IGF2 DRIVES IGF ONCOGENIC SIGNALING IN HCC AND EMERGES AS A POTENTIAL TARGET FOR THERAPIES

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Background and Aims

Hepatocellular carcinoma (HCC) is the 16th absolute cause of death world-wide and accounts for 90% of all liver cancers. IGF signaling has a relevant role in the pathogenesis of HCC and elucidation of its key molecular drivers is important to overcome the poor therapeutic results obtained so far by targeting this pathway in HCC.

We aimed to **a)** explore the oncogenic potential of IGF2 in genetically modified animal models, **b)** elucidate the mechanism responsible for its overexpression in human HCC patients and **c)** assess the efficacy of molecular therapies against this target.

Methodology

- In order to evaluate the oncogenic potential of IGF2 in HCC, a mouse model was generated using transposon-based delivery of IGF2 into the liver through hydrodynamic tail vein injection. The role of IGF2 in tumor initiation and tumor progression was assessed.
- A cohort of 228 HCCs was characterized analyzing gene expression, exomic mutations, DNA copy number and methylation status with a focus on the IGF pathway. Activation of the IGF2 promoters (P1-P3) were additionally assessed by qRT-PCR.
- Therapeutic potential of the monoclonal antibody BI836845 (Boehringer Ingelheim), selective for IGF ligands, was studied *in vitro* in 2 HCC cell lines overexpressing IGF2 (Hep3B, Huh7) and 2 with normal expression (PLC5, SNU449) by analyzing cell survival, proliferation and pathway activation.

Results

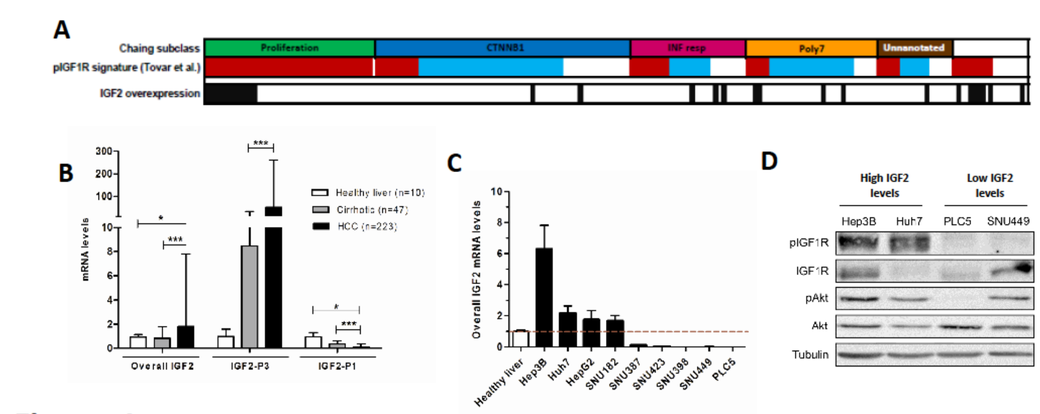

Figure 1. Role of IGF2 in human HCC. A) In our cohort of 228 HCCs, 15% of patients show IGF2 overexpression (FC>2). **B)** IGF2 expression is significantly higher in tumoral tissue than in cirrhotic adjacent tissue or healthy liver samples. This event is associated with the reactivation of the fetal promoter P3 and the inactivation of the adult promoter P1. **C)** 2/9 HCC cell lines screened overexpressed IGF2. **D)** Cell lines with high levels of IGF2 show activation of the IGF pathway, in contrast to cell lines expressing low levels of IGF2. * p<0.05, **p<0.01, ***p<0.001

