Long non-coding RNA GAS5 and miR-126-3p as molecular biomarkers of response to sorafenib in human cancer cells.

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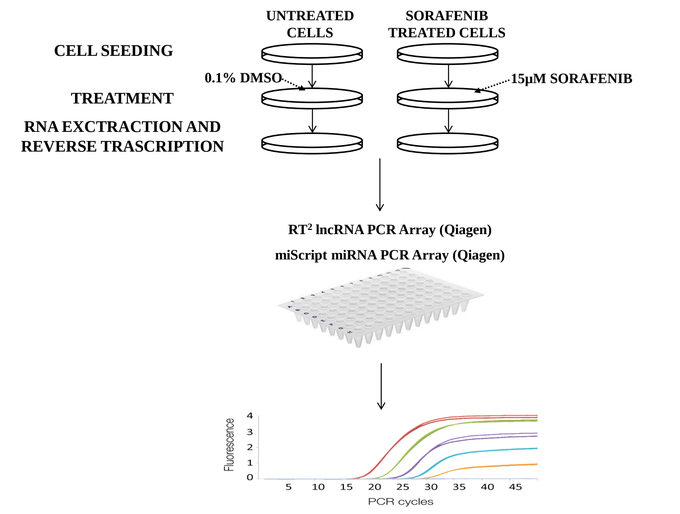
INTRODUCTION. Hepatocellular carcinoma (HCC) is a heterogeneous malignant tumor, often diagnosed at an advanced stage, with a poor prognosis, as well as the sixth world cancer cause of death. For the treatment of HCC in advanced unresectable stage, the FDA (Food and Drug Administration) approved the drug Sorafenib (Nexavar®). It is a multikinase inhibitor that may excert its antitumor activity on a multi-omics scale. Among the effects induced by sorafenib there have been observed variations in global mRNA and protein expression as well as dysregulation of ncRNAs. To better understand sorafenib-related molecular mechanisms, we profiled the expression of a panel of ncRNAs in a sorafenib-treated HCC cell line. Among the most modulated ncRNAs, we observed the dysregulation of the lncRNAs GAS5, HOTTIP and HOXA-AS2 and the miR-126-3p in different *in vitro* cancer cell models of HCC, renal (RCC) and breast carcinoma (BC).



Experimental workflow. We employed the RT² lncRNA PCR Array Human lncFinder (Qiagen) and miScript miRNA PCR Array Human Liver miFinder (Qiagen) to analyze the expression profile of 84 different disease- or pathway- focused lncRNAs and miRNAs in HCC cell line (HA22T/VGH) treated and untreated with 15 µM sorafenib for 24 hours.

Table 1. Differential expression levels of lncRNAs in sorafenib-treated HA22T/VGH cells *vs* untreated with 0.1% DMSO.

Gene symbol	Fold Change *	Regulation	p-value
BCYRN1	0.08	DOWN	0.005632
BDNF-AS	0.45	DOWN	0.022118
BOK-AS1	0.46	DOWN	0.043302
CDKN2B-AS1	0.24	DOWN	0.003393
DISC2	0.25	DOWN	0.008117
FAS-AS1	1.57	UP	0.008938
FTX	0.11	DOWN	0.004019
GAS5	2.69	UP	0.008294
GNAS-AS1	0.43	DOWN	0.015903
HEIH	2.16	UP	0.024813
HOTAIR	0.66	DOWN	0.049147
HOTTIP	0.28	DOWN	0.000035
HOXA11-AS	0.43	DOWN	0.012825
HOXA-AS2	0.09	DOWN	0.018223
IPW	0.60	DOWN	0.049269
LUCAT1	0.29	DOWN	0.045304
NEAT1	0.23	DOWN	0.009658
NRON	0.24	DOWN	0.013959
OIP5-AS1	0.38	DOWN	0.010743
PCAT1	0.15	DOWN	0.043572
TERC	0.43	DOWN	0.006213
TMEM161B-AS1	0.20	DOWN	0.004161
TUG1	0.24	DOWN	0.001951

