Evaluation of expression of genes potentially regulated by DNA methylation during Wilms tumors progression

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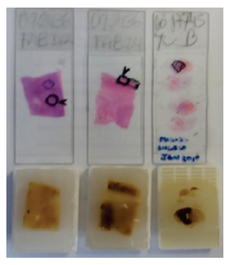
ABSTRACT

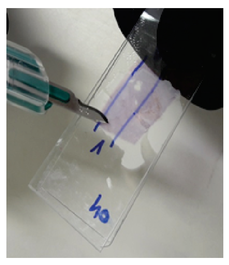
Wilms tumors (WTs) are embryonic tumors composed by blastemal, epithelial and stromal components[1]. In pre-treated tumors, blastemal predominance is associated with high risk of relapsing[2]. Relapse is observed in 20-25% of patients with WT[3]. DNA methylation dictates cellular plasticity, including the epithelial-mesenchymal transition, which is the first step for the formation of metastasis[4]. In a previous study, we observed that methylation profiles vary significantly between NK, WT and metastasis revealing a 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 a hypermethylated state in advanced cancers. Moreover, 36 regions located next to 28 genes were differentially methylated (DMRs) between WT and NK.

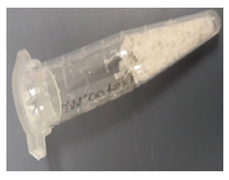
OBJECTIVES

To characterize gene expression profiles in matched WT, NK and metastatic samples to correlate with the alterations observed in DNA methylation patterns.

MATERIALS AND METHODS







Wilms tumor (2003-2014)
11 paired samples (NK, WT, MET)

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Manual microdissection (FFPE samples)

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RNA extraction (*Recover All™ Total Nucleic Acid Isolation Kit for FFPE* - ®Life Technologies) / Quantification and quality assessment by Spectrophotometry *(Nanodrop® ND-2000)*

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Evaluation of mRNA integrity by qRT-PCR

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Library preparation *(Truseq RNA Access library prep kit – Illumina)*

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Next Generation Sequencing *(HiSeq 2.500 – Illumina)*

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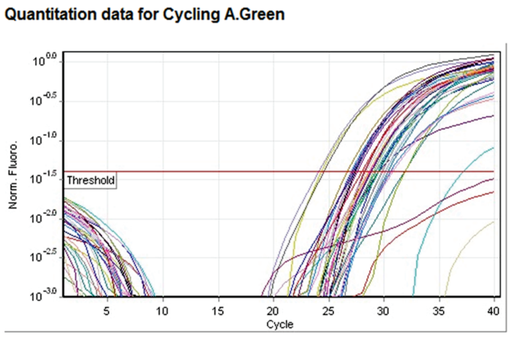
Data analysis and correlation of methylation and gene expression data



PRELIMINARY RESULTS

Paraffin blocks from the same patients were reviewed and blastemal areas, when available, next to the areas from which DNA was extracted were selected for total RNA extraction. Quality of samples was evaluated by qRT-PCR for gene *GAPDH* that amplified a sequence of 114pb. Table 1 shows the cycle threshold from qRT-PCR suggesting that all samples had RNA with enough quality to proceed to mRNA sequencing.

Sample ID	Gender	Age at diagnoses	Stage	Year	Ct*
T055 NK	F	3	III	2012	29.64
T055 WT					24.27
T055 MET					28.53
T057 NK	M	7	II	2012	28.91
T057 WT					27.60
T057 MET					26.92
T083 NK	M	2	III	2011	31.48
T083 WT					30.22
T083 MET					29.67
T091 NK	F	3	II	2010	28.75
T091 WT					27.42
T091 MET					37.13
T094 NK	M	5	I	2010	29.43
T094 WT					27.62
T094 MET					27.80
T101 NK	M	3	I	2009	27.63
T101 WT					27.92
T101 MET					31.76
T124 NK	F	5	II	2003	22.36
T124 WT					28.93
T124 MET					28.28
T133 NK	F	3	II	2013	28.94
T133 WT					26.02
T133 MET					33.14
T135 NK	M	9	I	2007	33.23
T135 WT					31.30
T135 MET					28.30
T137 NK	M	6	I	2008	32.97
T137 WT					29.25
T137 MET					32.82
T169 NK	M	4	I	2011	33.64
T169 WT					28.55
T169 MET					26.63

* Cycle threshold

PERSPECTIVES

Next, the libraries of all samples will be prepared for sequencing. The library fragments are short (30-400 bp), suitable for degraded RNAs. After sequencing, expression data will be analyzed and correlated with methylation data.

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