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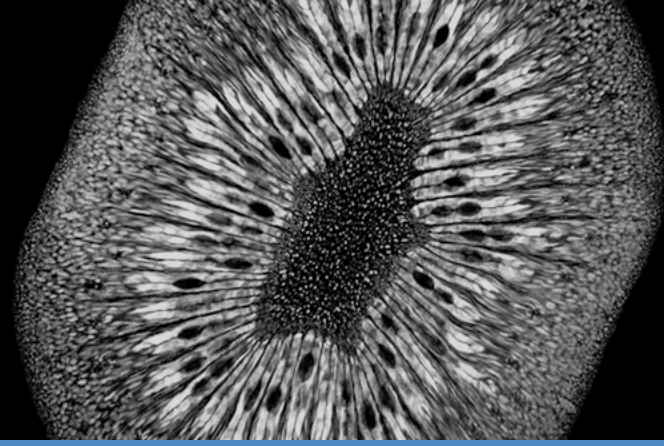
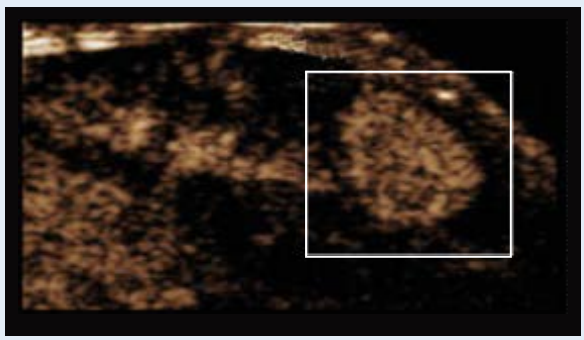


Ultrasound of hepatocellular carcinoma



Contrast-enhanced ultrasound (CEUS) using PolySon™ L to characterize hepatic tumors in a mouse model of hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and the second leading cause of cancer deaths worldwide[1]. HCC is a type of highly vascular tumor, and therefore accompanied by major vascular changes. The healthy liver has a dual blood supply, deriving ~80% of its blood from the portal vein and ~20% from the hepatic artery. As HCC nodules develop, this "normal" combined portal/arterial blood supply decreases and is replaced by an "abnormal" supply from arterial tumor vessels. These pathological changes in blood flow can be observed via diagnostic imaging and, in the clinic, several complementary imaging techniques are used, namely ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI)[2]. HCC tumors show a typical contrast enhancement pattern where, compared to liver parenchyma, they are first hyperdense during the arterial phase (wash-in) and then hypodense in the portal venous phase (wash-out). The presence of this characteristic enhancement pattern (wash-in with subsequent wash-out) is considered the definitive imaging feature of HCC and is recommended in the guidelines by various associations for liver studies[3]. In this work we develop a contrast-enhanced ultrasound (CEUS) method involving the use of PolySon™ L to detect hepatic focal lesions in a transgenic mouse model of HCC. Using the same imaging criteria of arterial phase wash-in and portal phase wash-out, we show that this method allows the detection of hepatic lesions associated with HCC, potentially facilitating the monitoring of therapy.

Materials and methods

Experiments were performed on isoflurane-anaesthetized Hepatitis B virus (HBV)-transgenic mice (n=5, 8-10 weeks old), which previously exhibited HCC tumors on MRI scans (details reported elsewhere[4]). Non-invasive monitors were used throughout to assure adequate oxygenation, ventilation and normal body temperature. Ultrasound of the murine livers was performed using a Vevo 2100 system (FUJIFILM, VisualSonics, Toronto, Canada), equipped with a high-frequency solid-state transducer at a 40 MHz center frequency providing axial resolution down to 30 µm. Initially, B-mode and Color Doppler imaging was conducted for identification of the target lesions. While keeping the transducer in a stable position, the imaging mode was then changed to contrast-specific imaging and CEUS was performed using the ultrasound contrast agent PolySon™ L (Viscover™, *nanoPET* Pharma GmbH, Berlin, Germany). The agent was first diluted 1:2 in 0.9% NaCl and the mice were then administered with a total of 200 µl via tail vein injection. Continuous scanning was performed over a period of 100 s to allow assessment of the arterial and portal venous phases. Two regions of interest (ROIs) were drawn along the perimeter of the tumor and in the surrounding liver parenchyma and

