ANALYSIS OF microRNA EXPRESSION IN BLASTEMA COMPONENT IN WILMS TUMOR

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BACKGROUND

Wilms tumor (WT) is an embryonal kidney neoplasia affecting 1:10000 children. The pre-operative chemotherapy allows to evaluate tumor response to treatment, and the presence of blastemal predominance classifies the patient as high risk. Mutations in the microRNA processing genes (miRNAPGs) (*DICER, DROSHA, DGCR8*), that affect microRNA maturation, have been recently described in WT.

OBJECTIVE

To characterize the microRNA expression profile and the mutations in the miRNAPGs investigating *sensitive* and *resistant* blastema cells looking for biomarkers candidate of therapeutic response.

DESIGN AND OUTCOME MEASURES

Cases have been selected among patients enrolled from 2003 to 2014 at INCA, treated according to the SIOP-2001 study and reviewed by the same pathologist (PASF). DNA and RNA were extracted from the blastema component of 20 FFPE cases, 8 blastemal predominant WTs (defined as *resistant* cases) and 12 regressive/mixed histologies (defined as *sensitive* cases). After quality control steps, 20 RNA samples were hybridized on the GeneChip® miRNA 4.0 array (Affymetrix®). Raw data were normalized using a robust multi-array average (RMA) and as criteria for differential expression of sncRNAs, including microRNAs, p-value <0.005 was used. All analyses have been carried out by limma (*Linear Models for Microarray*) package using RStudio software. We also sequenced the miRNAPGs genes in 58 DNA samples, corresponding to the 20 selected patients, in order to evaluate the presence of mutations and a possible genetic heterogeneity of these tumors. We investigated the hotspot mutations of the *SIX1, SIX2* genes and the regions spanning previously described mutations of the miRNAPGs.

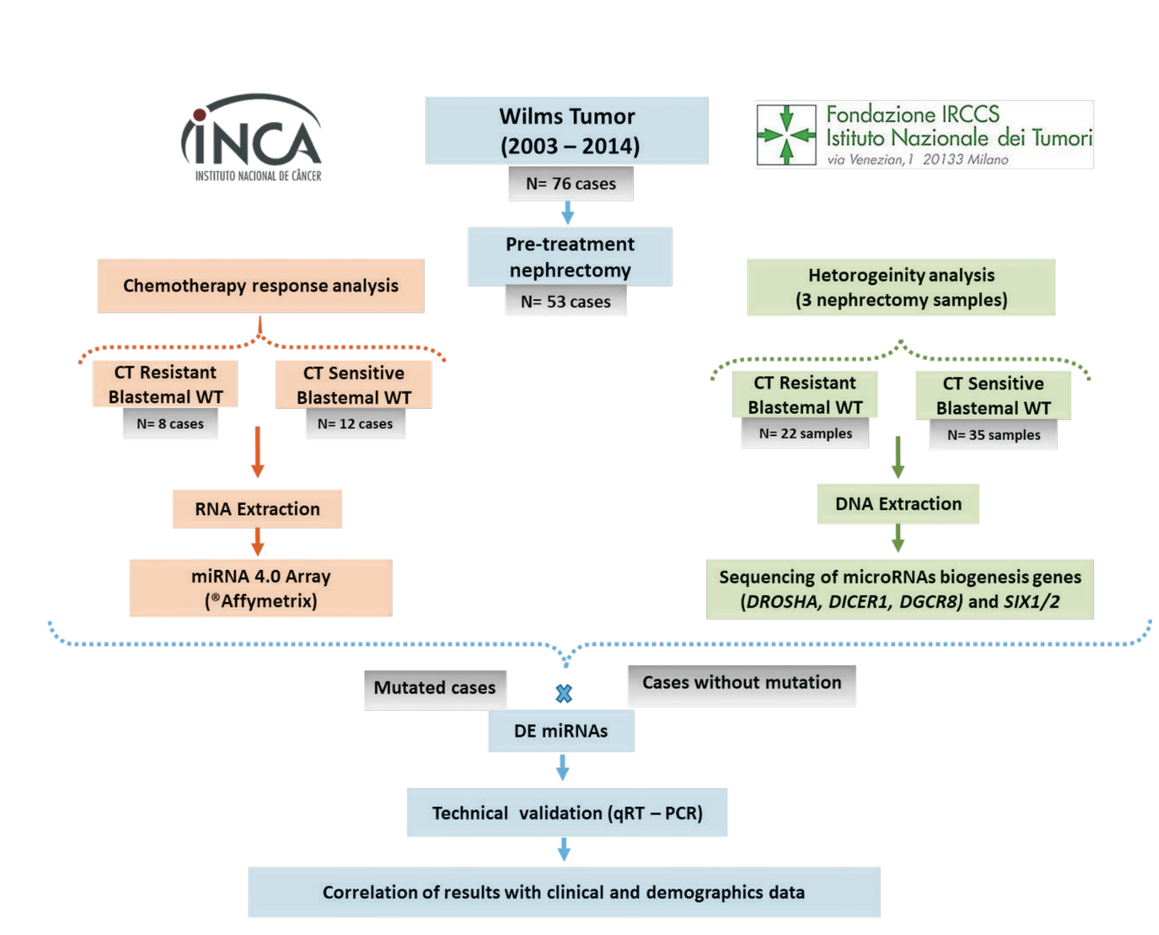

Figure 1: Fluxogram of chemotherapy response analysis developed on INCA and of heterogeneity analysis developed on INT (*Istituto Nazionale dei Tumori*).

