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RAC1 INHIBITION AS A NEW THERAPEUTIC STRATEGY FOR HCC.

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INTRODUCTION

In the last years the development of tyrosine kinase inhibitors and immunotherapy has expanded the treatment landscape of advanced hepatocellular carcinoma (HCC). However, there is still necessary to develop new systemic therapies for patients with advanced disease. Hyperactivation of the RHO GTPases, in particular RAC1, was pointed out as key mechanism in HCC development suggesting that it may be an attractive therapeutic target for this cancer type. Recently we developed a RAC1 inhibitors series been 1A-116 the leading compound. However, the therapy potential of RHO GTPases inhibitors in HCC remains unexplored.

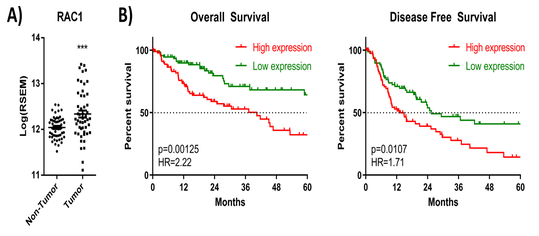
AIM

The aim of our work was to identify, and target RHO family members deregulated in HCC with a new series of RHO GTPases inhibitors.

MATERIAL & METHODS

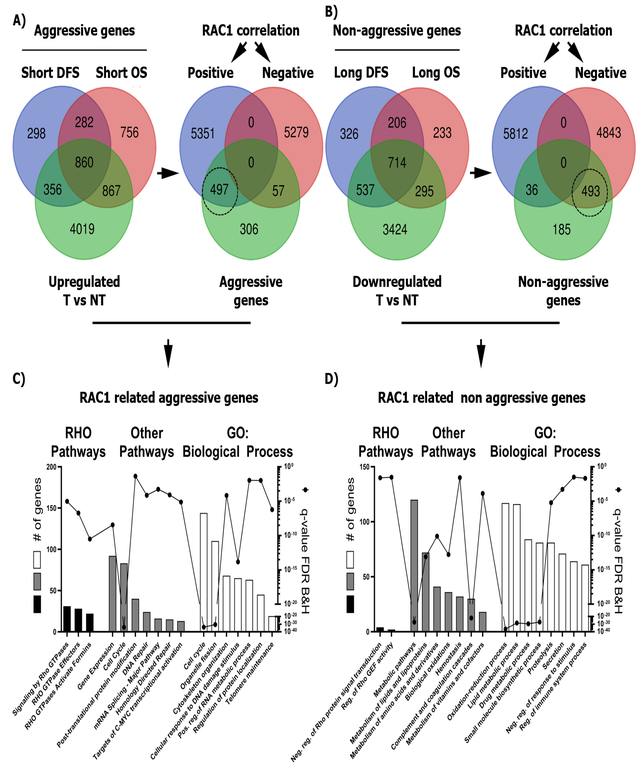
We studied expression deregulation, clinical prognosis, and transcription programs relevant to HCC using The Cancer Genome Atlas (TCGA). To develop the new series of RHO GTPases inhibitors we explored the chemical space surrounding guanidine inhibitors 1A-116 by a virtual screening. The therapeutic potential of RAC1 inhibitors in HCC was study in vitro and in vivo by MTT assay, flow cytometry and xenografts. The on-target effect was determined by a precipitation affinity assay (AP). RNA-Seq analysis and their correlation with HCC TCGA dataset were used to characterize the underlying mechanism upon RAC1 inhibition. The therapeutic effect of RAC1 inhibition on liver fibrosis was evaluated in vitro and in vivo in a mice model generated by thioacetamide (TAA) administration.

RAC1 expression is deregulated in patient with HCC and correlates with poor prognosis



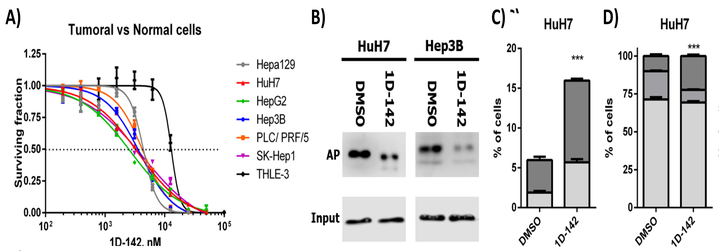
A. Expression levels of RAC1 within tumors are higher than those in NT adjacent tissue paired samples (n=50). ***paired t-test, p<0.05. **B)** Patients with high expression of RAC1 have significant worse prognosis in terms of OS and DFS in comparison with those expressing low levels; P-values (Cox-regression) and hazard ratio values are indicated in the figure.

RAC1 drives an oncogenic transcriptional program in HCC.



A) RAC1-related aggressive genes were defined by 1) overlapping upregulated genes in HCC with genes that correlate with shorter OS and DFS when are highly expressed (left panel). 2) overlap analysis of the aggressive genes with those that positively correlate with RAC1 expression. **B)** RAC1-related non-aggressive genes (doted circle) were defined as in "A" by overlapping genes that are downregulated in T vs. NT comparison, showing a better OS and DFS when highly expressed, and negatively correlate with RAC1 expression. **C-D)** Gene ontology analysis of RAC1-related aggressive (C) and non-aggressive (D) genes.