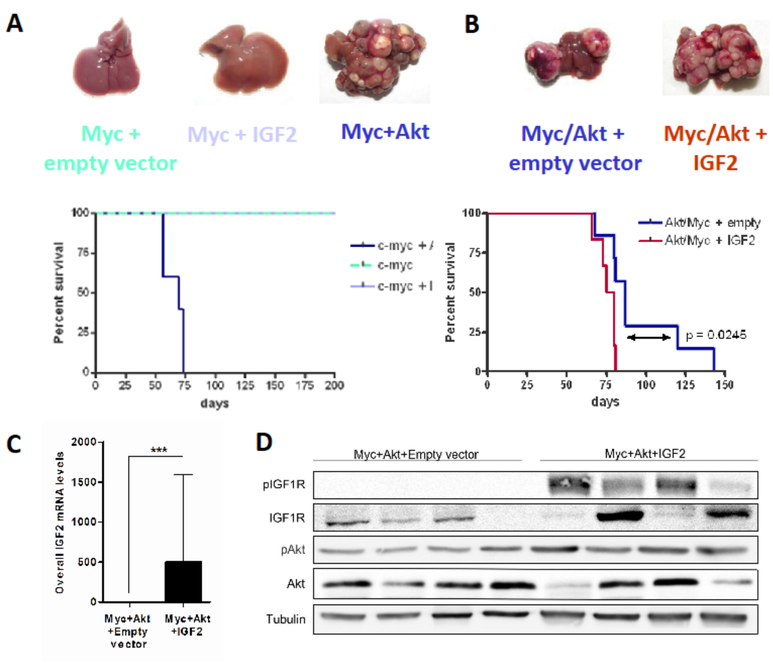

Figure 3. Oncogenic potential of IGF2 in a mouse model of HCC. A) Role of IGF2 in HCC tumor initiation evaluated through the delivery of IGF2+MYC in CDKN2A-/- knock-out mice. Injection of MYC+AKT induced liver tumors after 2 months and was used as a positive control. Mice overexpressing MYC+IGF2 or MYC alone did not show HCC development. **B)** Role of IGF2 in tumor promotion evaluated through the delivery of IGF2 along with MYC and AKT in wild-type mice. Mice overexpressing MYC/AKT+IGF2 showed significantly decreased overall survival. **C)** mRNA levels of IGF2 were significantly higher in tumors of mice injected with MYC/AKT+ IGF2. **D)** WB showing IGF pathway activation (pIGF1R) in mice overexpressing IGF2.

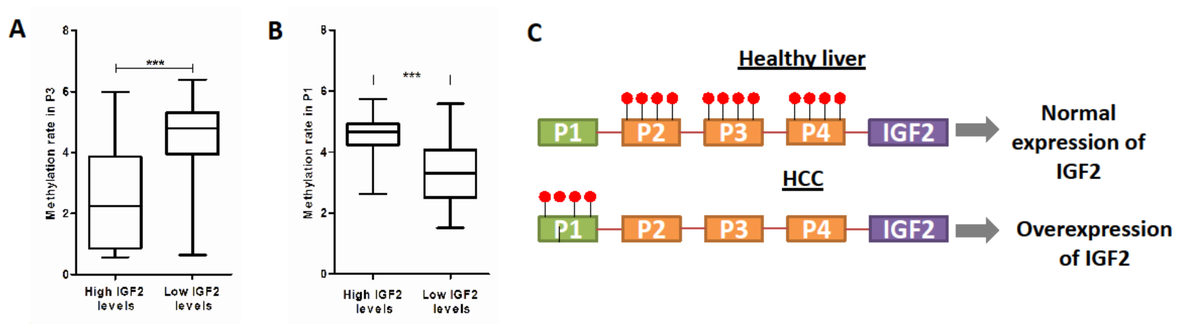

Figure 2. Mechanism of reactivation of IGF2 fetal promoters. A) HCC human samples expressing high IGF2 levels showed hypomethylation of the fetal promoter P3. **B)** HCC human samples overexpressing IGF2 showed hypermethylation of the adult promoter P1. **C)** Schematic representation of the aberrant methylation pattern in IGF2 promoters in 87% of HCC human patients.

