Genes controlled by DNA methylation are involved in Wilms tumor progression

de Sá Pereira, Bruna Maria[3],†; Guerra, João Victor Da Silva[3],†.; Cruz, Jéssica Gonçalves Vieira da[4]; Scherer, Nicole de Miranda[4]; Furtado, Carolina[3]; Montalvão de Azevedo, Rafaela[3]; Oliveira, Paulo Sérgio Lopes de[1]; Faria, Paulo[3]; Boroni, Mariana[4]; de Camargo, Beatriz[3]; Maschietto, Mariana[1,5]

[1]Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil.
[2]Graduate Program in Biosciences and Technology of Bioactive Products, Institute of Biology, University of Campinas, Campinas, SP, Brazil
[3]Brazilian National Cancer Institute (INCA), Rio de Janeiro , RJ, Brazil
[4]Bioinformatics an Computacional Biology Lab, Brazilian National Cancer Institute (INCa), Rio de Janeiro, RJ, Brazil
[5]Boldrini Children's Hospital, Campinas, SP, Brazil
Author email: brunamspereira@yahoo.com
†These authors contributed equally to this work

Background / Objectives

Wilms tumors (WTs) have a relapse rate of ~25%. In preoperative chemotherapy treated cases, blastemal predominant WT have a higher risk of relapse and capacity of forming distant metastasis. The aim of this study was to identify mechanisms involved with metastasis formation in WT.

Design / Methods

DNA and RNA were extracted from FFPE and hybridized in the *Infinium Illumina* 450K *Beadchip* arrays and sequenced by RNA-Seq (Illumina), respectively, for seven paired cases of normal renal cortex (NK), primary tumor (WT) and pulmonary metastasis (MET) samples. Both data were explored regarding their involvement with WT metastasis formation. Clinicopathological characteristics of the cases evaluated for both techniques are described in **Table 1**.

Table 1: Clinicopathological characteristics of the cases evaluated for DNA methylation and gene expression.

Sample (ID)	Age at diagnosis (years)	Gender	Histology (primary tumor)	Patient stage	Relapse site	Histology (metastasis)	Technique
1	3	F	Blastemal	III	Left lung	Mixed	RNA-Seq / 450k
2	7	M	Regressive	II	Left lung	Epithelial	RNA-Seq / 450k
3	5	M	Mixed	I	Left lung	Blastemal	RNA-Seq / 450k
4	3	F	Mixed	II	Right lung	Blastemal	RNA-Seq
5	3	F	Mixed	II	Right and left lung	Epithelial	450k
6	9	M	Mixed	I	Left lung	Mixed	450k
7	4	M	Regressive	I	Right lung	Blastemal	RNA-Seq
9	3	M	Mixed	I	Right lung	Blastemal	RNA-Seq / 450k
10	6	M	Mixed	I	Right lung	Mixed	RNA-Seq / 450k

Gender: female (F) and male (M).

Results

A linear Bayesian framework model identified 497 differentially methylated positions (DMPs) between groups that discriminated NK from WT, but MT samples were divided in two groups. Accordingly, methylation variance grouped NK and three MT samples tightly together and all WT with four MT samples that showed high variability. WT were hypomethylated compared to NK, and MT had a hypermethylated pattern compared to both groups **(Figure 1A and 1B)**. For a comprehensive insight into the variation in DNA methylation between the three groups, was applied Principal Component Analysis (PCA) to the full dataset. The principal component (PC) 1 and 2 explained 88.1% and 3.1% of the variance **(Figure 1C)**, respectively, which clearly separated NK and WT and both groups of metastases. This suggests that NK has a more stable epigenome than WT. The methylation patterns were in agreement with methylases and demethylases expression **(Figure 1D)**. Methylation data pointed to the existence of two groups of metastases. Gene expression profile of IGF2 and H19 was evaluated, once these genes are commonly founded altered in WTs **(Figure 2A)**. While hierarchical clustering analysis based on the expression of all 2.569 differentially expressed genes (DEGs) discriminated WT and MT from all NK samples **(Figure 2B)**, the hierarchical clustering based on the expression of 44 genes with a DMR (differentially methylated regions) located in their promoter region revealed two groups: one containing all NKs and three MTs and one containing all WT and four MTs **(Figure 2C and 2D)**. Methylation changes might be controlling expression of genes associated with WT progression. The 44 genes are candidates to be further explored as a signature for metastasis formation in WT