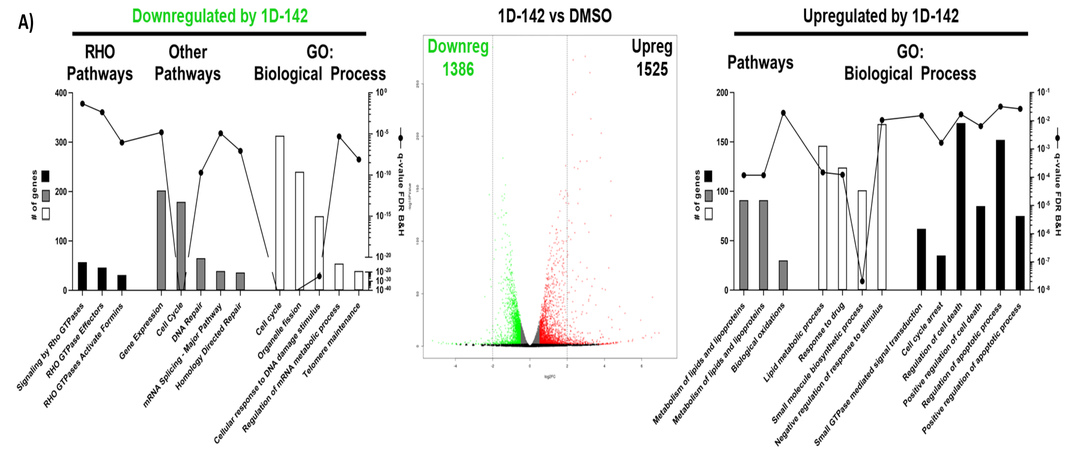
The RAC1 inhibitor 1D-142 exerts a potent antitumor effect against HCC



A) Dose-response curves for cell viability assessed in HCC cells and the hepatocyte cell line THLE-3 treated with 1D-142 by standard MTT assay. **B)** Block of RAC1 activation by 1D-142. RAC1-GTP was affinity precipitated (AP) from cellular extracts using PAK affinity beads. **C)** HCC cell death and **D)** Cell cycle arrest induced by 1D-142 (10 μM) after 24 h of treatment. ***p<0.001 vs. DMSO, by 2-way ANOVA.

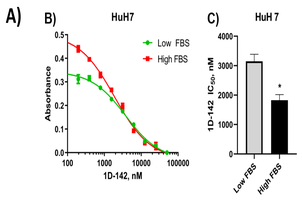
RESULTS

1D-142 reverses a RAC1-related oncogenic transcription program on human HCC cells.



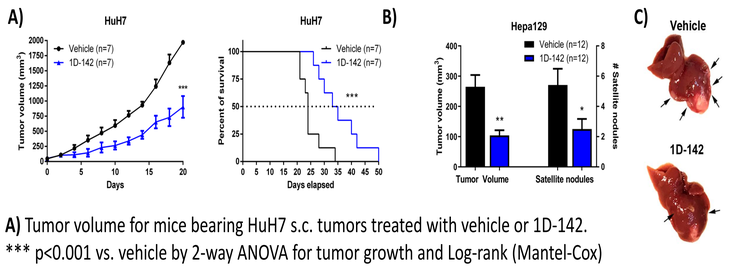
A) Volcano plot (middle panel), gene ontology (GO) and pathway Analysis of differential expressed genes [FDR = 0.05, Log2(FC)=+0.585] in HuH7 after 1D-142 treatment (10 μM) during 24 h. Gene expression was determined by RNA-Seq. The volcano plot highlights the upregulated (red) and downregulated (green) genes. The GO and Pathway Analysis was performed separately on downregulated (left) and upregulated (right) genes.

1D-142 antitumoral activity is stronger in highly proliferating HCC.



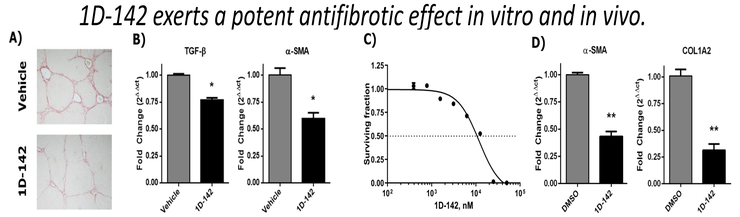
B) Cell viability dose response curves for HuH7 cells treated with 1D-142 and cultured in high (20%) or low (10%) FBS by standard MTT assay. C) Average of IC_{50} values obtained of 4 independent assays. *p<0.05, by paired t-test.

1D-142 exerts a potent antitumor effect on HCC in vivo.



A) Tumor volume for mice bearing HuH7 s.c. tumors treated with vehicle or 1D-142. *** p<0.001 vs. vehicle by 2-way ANOVA for tumor growth and Log-rank (Mantel-Cox) test for survival analysis. **B)** Tumor volume and satellite nodules number for mice bearing Hepa129 orthotopic tumors treated with vehicle or 1D-142. *p<0.05 and **p<0.01 vs vehicle-treated mice by t test. C) Representative photographs of livers of mice bearing Hepa129 orthotopic tumors. The arrows indicate satellite tumor nodules.

1D-142 exerts a potent antifibrotic effect in vitro and in vivo.



A-B) Liver fibrosis was induced by chronic TAA administration in BALB/c mice. Then mice were treated with vehicle or 1D-142. **A**) Microphotographs of liver sections stained with Sirius red. **B)** mRNA levels of liver fibrosis-associated genes. *p<0.05 by Mann-Whitney. **C)** Cell viability dose response curves for the hepatic stellate cell line CFSC-G2 treated with 1D-142. **D)** mRNA levels of liver fibrosis-associated genes in CFSC-G2 cells treated with 1D-142 (10 μM) or DMSO during 24 h. **p<0.01 vs. DMSO by Mann-Whitney.

CONCLUSION

The bioinformatic analysis of the HCC dataset of TCGA, allows identifying the RHO family of GTPases member RAC1 as a new therapeutic target for HCC. The targeted inhibition of RAC1 by 1D-142 resulted in a potent antitumoral effect in highly proliferative HCC established in fibrotic livers.

Disclosures: All authors declare that they have no conflict of interest.