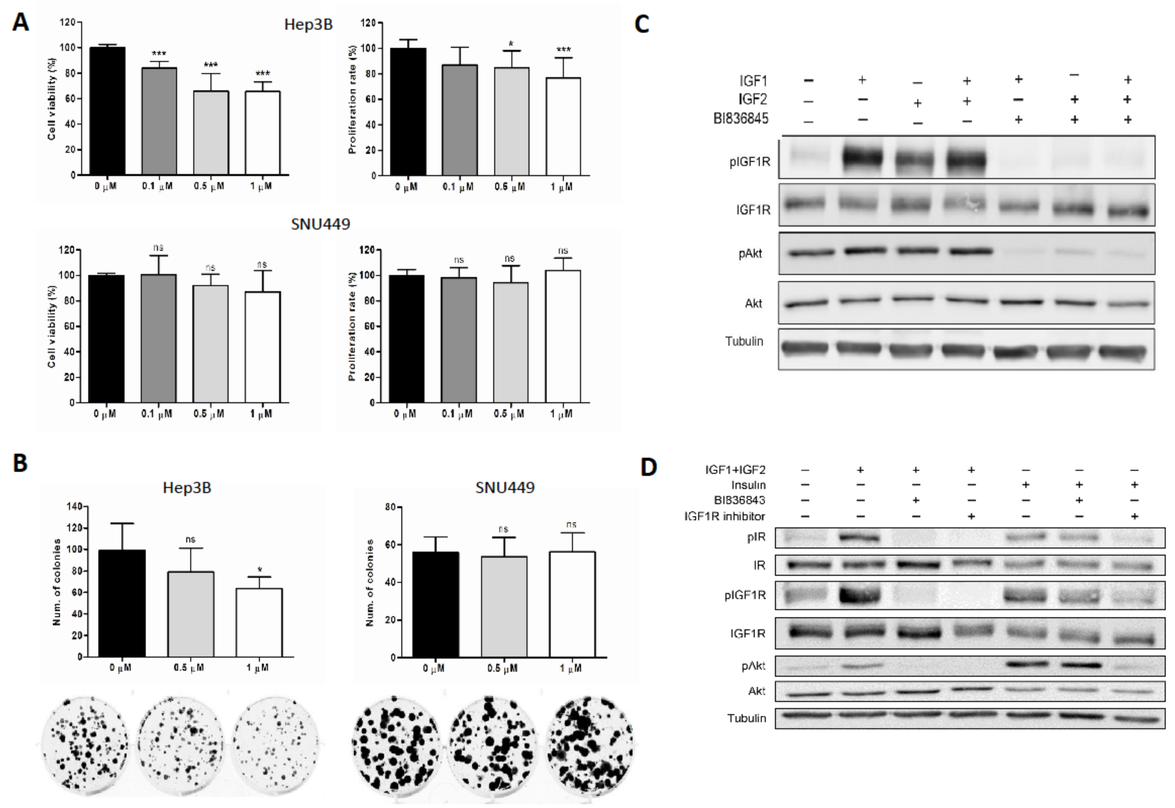

Figure 4. Antiproliferative potential of BI836845 in HCC cell lines. A) 1 µM of BI836845 reduced in 30% the viability and proliferation of cell lines overexpressing IGF2 (Hep3B cells), but not in cells with normal levels of IGF2 (SNU449). **B)** 1 µM of BI836845 significantly reduced colony formation capacity (36%) of Hep3B cell lines overexpressing IGF2, but not in SNU449 cells. **C)** Treatment with BI836845 impaired IGF ligand-mediated activation of the IGF pathway (pIGF1R) in BI836845-responsive cells. **D)** BI836845 inhibited IGF pathway activation without interfering with the effects of insulin on insulin receptor (IR). In contrast , inhibiting IGF1R with the IGF1R inhibitor (Lisitinib) interfered with IR activation , suggesting blocking of both receptors due to high structural homology.

Conclusions

1) IGF2 is overexpressed in 15% of HCC patients due to a switch in the activation of its promoters (inactivation of adult promoter P1 and reactivation of fetal promoters P2-P4).

2) The mechanism responsible for the switch in the activation of IGF2 promoters is associated with an aberrant methylation pattern (hypermethylation of adult-P1 and hypomethylation of fetal-P3) in 87% of cases.

3) IGF2 is able to accelerate the progression of HCC in GEMM models.

4) BI836845, a monoclonal antibody against IGF ligands, efficiently inhibits IGF1R activation in HCC cell lines overexpressing IGF2 and is able to reduce their proliferative potential *in vitro*, without interferring with insulin metabolic effects.

Institutions

Funding

HEPTROMIC
Boehringer Ingelheim