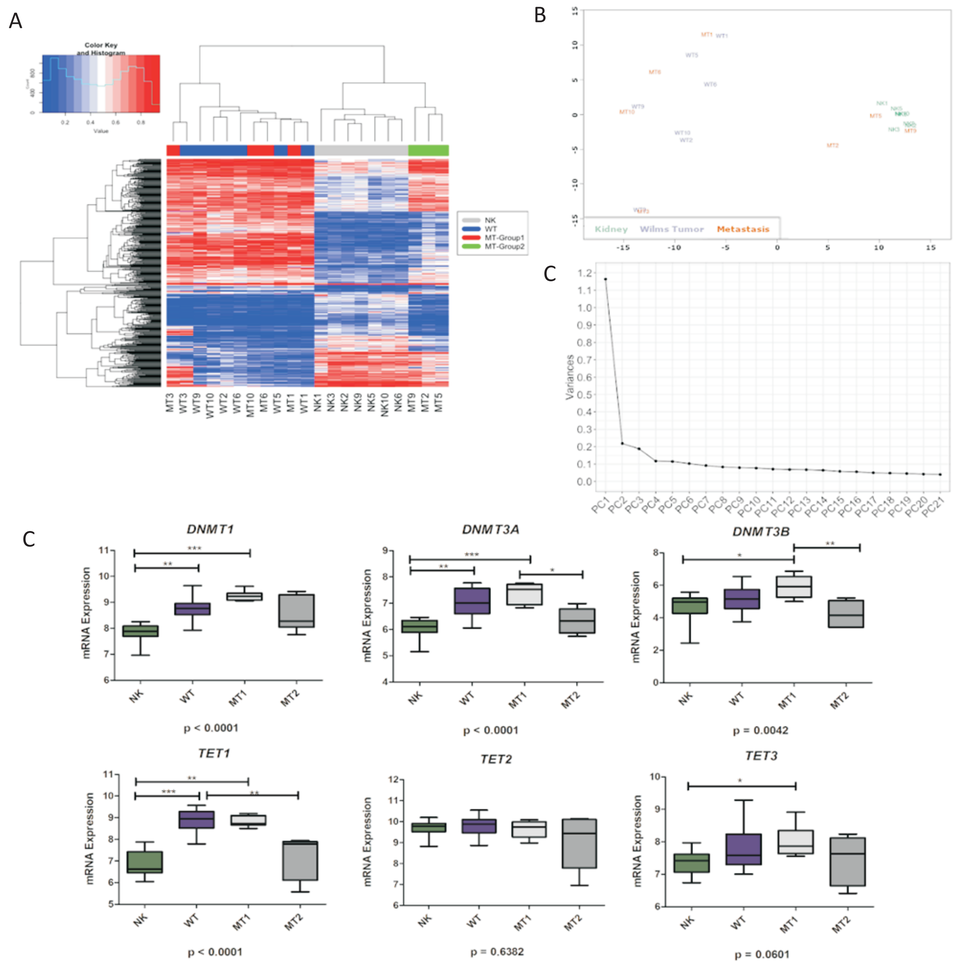

Figure 1. Methylation analyses in matched trios of normal kidney (NK), Wilms tumor (WT), and metastatic tissues (MT). (A) Hierarchical clustering (Euclidean distance with average linkage) of the 21 samples, based on methylation levels of the 497 differentially methylated CpG sites. Heatmap colors refer to methylation levels: unmethylated (blue), partially methylated (white), and methylated (red). (B) Multidimensional scaling of the top 1% most variable positions. (C) Variance in DNA methylation related to each principal component identified. (D) Boxplot representing expression levels (from RNAseq) for DNMT1, DNMT3A, DNMT3B, TET1, TET2, and TET3. Kruskal–Wallis test followed by Dunn post-test was applied: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.01$. NK: normal kidney (n = 7), WT: Wilms tumor (n = 7), MT1: metastasis (n = 4), MT2 (n = 3).

