Association between long interspersed nuclear element-1 methylation levels and relapse in Wilms tumors

de Sá Pereira BM; Montalvão-de-Azevedo R; Faria PA; de Paula Silva N; Nicolau-Neto P; Maschietto M, de Camargo B*; Soares Lima SC*.
*These authors contributed equally to this work.

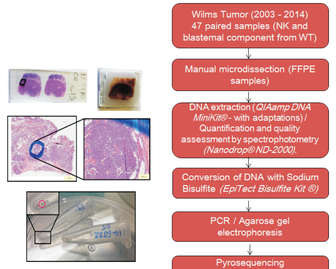
BACKGROUND

Wilms tumor (WT) is a curable pediatric renal malignancy, but there is a need for new molecular biomarkers to improve relapse risk–directed therapy. Somatic alterations occur at relatively low frequencies whereas epigenetic changes at 11p15 are the most common aberration. We analyzed long interspersed element-1 (LINE-1) methylation levels in the blastemal component of WT and normal kidney samples to explore their prognostic significance.

OBJECTIVE

Evaluate the methylation profile of LINE-1 in paired samples to WTs (blastemal component) and normal kidney.

METHODS



Wilms Tumor (2003 - 2014)
47 paired samples (NK and blastemal component from WT)

↓

Manual microdissection (FFPE samples)

↓

DNA extraction (*QIAamp DNA MiniKit®* - with adaptations) / Quantification and quality assessment by spectrophotometry *(Nanodrop® ND-2000)*.

↓

Conversion of DNA with Sodium Bisulfite *(EpiTect Bisulfite Kit ®)*

↓

PCR / Agarose gel electrophoresis

↓

Pyrosequencing *(Pyromark Q24, Qiagen)*

↓

Data analysis *(GraphPad Software Inc.)*

RESULTS

WT samples presented a hypomethylated pattern at all five CpG sites compared to matched kidney samples; therefore, the averaged methylation levels of the five CpG sites were used for further analyses. WT presented a hypomethylation profile (median 65.0%, 47.4–73.2%) compared to normal kidney samples (median 71.8%, 51.5–77.5%; $p < 0.0001$). No significant associations were found between LINE-1 methylation levels and clinical–pathological characteristics. As shown in Figure 1A, we observed that LINE-1 methylation levels were lower in tumor samples from patients with relapse (median methylation 60.5%) compared to patients without relapse (median methylation 66.5%; $p = 0.0005$), and a receiving operating characteristic curve analysis was applied to verify the ability of LINE-1 methylation levels to discriminate WT samples from these patients. Using a cut-off value of 62.71% for LINE-1 methylation levels, the area under the curve was 0.808, with a sensitivity of 76.5% and a specificity of 83.3% (Figure 1B). Having identified differences in LINE-1 methylation between WT samples from patients with and without relapse in this cohort, we evaluated other prognostic factors using a logistic regression model (Figure 2).

This analysis showed that in risk stratification, LINE-1 methylation level was an independent variable for relapse risk: the lower the methylation levels, the higher the risk of relapse. The logistic regression model indicated a relapse risk increase of 30% per decreased unit of methylation (odds ratio 1.30; 95% confidence interval 1.07–1.57). In other words, a decrease of 1% in the percentage of methylated cells measured by pyrosequencing is associated with an increase of 30% in relapse risk.

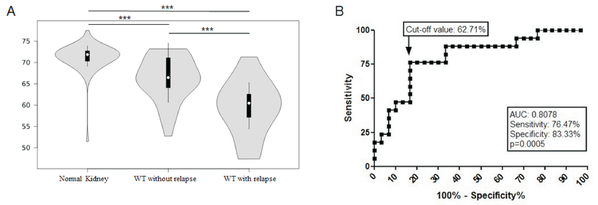


Figure 1. LINE-1 methylation profile in WT according to relapse status (A) Violin plots showing LINE-1 methylation levels in normal kidney and WT samples grouped according to relapse status. The curve is estimated by a kernel density and is proportional to the number of samples. Internal boxplots include methylation levels within the 25% and 75% interquartiles, with bars indicating 1.96 × standard deviation and white dots representing the median methylation values. ***$p < 0.0001$, Kruskal–Wallis test. **(B)** ROC curve of LINE-1 methylation for discrimination of WT samples without relapse and WT samples with relapse ($p < 0.001$).

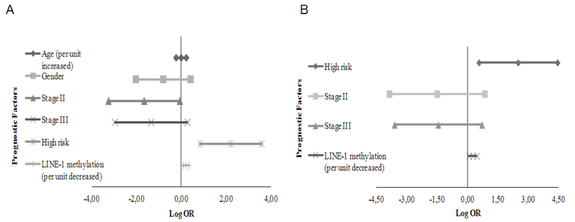


Figure 2. Estimated risk of relapse in WT patients according to prognostic factors and LINE-1 methylation levels. (A) Crude risk estimates; to analyze gender as a prognostic factor, we used 'male' as the reference category. **(B)** Adjusted risk estimates: risk classification was adjusted by stage and LINE-1 methylation levels; stage was adjusted by risk classification and LINE-1 methylation levels; and LINE-1 methylation levels were adjusted by stage and risk classification.

CONCLUSION

Our results reinforce previous data showing a global hypomethylation profile in WT. LINE-1 methylation levels can be suggested as a marker of relapse after chemotherapy treatment in addition to risk classification, helping to guide new treatment approaches.

Acknowledgement

FAPERJ FAPESP CNPEM LNBIO

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

INCA SUS MINISTÉRIO DA SAÚDE BRASIL