* Sorafenib-treated cells *vs* Untreated

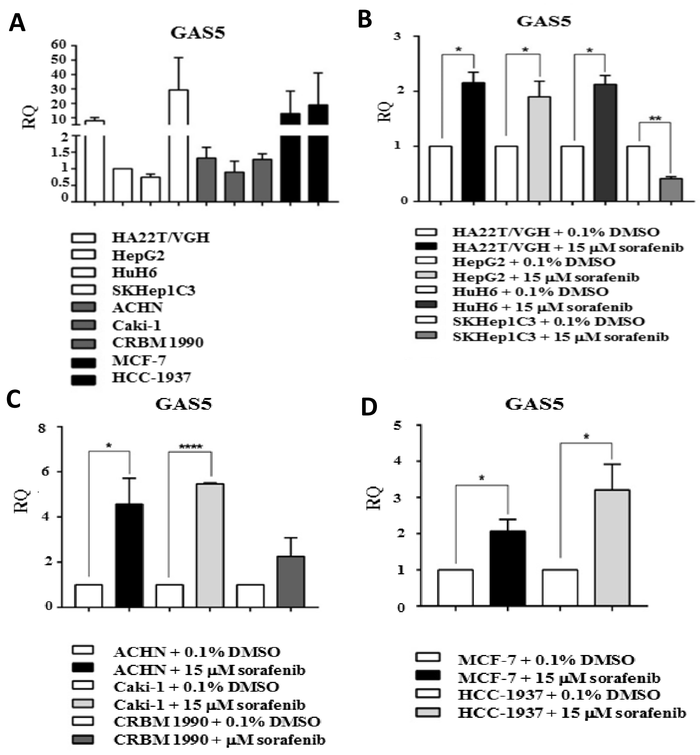
Dysregulated lncRNA. 23 lncRNAs resulted significantly dysregulated in sorafenib-treated HA22T/VGH cells compared to cells treated with DMSO. In particular, 3 lncRNAs were up-regulated while 20 were down-regulated.

Table 2. Differential expression levels of miRNAs in sorafenib-treated HA22T/VGH cells *vs* untreated with 0.1% DMSO.

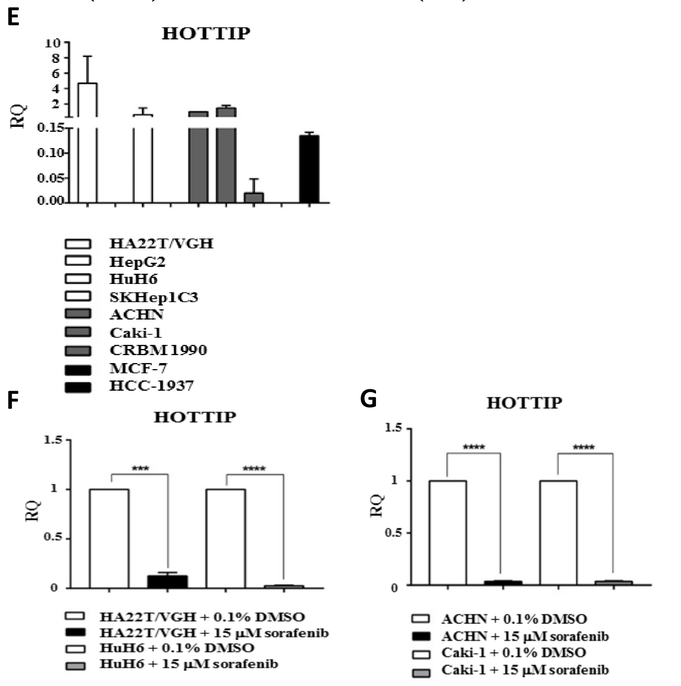
miR-	Fold Change *	Regulation	p-value
hsa-miR-126-3p	0.44	DOWN	0.021598
hsa-miR-148a-3p	0.75	DOWN	0.030670
hsa-miR-16-5p	0.82	DOWN	0.049102
hsa-miR-27b-3p	0.86	DOWN	0.015058
hsa-miR-3183	3.35	UP	0.002017
hsa-miR-3907	2.87	UP	0.022169
hsa-miR-4454	0.89	DOWN	0.039167
hsa-miR-4516	2.43	UP	0.029799

* Sorafenib-treated cells *vs* Untreated

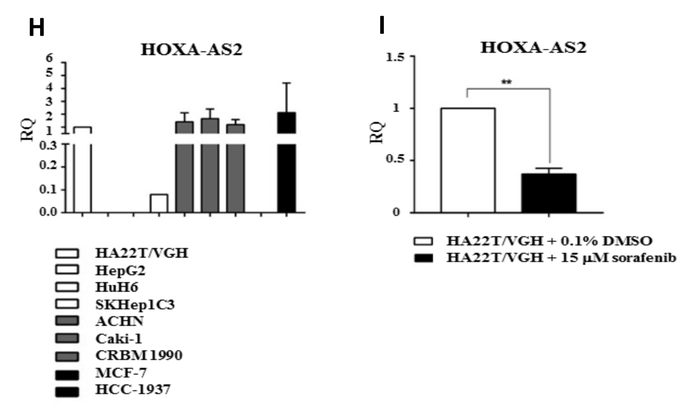
Dysregulated miRNA. 8 miRNAs resulted significantly dysregulated in sorafenib-treated HA22T/VGH cells compared to cells treated with DMSO. In particular, 3 were up-regulated and 5 down-modulated.



