A panel of pediatric liver cancer patient-derived xenografts to improve stratification of children with hepatoblastoma

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INTRODUCTION

Despite being the predominant type of pediatric liver malignances, hepatoblastoma (HB), with a world-wide incidence of 1 case per million persons per year, is a rare tumor. The high rate (> 60 %) of β-catenin activating mutations places HB as one of the human tumors most tightly associated with activation of the Wnt/β-catenin pathway. Evidence for (epi)genetic origin of HB is provided by its association with congenital anomalies, Beckwith-Wiedemann syndrome, and familial adenomatous polyposis, a disorder caused by germline mutation of APC, involved in β-catenin degradation. Like other rare diseases, rare cancers are particular challenging due to their low incidence, particularly for the identification of novel therapies. HCC, fibrolamellar carcinoma (FLC), and transitional liver cell tumors (TLCT), which combines histological features of HB and HCC, also arise in children and adolescents, at a lower extent though. Sporadically, very rare forms of liver tumor likely of non-epithelial origin such as rhabdoid tumor or hepatic sarcoma also occur. The rarity and the heterogeneity of childhood liver cancers hamper the development of reliable research tools that recapitulate each disease.

STRATEGY AND METHODS

To tackle this issue, in collaboration with pediatric oncologists, surgeons and pathologists of the International Childhood Liver Tumour Strategy Group (SIOPEL), we have launched a program aimed at the constitution of a preclinical panel of liver cancer patient-derived xenografts (PDXs). From May 2010 to January 2013, 42 tumors from resected liver cancer samples have been grafted by interscapular implantation of tumor fragments in athymic nude mice directly post-surgery. These tumors included 35 hepatoblastomas (HB), 1 HB/HCC transitional tumor, 1 late-occurring lung metastasis with HCC-features from a patient operated of HB 10 years earlier, 2 hepatic sarcomas, 2 fibrolamellar carcinoma, and 1 rhabdoïd tumor.

Post-surgery tumor specimens were transplanted in the interscapular region or into the renal capsule of nude or NOD/SCID mice (Figure 1). Tumor growth was observed with a latency period of 1 to 6 months. Tumor xenografts were amplified by serial transplantation, and tissue samples were retained at each passage for comparison with the patient's tumor (MF).

From these grafts we could develop 12 HB PDXs (overall 39% take rate), 1 TLCT, 1 HCC model, and 1 rhabdoïd tumor PDX.

For the HB models established, we observed that successful grafting is observed for the majority of specimens from recurrent tumor (6/8, 75%), whereas for primary tumors it was strongly reduced (9/32, 28.1%) (Table1). All models were established upon written informed consent by the children's families, and in compliance with all requirements in terms of animal facility qualification and of experimental procedures approval by National Animal Ethics committee and in compliance with EU legislation on the protection of animals used for scientific purposes.

All PDX models, except for HB-214 and RT-001 that are still ongoing, have been profiled by RNA-seq as well as the parental tumor and the adjacent healthy tissue. In addition, all PDXs, and parental tumors, except for RT-001, have been profiled by Affymetrix HTA gene chip and cytoSCAN HD aCGH. Instead, RT-001 was previously characterized by next generation exon sequencing of the 73 genes most frequently mutated in cancer according to the COSMIC database, together with HB-213, HB-214 and HB-217, thanks to an internal sequencing program run by XenTech on its whole PDX panel.

Immunohistochemistry has been performed on the whole panel (Table 3) that includes all the diagnostic markers available to assist clinical decisions. The results have been compared with the histological phenotype of the tumors of origin, and we observe and overall selection of the most undifferentiated tumor components growing on PDXs.

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PDX ID	Age (months)	Sex	Tumor Type	Tumor origin	Risk	Pre-surgical chemotherapy	Distant metastasis at diagnosis	Aggressive features
HB-213	19	F	HB	Primary	H	+	+	
HB-214	30	F	HB	Primary	H	+	+	SCUD
HB-217	24	M	HB	Local Recurrence		+	-	
HB-229	54	M	HB	Local Recurrence		+	+	AFP-negative
HB-232	6	M	HB	Primary	S	+	-	
HB-233	16	M	HB	Primary	S	+	-	SCUD
HB-236	8	F	HB	Primary	S	+	-	
HB-238	110	F	HB	Local Recurrence		-	-	
HB-239*	113	M	HB	Primary	H	+	-	SCUD
HB-243	52	M	HB	Local Recurrence		+	-	
HB-244*	114	M	HB	Local Recurrence		+	-	
HB-252	14	F	HB	Primary	S	+	-	
RT-001	24	F	MRT	Primary	na	+	-	Rhabdoid
HC-001	276	M	HCC	Lung metastasis		-	-	HCC
TT-001	42	F	TLCT	Primary	H	+	+	HCC features

