Orthotopic model of Hepatocellular Carcinoma (HCC) using Huh-7 and HepG2 cells: Application of micro-CT in detection of cancer progression

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and was listed as the third cause of death by cancer in 2020 (WHO). Hepatocarcinogenesis is a multistep process and to date it is still poorly understood. HCC occurs almost entirely in patients with underlying chronic liver disease and cirrhosis. Pre-clinical animal models play an important role in unveiling cancer biology which can facilitate development and testing of new antineoplastic drugs.

The aim of this study was to establish an orthotopic model of HCC utilizing two cell lines Huh-7 and HepG2, which could be effectively used to study efficacy of new potential treatments.

Materials and Methods

Female NMRI (NMRI-Foxn1nu; Janvier, France), 7-9 weeks old were used in the study. Mice were weighed two times per week to follow up on their well-being. Two cancer cell lines were used: HepG2 and Huh-7. In the intrahepatic (ih-) model cancer cells were inoculated directly into the left lobe of the liver, whereas in the intrasplenic (is-) model cells were injected via the spleen followed by splenectomy. *In vivo* tumor size was assessed using Bruker SkyScan 1276 High-Resolution Micro-CT Scanner (Bruker micro-CT). To visualise liver and increase tissue contrast ExiTron Nano 6000 (Viscover™) was used. The contrast agent was administered once during the study according to manufacturer's instruction. The *in vivo* measurements were performed using one field of view (FOV) and an image pixel size of 18 µm. Each *in vivo* scanning was completed within 12 minutes and estimated radiation dose was kept under 500 mGy at each imaging session. In the follow up study, serum alpha-fetoprotein was measured using Quantikine® ELISA kit.

Mice were sacrificed individually according to the predefined criteria. At sacrifice, in case tumors were protruding out of the liver, ex vivo measurements were obtained using electronic caliper.

Study design

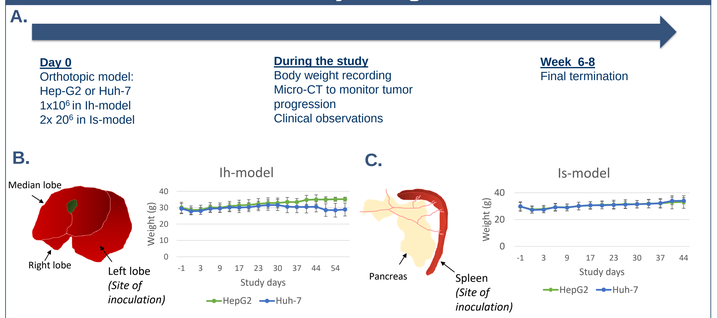

FIGURE 1. A. Schematic workflow of the orthotopic model**.** Mouse well-being was followed for 10 weeks by observing changes in body weight and clinical status. **B.** Graphic representation of the intrahepatic model. Site of inoculation is indicated with an arrowhead. Body weight growth curves (g, mean ± SEM) are presented. **C.** Graphic representation of the intrasplenic model. Site of inoculation is indicated with an arrowhead. Body weight growth curves (g, mean ± SEM) are presented.