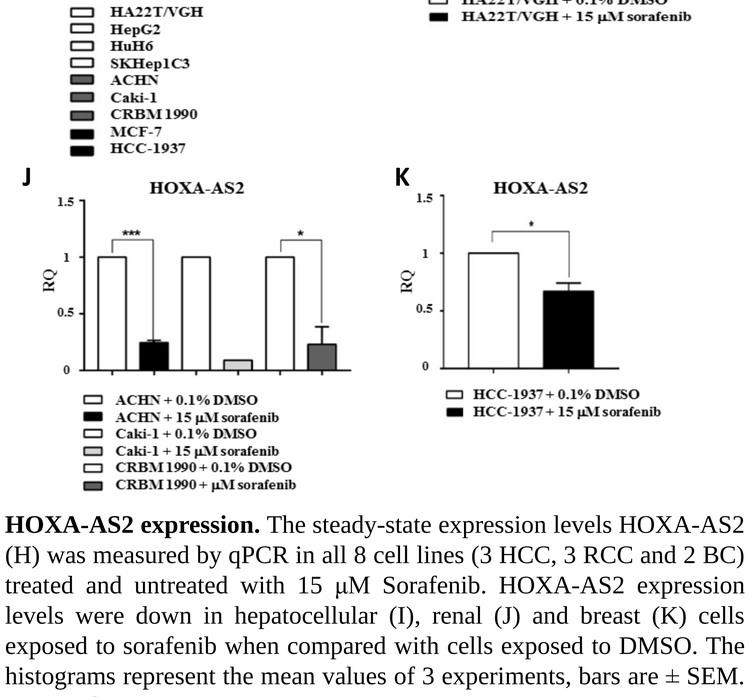
GAS5 expression. The steady-state expression levels of GAS5 (A), were measured by qPCR in 8 cell lines (3 HCC, 3 RCC and 2 BC) treated and untreated with 15 µM Sorafenib. GAS5 was up-regulated in all HCC cell lines except of SKHp1C3 (B), and in all renal cells (C) and in all breast (D) cancer cells treated. The histograms represent the mean values of 3 experiments, bars are ± SEM. Unpaired t-test; *p<0.05, **p<0.01, ***p<0.001; ****p<0.0001.



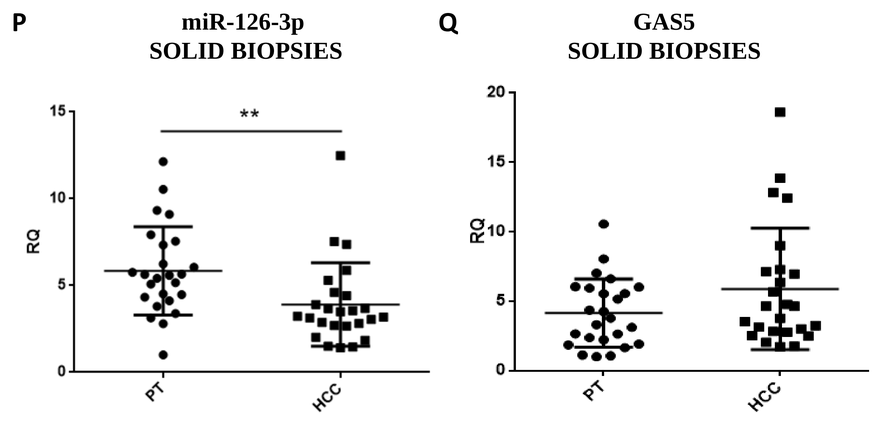
HOTTIP expression. The steady-state expression levels of HOTTIP (E) was measured by qPCR in all cell lines (3 HCC, 3 RCC and 2 BC) treated and untreated with 15 µM Sorafenib. HOTTIP resulted down-modulated in either HCC (F) and RCC (G) cells treated with 15 µM sorafenib. The histograms represent the mean values of 3 experiments, bars are ± SEM. Unpaired t-test; *p<0.05, **p<0.01, ***p<0.001; ****p<0.0001.



