Characterization of methylation profile during progression of Wilms tumors

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Abstract

Wilms tumors (WTs) are the most common renal tumors with a relapse rate of ~25%, from which long-term survival remains approximately 50%. The ability of relapsing is associated with resistance to chemotherapy and capacity of forming distant metastasis. Because in DNA methylation has being correlated with tumor cells plasticity, we purposed to characterize DNA methylation changes in normal kidney (NK), WT and distant metastasis to identify the mechanisms involved with tumor progression and resistance.

Key words:

Wilms Tumor, DNA methylation, metastasis.

Introduction

Wilms tumors (WTs) are embryonic tumors of the kidney composed by blastemal, epithelial and stromal components. In pre-treated tumors, blastemal predominance is associated with high risk of relapsing. Heterogeneity is also observed at molecular level, although its impact remains to be addressed. DNA methylation dictates cell plasticity including the epithelial-mesenchymal transition (EMT), which is the first step for the formation of metastasis. Mutations in known genes are found in overlap in up to 30% of the cases, and loss of imprinting (LOI) of 11p15, resulting in *IGF2* overexpression, is present in 70% of cases. In addition, WTs present changes in DNA methylation with tumor-specific methylated DNA being released into the blood circulation, which could be used to improve the accuracy of determining tumor response to treatment and relapse.

To identify the mechanisms involved with metastasis formation in WT, we performed comprehensive DNA methylation analyzes in matched WT, NK and metastasis.

Results and Discussion

From 110 sporadic WT cases treated in the National Institute of Cancer (INCA) between 2003 and 2014, we selected 10 matched trios (NK, WT, metastasis) with confirmed histology in formalin fixed paraffin embedded samples. Bisulfite-converted DNA was hybridized to the *Infinium HumanMethylation450 BeadChip* (Illumina). Seven matched trios passed all quality control steps and IDAT files were processed using *Minfi* package and normalized with SWAN method. Data were corrected for cell heterogeneity using *reFACToR* package. Considering the global methylation pattern, we observed that WT is hypomethylated compared to NK, and both are hypomethylated in relation to metastasis.

Limma was used to generate an empirical Bayesian framework linear model considering intra-patients and inter-groups comparisons. There were common 146 differentially methylated positions (DMPs, $\text{adjP} < 0.05$) between Tumor-Kidney (total of 296 DMPs) and Metastasis-Kidney (total of 2,620 DMPs) comparisons, with 37 hypomethylated and 109 hypermethylated CpG sites.

Metastasis presented more subtle changes in relation to the tumors. Therefore, we applied a direct comparison between metastasis and WT, using $p < 0.05$ and methylation changes $> 10\%$ as parameters. There were 2,448 DMPs, with most of the 1,126 hypomethylated CpGs located in open sea and most of the 1,322 hypermethylated CpGs located in CpG islands and N-Shelves, which are related to repression of transcription. The 2,448 DMPs were related to 1,304 genes, enriched within signaling pathways related to EMT, metabolism and immune system as well as MAPK and Wnt signaling pathways ($\text{adjP} < 0.05$ and 10 genes minimum). These genes are also enriched in several types of cancers, including renal tumors ($\text{adjP} < 0.05$). Among others, alterations in *PAX2*, *WT1* and *IGF2* were previously described in WT as well as their involvement in early nephrogenesis.

Conclusions

Methylation profiles vary significantly in NK, WT and metastasis revealing an hypermethylated state for metastatic samples. CpG sites more methylated in metastatic samples are related to repression of transcription, in agreement with other studies that described an hypermethylated state in advanced cancers. We found that genes associated to the DMPs are involved with cellular pathways associated with EMT, and consequently, metastasis. As a proof of concept, genes with a role in nephrogenesis and disrupted in WT appeared as differentially methylated in our analysis.

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Scott, R.H. et al. Stratification of Wilms tumor by genetic and epigenetic analysis. *Oncotarget*. **2012**, 3(3), 327–335.

Dome J. S. et al. Advances in Wilms tumor treatment and biology: progress through international collaboration. *Journal of Clinical Oncology*. **2015**, 33 (27), 2999–3007.

<http://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0434-y>

Kiesslich, T. et al. Epigenetic control of epithelial-mesenchymal-transition in human cancer. *Molecular and clinical oncology*. **2013**, 1(1), 3–11.