Circulating ncRNAs as promising non-invasive molecular biomarkers of HCC

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INTRODUCTION. Human hepatocellular carcinoma (HCC) is the most frequent primary tumor of the liver and it is the third cause of cancer-related deaths. The prognosis of HCC is poor and thus the identification of novel molecular biomarkers for the early diagnosis in at-risk patients is needed. Circulating ncRNAs (including IncRNAs and miRNAs more and less than 200 nt long respectively) have been detected in different human body fluids, including serum, plasma and urine. In order to identify promising circulating diagnostic molecular biomarkers, we measured the levels of selected ncRNAs in plasma from HCC patients. In particular, we evaluated the levels of plasma circulating miR-23b-3p and miR-126-3p that we previously found significantly down-regulated in primary HCCs and the levels of plasma circulating IncRNA GAS5 since it resulted up-regulated following sorafenib treatment of a panel of cancer cells (HCC, breast and renal cancers).

miR-23b-3p levels are down-regulated in plasma of HCC patients

The levels of plasma circulating miR-23b-3p measured by digital-droplet PCR (ddPCR) were significantly lower in HCC patients (n=25) respect to healthy subjects (n=37)

miR-126-3p levels are up-regulated in plasma of HCC patients

The levels of circulating miR-126-3p were significantly higher in HCC patients compared to controls (upper graph). The plotting was made on the average raw cycle thresholds (Ct) since there are no established endogenous miRNAs acting as normalizers for plasma miRNAs. The average Ct values of the spike-in cel-miR-39 (lower graph) were the same in the 2 groups demonstrating a good and a constant performance of RT and qPCR reactions.

IncRNA GAS5 levels are down-regulated in plasma of HCC patients

In order to assess whether GAS5 can be considered a circulating diagnostic molecular biomarker for HCC, we determined its levels in the plasma by qPCR. The expression levels of GAS5 were significantly lower in HCC patients compared to controls.

Variation of miR-23b-3p and miR-126-3p expression evaluated by ddPCR and qPCR in plasma of HCC patients during the treatment with sorafenib

Flowcharts of patient history and their treatment. The objective was to measure the expression variation of miR-23b-3p (by ddPCR) and miR-126-3p (by qPCR) in liquid biopsies (plasma) of HCC patients during the course of treatment with Sorafenib, an oral multikinase inhibitor used to treat the unresectable HCC. Currently, six patients with advanced HCC were analyzed.

CONCLUSIONS. The levels of plasmatic circulating miR-23b-3p and miR-126-3p were significantly lower and higher respectively in HCC patients respect to healthy subjects, while the levels of IncRNA GAS5 were significantly lower in HCC patients respect to healthy subjects. Sorafenib treatment promoted variations in plasmatic levels of miR-23b-3p and miR-126-3p during the treatment of patients with advanced HCC. Our results contribute to identify potential novel non-invasive biomarkers of diagnosis and response to therapy in liquid biopsies of HCC patients.