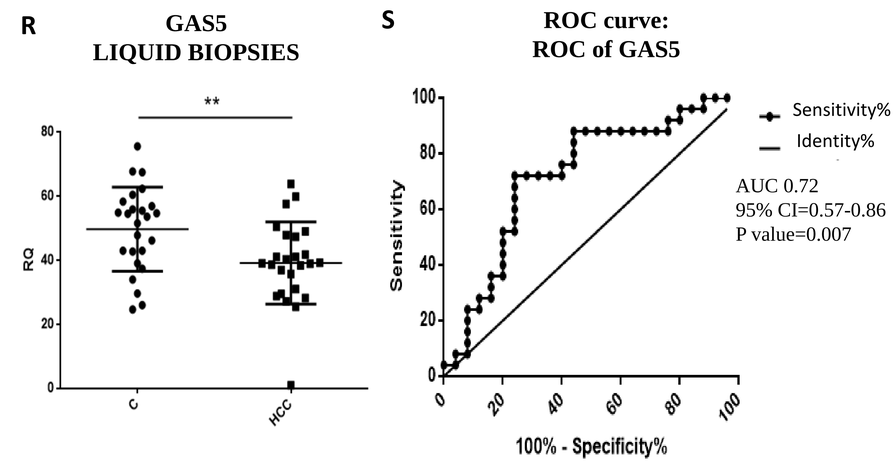
HOXA-AS2 expression. The steady-state expression levels HOXA-AS2 (H) was measured by qPCR in all 8 cell lines (3 HCC, 3 RCC and 2 BC) treated and untreated with 15 µM Sorafenib. HOXA-AS2 expression levels were down in hepatocellular (I), renal (J) and breast (K) cells exposed to sorafenib when compared with cells exposed to DMSO. The histograms represent the mean values of 3 experiments, bars are ± SEM. Unpaired t-test; *p<0.05, **p<0.01, ***p<0.001.



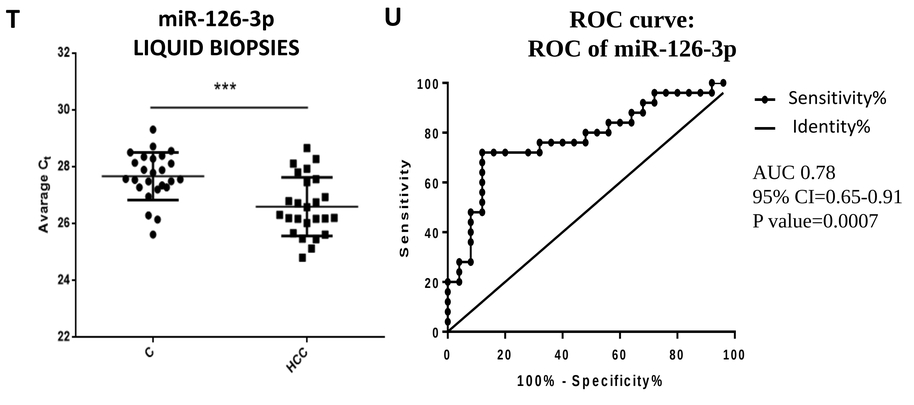
miR-126-3p expression. The steady-state expression levels of miR-126-3p (L) was measured by qPCR in all cell lines (3 HCC, 3 RCC and 2 BC) treated and untreated with 15 µM Sorafenib. miR-126-3p was down-modulated following sorafenib treatment in all the cancer cell lines used except of Caki-1 where it was up-regulated (M-O). The histograms represent the mean values of 3 experiments, bars are ± SEM. Unpaired t-test; *p<0.05, **p<0.01, ***p<0.001.



Expression levels of miR-126-3p and GAS5 in solid biopsies. The expression levels of GAS5 and miR-126-3p were measured by qPCR in 25 HCC solid biopsies and their corresponding peritumoral counterpart (PT). miR-126-3p levels were significantly down-modulated in HCC compared to PT. On the other hand GAS5 levels showed a trend of up-regulation in HCC compared to PT. Two-tailed, paired t-test; *p<0.05, **p<0.01, ***p<0.001.



Plasma circulating levels of GAS5. The expression levels of GAS5 were measured by qPCR in 25 plasma of healthy subjects and HCC patients. HCC patients plasma showed significantly lower levels of GAS5 compared to healthy control (R). ROC curve analysis of plasma GAS5 (S). AUC: area under the ROC curve; CI: confidence interval. Two-tailed, unpaired t-test; *p<0.05, **P<0.01, *** p<0.001.



Plasma circulating levels of miR-126-3p. The expression levels of miR-126-3p were measured by qPCR in 25 plasma of healthy subjects and HCC patients. HCC patients plasma showed higher levels of miR-126-3p compared to healthy controls (T). ROC curve analysis of plasma miR-126-3p (U). AUC: area under the ROC curve; CI: confidence interval. Two-tailed, unpaired t-test; *p<0.05, **P<0.01, *** p<0.001.

CONCLUSIONS. Over the last decades efforts have been carried out to make sorafenib a more effective drug in the management of different solid tumors as well as to identify novel molecular biomarkers of response. It is the first line-treatment for the management of unreseactable HCC and a therapeutic option for advanced RCC. Promising results are also emerging on the efficacy of sorafenib in combination with gemcitabine and/or capecitabine in BC clinical trials. This study highlighted the capability of sorafenib to modulate the expression of a wide range of ncRNAs. Specifically, GAS5 and miR-126-3p were involved in the response to sorafenib of different cancer cell types. Indeed they could be considered as useful and good diagnostic biomarkers of HCC in liquid biopsies.