these genes highlights a

pool of 37 different miRNAs reported to regulate the expression of 50 out of 80 genes and interestingly, among these genes, 7 are involved in the regulation of the p53 signaling pathway. The hypothesis that we are evaluating is that, upon a global DNA demethylation, the pervasive activation of TEs interferes with miRNAs regulatory dynamics resulting in the upregulation of specific groups of genes.

Info

1. “A unified classification system for eukaryotic transposable elements” (Wicker et al., Nature Reviews Genetics 2007)
2. “Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition” (Muotri et al., Nature 2005)
3. “Roles of Transposable Elements in the Different Layers of Gene Expression Regulation” (Drongitis et al., Int J Mol Sci 2019)
4. “The DNA methyltransferase family: a versatile toolkit for epigenetic regulation” (Lyko, Nature Reviews Genetics 2017)
5. “Activation of neuronal genes via LINE-1 elements upon global DNA demethylation in human neural progenitors” (Jonsson et al., Nature Communications 2019)
6. “STAR: ultrafast universal RNA-seq aligner” (Dobin et al., Bioinformatics 2013)
7. “HTSeq—a Python framework to work with high-throughput sequencing data” (Anders et al., Bioinformatics 2015)
8. “SQuIRE reveals locus-specific regulation of interspersed repeat expression” (Yang et al., Nucleic Acids Research 2019)
9. “Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2” (Love et al., Genome Biol 2014)
10. “BEDTools: a flexible suite of utilities for comparing genomic features” (Quinlan et al., Bioinformatics 2010)
11. “g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update)” (Raudvere et al., Nucleic Acids Research 2019)
12. “The piRNA Pathway Guards the Germline Genome Against Transposable Elements” (Toth et al., Adv Exp Med Biol 2016)
13. “L1 drives IFN in senescent cells and promotes age-associated inflammation” (De Cecco et al., Nature 2019)
14. “LINE-2 transposable elements are a source of functional human microRNAs and target sites” (Petri et al., Plos Genetics 2019)

Figure

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Availability	-

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