RESULTS

We identified 6 mutations in SIX and miRNAPGs out of 20 cases, 3 in blastema predominant/*resistant* (37.3%) cases and 3 in regressive histology/*sensitive* (25%) cases. While we were not able to find sncRNAs differentially expressed between tumors classified as *resistant* and *sensitive* blastema (Figure 2), 92 sncRNAs were differentially expressed between mutated and wild-type cases (Figure 3). Of these, 52 (56.5%) sncRNA were upregulated and 40 (43.5%) were downregulated in mutated WT cases. Sixty-two (67.4%) were mature microRNAs. Downregulation of some members of let-7 microRNA family was identified in mutated cases.

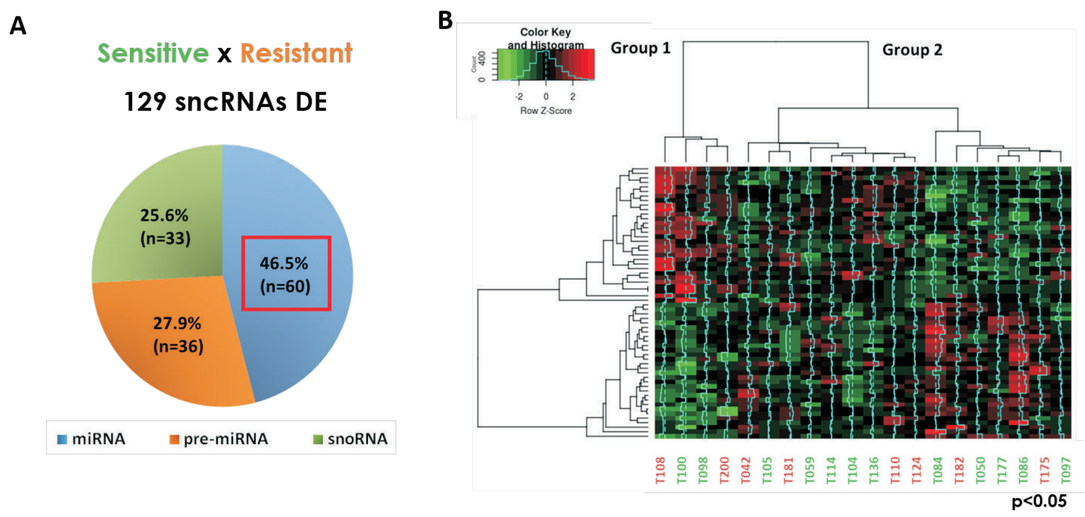

Figure 2: A. sncRNAs differentially expressed (DE) between blastema *sensitive* cases and blastema *resistant* cases; B. Unsupervised hierarchical clustering (Euclidian distance, complete linkage) of the 20 samples based on expression of the 60 microRNAs DE. The heatmap shows microRNAs with high expression in red, miRNAs with low expression in green.

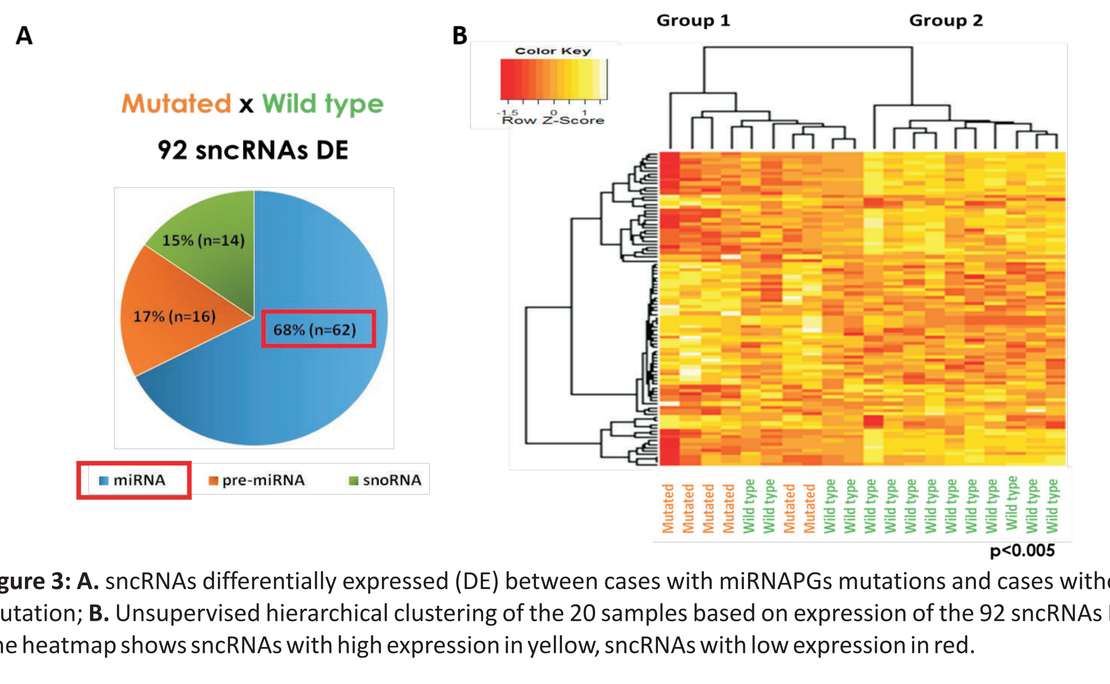

Figure 3: A. sncRNAs differentially expressed (DE) between cases with miRNAPGs mutations and cases without mutation; **B.** Unsupervised hierarchical clustering of the 20 samples based on expression of the 92 sncRNAs DE. The heatmap shows sncRNAs with high expression in yellow, sncRNAs with low expression in red.

PERSPECTIVES

Validation of the differentially expressed microRNAs and their targets and correlate all the results with clinical and sociodemographic data will be perform.

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