Transcriptional study in hepatoblastoma patients points to a new tumor stratification, oncogenic mechanism and treatment

Katarzyna B. Hooks1,2, Jérôme Audoux1,4, Helena Fazli1,2, Sarah Lesjean1,2, Tony Ernault1,6, Nathalie-Dugot Senant2, Thierry Leste-Lasserre3, Martin Hagedorn1,2, Benoît Rousseau5, Coralie Danet9, Sophie Branchereau5, Laurence Brugières11, Sophie Taque12, Catherine Guettet11, Monique Fabre11, Anne Rullier11, Marie-Annick Buendia11, Thérèse Commes11, Anne-Aurélie Raymond11,2 and Christophe F. Grosset11,2

1Univ. Bordeaux, Inserm, GREMI, U1053, 33076 Bordeaux, France; 2Faculté de Médecine, Université de Bordeaux, 33076 Bordeaux, France; 3Institut de Biologie Computationnelle, Université Montpellier, Montpellier, France; 4Physiopathologie et traitement des maladies du foie, Inserm, UMR2113, Hôpital Paul Brousse, Hépatobiliary Centre, 94800 Villejuif, France; 5Univ. Paris Saclay, 94800 Villejuif, France; 6Physiopathologie de l'Inst. des Neurosciences, UMR, 94800 Villejuif, France; 7Institut d'Onco-hépatologie, Hôpital de Créteil, 94800 Villejuif, France; 8Institut d'Onco-hépatologie, Hôpital de Créteil, 94800 Villejuif, France; 9Magendie, U1215, Faculté de Médecine, Université de Bordeaux, Bordeaux, France; 10Hôpital Bichat, La Kerménie-Bichat, Paris, France. 11Institut de Cancer, Gustave Roussy Institute, Villejuif, France. 12Hôpital Universitaire de Rennes, France. 13Hôpital Necker, Paris, France. 14Hôpital Universitaire de Bordeaux, France.

Background and objectives

Hepatoblastoma (HB) is the most common pediatric liver cancer that develops on a normal liver (NL). HB diagnosis and tumor staging include histopathological assessment of biopsies, radio-imaging and alpha-fetoprotein levels. Treatment of HB associates chemotherapy (CT) and surgical resection of the primary tumor leading to an overall patients survival of 70-80%. However, the outcome is less satisfactory for patients with high-risk tumors, poor response to CT and/or lung metastasis. New biological study should bring new information and treatment options.

Results

Figure 1: Transcriptional study of 24 HB and matching NL samples.
(A) We compared polyA+ sequencing of HB (T) and matching normal liver tissue (NT) from 24 patients and two HB cell lines (CL). A previous study performed by Cairo, Arnesson and coworkers identified two main molecular profiles in HB, discriminated by a 16-gene signature: C1 for good prognosis and C2 for poor prognosis (PMid: 19003588). (B) PCA analysis confirmed existence of these two groups and divided poor-prognosis C2 tumors in two subgroups: C2A and C2B.

Figure 2: Transcriptional study established a 4-gene signature distinguishing 3 tumoral subgroups, instead of 2. (A) Using Rseq data we designed a 4-gene signature distinguishing normal liver and 3 groups of HB. (B) Our results were further validated by RT-qPCR on an enlarged collection of 78 samples (9) and confirmed at the protein level by immunostaining on formalin-fixed paraffin-embedded tissues (C). TOP2A and VM could constitute new biomarkers for poor-prognosis C2A and C2B tumors, respectively.

Figure 3: Pathways deregulated in HB subgroups.
(A) Top: significant Gene Set Enrichment Analysis (GSEA) Hallmarks for NT and each tumor subgroup. (B-C) The Fanconi Anemia pathway (FA) is over-activated in C2A group and many FANC factors are overexpressed in proliferative C2A tumors.

Figure 4: Effect of proteasome inhibitors on HB cells viability.
(A) MTS cell proliferation assay performed on HepG2 (left panel) and HepG2 (right panel) cells after addition of cisplatin (CP), bortezomib (B), combination of the two (CP+B), MG132 and combination of MG132 with cisplatin (CP+MG132). (B) Sulfhydrylamine B cell density determination assay performed on HepG2 (left panel) and HepG2 (right) cells after addition of CP, B or combination of the two. (C) Apoptosis assessed by Annexin V and 7AAD staining in HepG2 (top panel) and HepG2 (bottom panel) cells after addition of CP, B or combination of the two. (D) Cisplatin induced DNA damage in HepG2 cells treated either with CP, B or combination of the two. (E) Mouse bearing HB xenografts were treated with CP, B or combination of the two. Tumor volumes were used as readout for tumor progression and treatment efficiency.

Figure 5: Effect of bortezomib on Fanconi Anemia pathway and DNA repair.
(A) Western blot analysis of the H2AX phosphorylation at serine 139 (pH2AX) at different time points after γ-irradiation with or without bortezomib treatment (B) performed on whole extract of HepG2 and HepG2 cells. (B) Protein expression and ubiquilinization of FANC D2 and 5ANKI 4hr after γ-irradiation (IR) and bortezomib treatment (B) analyzed by Western blot on whole cell extracts from HepG2 or HepG2 cells. (C) Top panel: Representative images of FANC D2 and FANC D4 in HepG2 cells. Sixteen hours after bortezomib treatment, cells were irradiated and 30hr later analyzed by immuno-fluorescence (bars = 20 μm). The graphs on the bottom panel represent the mean number of foci per cell (±SD) (n=200 cells were counted per experiment).

Conclusions and references

We designed an easy-to-use 4-gene signature for HB stratification amenable in clinic.

- Fanconi Anemia pathway plays a key role in HB carcinogenesis and chemoresistance.
- Proteasome inhibitors efficiently eliminates C2A HB cells.
- Proteasome inhibitors could be used for HB treatment in combination or not with cisplatin when facing patients with lung metastasis or high-risk tumors with TOP2A+ cells → Theranostic biomarker.