Table 1: list of liver cancer PDXs established

PDX ID	73-gene mutation*	RNA-seq	HTA 2.0 array	CytoSCAN HD array	Patient/PDX histology and IHC**	β-catenin status	Molecular features (other than β-catenin/INI1 status)	Circulating AFP Patient/PDX
HB-213	+	+	+	+	+	G34V	MSH2 G322D variant	+/+
HB-214	+	-	+	+	+	exon 3 del	PBRM1 mutation	+/+
HB-217	+	+	+	+	+	exon 3 del	AKAP9 mutation	+/+
HB-229	-	+	+	+	+	exon 3 del	Chr 8 gain, 4q loss	-/-
HB-232	-	+	+	+	+	wt	Chr 2p and 20 gain	+/na
HB-233	-	+	+	+	+	exon 3 del		+/na
HB-236	-	+	+	+	+	exon 3 del		+/na
HB-238	-	+	+	+	+	exon 3 del	Chr 20 gain, 4q loss	+/na
HB-239	-	+	+	+	+	exon 3 del	Chr 2p and 20 gain, 4q loss	+/+
HB-243	-	+	+	+	+	exon 3 del		+/+
HB-244	-	+	+	+	+	exon 3 del	Chr 2p, 8 and 20 gain	+/+
HB-252	-	+	+	+	+	exon 3 del		+/na
RT-001	+	-	-	-	+	wt	INI1 loss; ATM R2854C variant	-/-
HC-001	-	+	+	+	+	wt		+/+
TT-001	-	+	+	+	+	D32Y		+/+

Table 2: list of molecular characterization and main molecular features of the liver cancer PDXs established

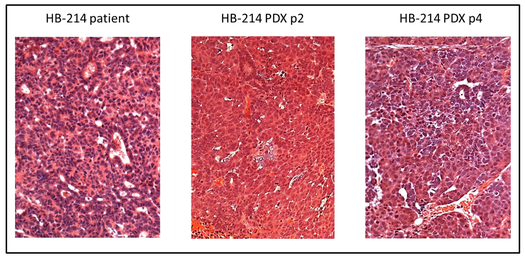

Figure 1: comparative histology of patient's and PDX tumors

RESULTS

All PDXs maintain the histological features of primary human tumors (Fig 1), and the heterogeneity of AFP levels in mouse blood correlate with that observed in patients (Table 2). Comparative analysis of the clinical parameters associated to tumors from which PDX could or could not be established has been performed. Among HB PDXs models that have been established several are resistant to cis-platinum standard of care (Fig.2). This is probably due to selection of resistant tumor cells during the administration of neo-adjuvant therapy cycles since most of these patients showed objective tumor response prior to surgery. In vivo anti-cancer screening in histologically different HB/HCC/TLCT PDX subtypes identified the combination irinotecan/temozolomide as a promising second line combination for a subset of liver cancer PDXs (Fig 3). When tested on PDXs from primary and recurrent tumor from the same patient, we observed different response, warranting on the need of predictive markers to help with therapeutic indications.

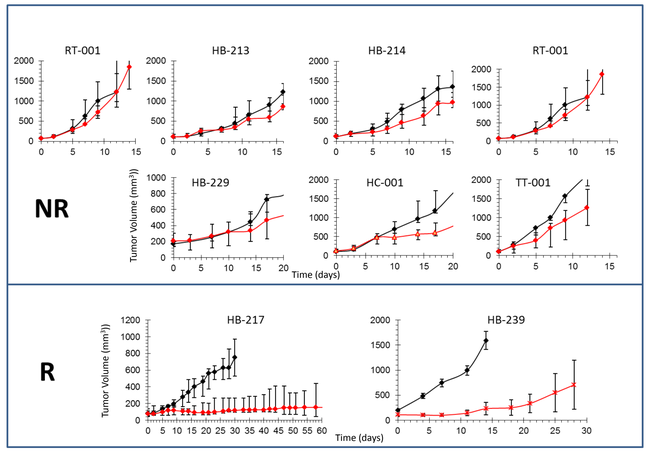

Figure 2: PDX response to cisplatin (5mg/Kg, q3wk). Black line: control group; red line: treated group. For each group at least 6 mice were included. R= responders; NR=non-responders.