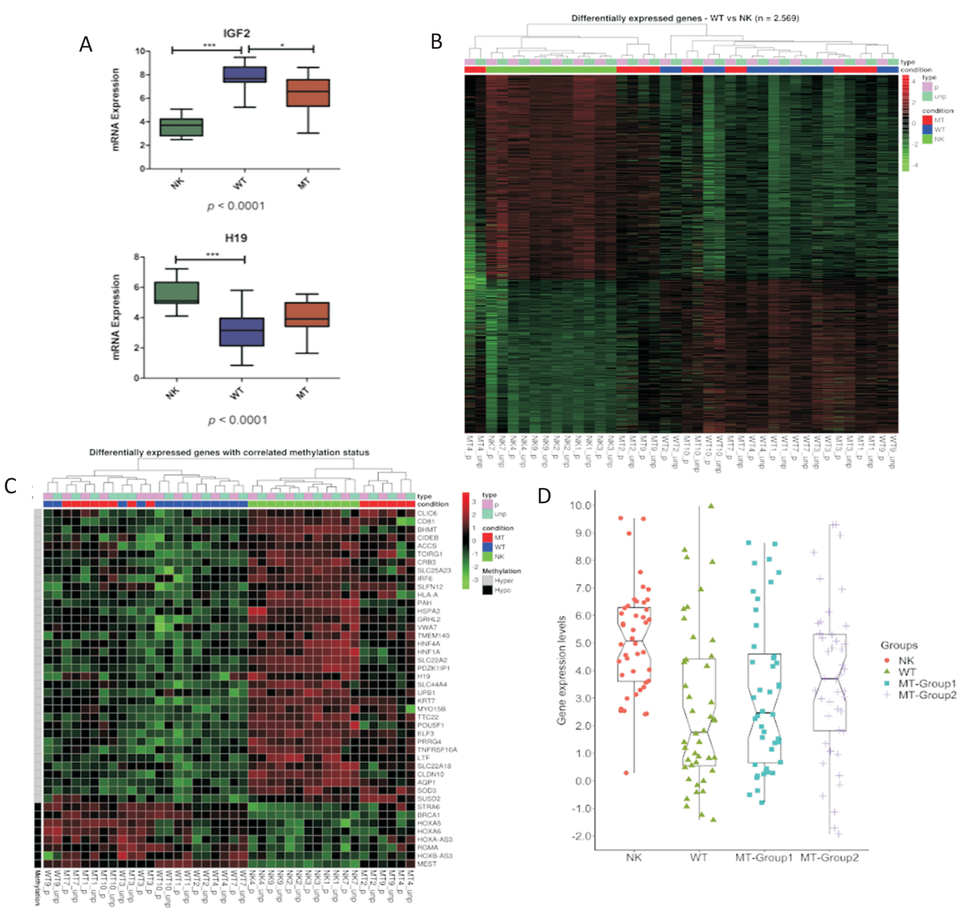

Figure 2. Genes controlled by DNA methylation. (A) Boxplot of expression levels across the groups (NK, WT, and MT) for IGF2 and H19, commonly altered in WT (ANOVA and Tukey's Multiple Comparison Test; p-value < 0.05) (B) Hierarchical clustering (distance was measured as 1-Pearson correlation coefficient with complete linkage) of the paired seven cases (21 samples), based on expression levels of the (B) 2569 differentially expressed genes (DEGs) and (C) 44 genes controlled by methylation. Heatmap colors refer to expression levels Z-score transformed: lower expression (green), median levels partially methylated (black), and highly expressed (red). (D) Boxplot of expression levels across the groups (NK, WT, MT-Group1, and MT-Group2) for 44 genes controlled by methylation. NK: normal kidney (n = 7), WT: Wilms tumor (n = 7), MT: metastasis (n = 7), MT-Group1: metastasis group 1 (n = 4), MT-Group2: metastasis group 2 (n = 3).

Conclusion

We suggest that DNA methylation might be involved in the control of genes related to metastasis formation.

Financial support /Acknowledgments

FAPERJ CNPq FAPESP

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

INCA SUS MINISTÉRIO DA SAÚDE PÁTRIA AMADA BRASIL GOVERNO FEDERAL