Application of Micro-CT

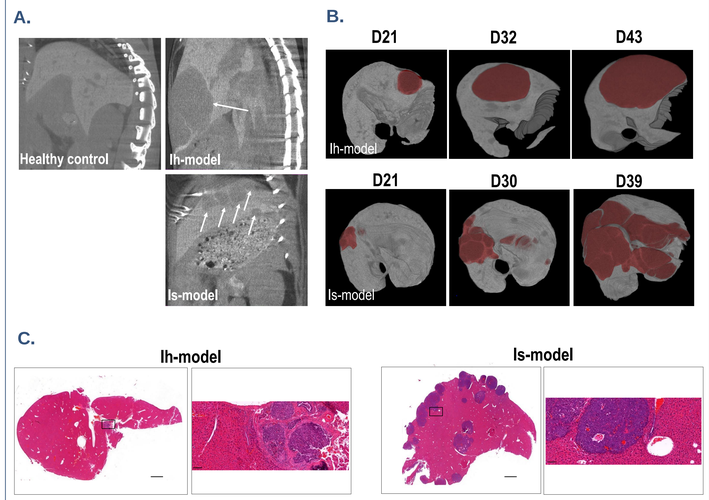

FIGURE 2. A. Tumor presentation *in vivo* (liver side-view), Liver was visualized using contrast agent Exitron 6000 Nano. A single nodule is visible in the HepG2 ih-model, versus multiple small tumor nodules seen in the is-model (HepG2). Similar tumor presentation was observed when Huh-7 cells were used. Healthy control is shown for a comparison. B. Visualization of tumor progression by 3D reconstructions. Timeline development of both ih-and is-model are shown. C. Representative images of HE staining. From left to right, ih-model (scale bar 2000 µm), 10x objective (scale bar 100 µm), is-model (scale bar 2000 µm), 10x objective (scale bar 100 µm).

Tumor growth correlation to AFP

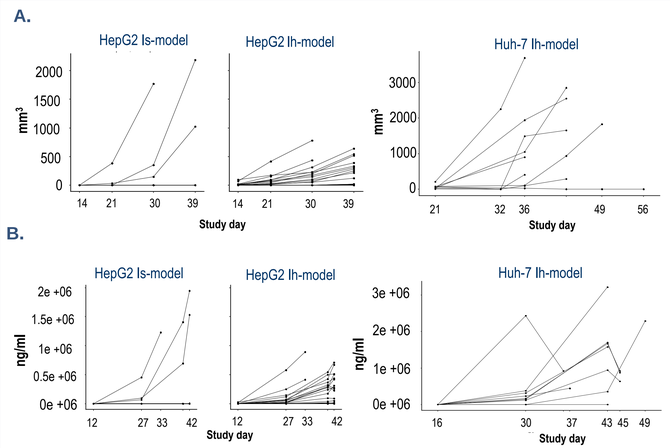

FIGURE 3. A. *In vivo* tumor size (mm^3). Images obtained on multiple occasions were manually segmented and individual region of interest (ROI) for each tumor were drawn. The volume of interest (VOI) was saved for each ROI and was later used in analysis. The tumor size for individual study subject (mm^3) is presented. Tumor take in the ih-model varied between 53-85% and between 15-46% in the is-model (presented data reflects only the studies where AFP was measured). **B.** Measured in a follow-up study, levels of tumor marker alpha-fetoprotein were increasing in animals with tumor nodules detected *in vivo* in both models (ih, is). Levels of AFP did not increase in animals with no detected tumors.

Clinical observations

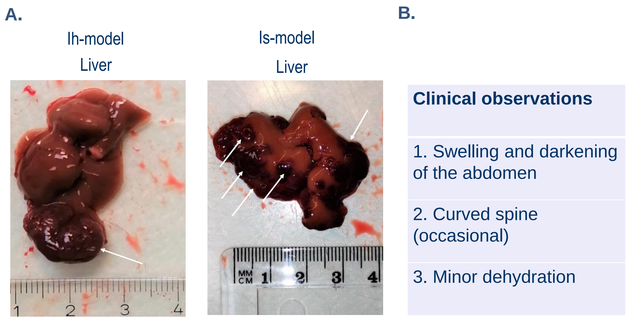

FIGURE 4. *Ex-vivo* tumor presentation. A. At sacrifice tumor location was confirmed to be in the liver in both ih- and is-models. In the ih-model the tumor formation was confined to one location (arrow), whereas in the is-model tumor formation was wide-spread across the whole organ. For presentation purpose only few changes are indicated with an arrow. B. Major clinical observations made during in-life phase are listed. Animals did not present major clinical symptoms before end-stage disease.

Summary

- Tumor take rate in the ih-model was to 85%. However, it was less than 50% in the is-model.
- The mice did not present any acute symptoms before end stage disease.
- Intrahepatic inoculations led to more uniform and measurable tumor nodules both *in vivo* and *ex vivo* compared to intrasplenic inoculations, where tumors were dispersed across liver forming multiple nodules.
- In a follow –up study animals with increasing tumor volume showed increasing amounts of alpha-fetoprotein in serum samples in both models.
- Micro-CT was a useful tool in tracing tumor development and disease progression

Conclusions

In summary, an orthotopic model using intrahepatic approach of cancer cell implantation was established. Application of micro-CT enabled following tumor growth and recognition of cancer spread in the abdomen. Altogether this provides a promising tool contributing to the development of novel therapies targeting HCC *in vivo*.

Acknowledgements

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