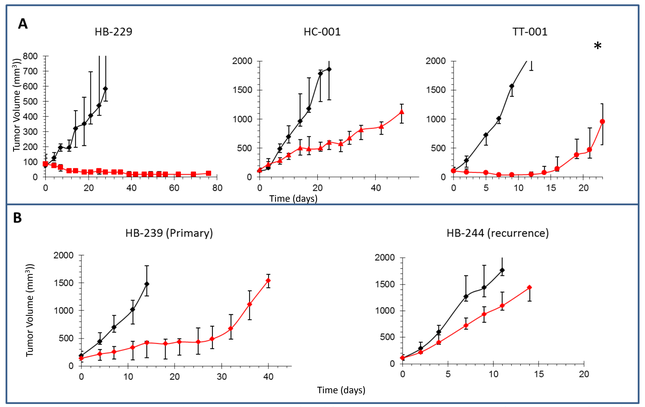

Figure 3: PDX response to irinotecan/temozolomide (Irinotecan 10mg/Kg q5dx5 + Temozolomide 68mg/Kg qdx5). Black line: control group; red line: treated group. For each group at least 6 mice were included. A: PDXs representing aggressive liver cancer tumors; B: treatment was administered to two PDXs from the same patient.
*=Irinotecan 40 2qwk + Temozolomide 68 qdx5 (treatment arrested at day 10 due to toxicity)

PDX ID	ARG1	AFP	GPC3	GS	β-Catenin	EpCAM	CK7	CK19	CD56	Ki67	p53	Cyclin D1	Vimentin	INI1
HB-213	na	20%/C/I2	60%/C/I3	100%/C/I3	90%/N&C/I2	100%/M/I3	na	na	na	60%/N/ I3	80%/N/I3	na	na	na
HB-214	na	90%/C/ I2-3	80%/C/I3	70%/C/I3	100%/N&C/I3	30%/M/I1-2	na	na	na	90%/N/I3	80%/N/I3	na	na	na
HB-217	na	100%/M/I1-3	90%/M&C/I1-3	100%/C/I3	100%/N&C/I3	100%/M/I3	na	na	na	80%/N/I3	40%/N/I3	na	na	na
HB-229	na	negative	negative		100%/N&C/I3				negative	80%/N/I3	90%/N/I2		40%/C/I3	positive
HB-232	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing
HB-233	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing	ongoing
HB-236	5%/C/I3	90%/C/I1-2	50%/C/I2	40%/C/I-i2	100%/N&C/I2	100%/M/I3	80%/M/I3; 30%/C/I2	80%/M/I3; 30%/C/I2	90%/M/I2; 10%/C/I2	80%/N/I3	1%/N/I3	20%/N/ I1-2	na	na
HB-238	70%/C/I2	/60%C I2	/60%.I1/12	/20%C/I1	100%/N&C/I2	100%/M/I2-3	80%/M/I2	100%/M/I3	70%/M/I2-3; 20%/C/ I1-2	80%/N/I3	70%/N/I1-I2	50%/M/I1-2	na	na
HB-239	80%/C/I1-2	100%/C/I2	100%/M&C/ I2-3	70%/C/ I1-2	100%/N&C>M/I2-3	na	100%/C/I2	100%/C/I3	100%/C/I2-3	70%/N/I2	50%/N/I1-3	50%/N&C/I2	na	na
HB-243	70%N&C/I3	60%/C/ I2-3	70%/C/ I2-3	40%/C/I2	100%/N&C/I2-3	100%/M/I2-3	negative	30%/M/I3	negative	70%/N/I2-3	90%/N/I2-3	60%/N&C/I2-3	na	na
HB-244	50%/C/I3; clone 15%/C/ I0-1	100%/C/I3	100%/C/I3	50%/C/I1-2	100%/N&M>C/I3	na	20%/C/I3; clone 10%/C/I1	10%/C/I1	90%/M/I1	90%N/I3	30%/N/I1-3	80%/N&C/I2-3	na	na
HB-252	100%/M/I3; 10%/C/I2	90%/C/I2	5%/C/ I1-2	5%/C/I1-2	100%/N&C/I3	100%/M/I2-3	30%/C/I1/i2; 80%/M/I1	30%/C/I1-2; 80%/M/I1	100%/M/I2; 30%/C/I2	80%/N/ I3	60%/N/I1-3	30%/N/I1-2	na	na
RT-001	na	na	na	na	na	na	na	na	na	na	na	na	na	negative
HC-001	na	negative	100%/C&M /i2	30%/C/I2	100%/N&C/I3	10%/M&C/ I2	na	<1%/M/ I2	negative	50%/N/ I3	80%/N/I2	na	negative	na
TT-001	na	80%/C/ I1	negative	10%/C/I1	100%/N&C/I3	100%/M/I3	na	na	negative	90%/N/I3	15%/N/I1	na	negative	na

Table 3: liver cancer PDXs characterization by IHC. Legend: % positive cells/cellular localization (C=cytoplasm; M= membrane; N=nucleus)/I=intensity (0 to 3)

CONCLUSIONS AND PERSPECTIVES

Development of a panel of childhood liver tumor PDXs will endow the scientific community with an innovative and versatile research tool that will decisively contribute to improve our understandings on pediatric liver malignancies. These models constitute an unperishable reservoir of biological samples that strongly recapitulate the human tumor biology, and they can be used in several research domains such as functional genomics, cancer stem cell biology and pharmacogenomics, notably for the identification of Wnt/β-catenin inhibitors. In the long run, improved knowledge in all these research fields will be translated in improved cures for kids.