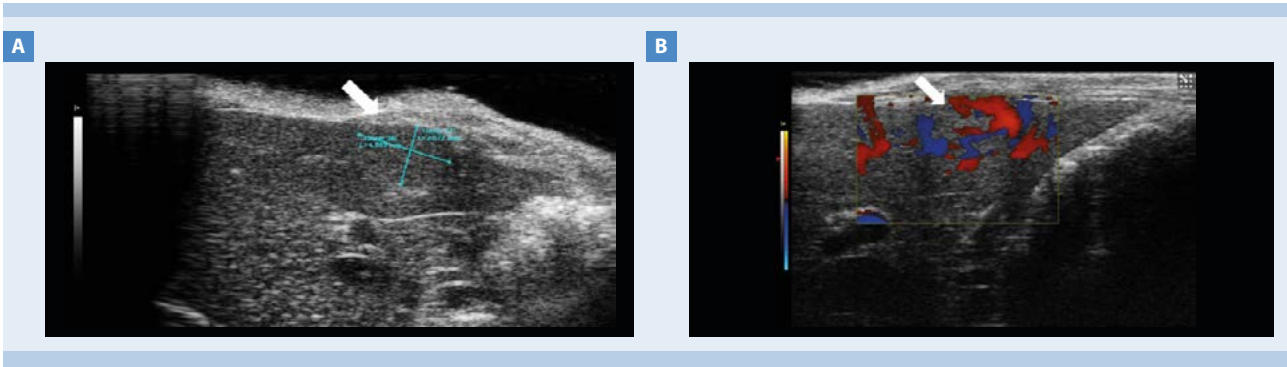


Figure 1: Ultrasound images of the left liver lobe of an HBV-transgenic mouse prior to contrast agent injection.
A. Conventional B-mode image and **B.** Color Doppler image of a liver nodule measuring 4 x 2.5 nm (white arrows). In the B-mode image, the nodule displays the typical isoechoic appearance while the Color Doppler image depicts tumor vascularization.

the resulting data was analyzed using VevoCQ™ software (FUJIFILM, VisualSonics, Toronto, Canada) for quantification of peak enhancement (PE), rise time (RT), time to peak (TTP) and wash-in rate (WiR).

Results and discussion

Prior to CEUS, conventional B-mode and Color Doppler imaging was performed on the animals for identification of the target lesions associated with HCC. B-mode imaging revealed a predominant isoechoic appearance of liver nodules (Fig. 1A), and Color Doppler imaging enabled clear depiction of tumor vascularity, a typical feature of HCC (Fig.1B). Thereafter, PolySon L was administered and the mice underwent CEUS. During CEUS, a dual image display format was used where the B-mode fundamental image and the contrast-specific image are displayed side-by-side. This enables the lesion to be kept in the imaging plane, and simultaneously enables evaluation of the feasibility of the CEUS method to increase the conspicuity of lesions. In the arterial phase, the nodules presented the typical wash-in, appearing as hyperechoic (Fig. 2A). This phase was followed by evident wash-out in the portal venous phase where the lesions became hypoechoic compared to the surrounding liver parenchyma (Fig 2B).
To visualize the complete wash-in/wash-out pattern of HCC nodule enhancement, continuous scanning was performed on HCC nodules over a period of 100 s (Fig. 3). In the CEUS images (Fig. 3A), the wash-in phase was generally found to occur during the first few seconds after injection of PolySon L and was characterized by a significantly strong enhancement of the nodules compared to that of healthy liver tissue. Thereafter, equalization of the enhancement in the nodule and liver was detected, which was subsequently followed by initiation of the wash-out phase where the nodules started to become hypoechoic in comparison to the surrounding liver. Analysis of the time-dependent ultrasound signal intensity enabled determination of various parameters namely peak enhancement, rise time, time to peak and wash-in rate (Fig. 3B). The wash-in/wash out pattern was generally observed within 90 seconds after contrast agent injection and proved the presence of moderately differentiated HCC, a finding that was later confirmed by histopathology[4].
To assess whether the results obtained during CEUS of HCC depended on the route of intravenous delivery of PolySon L, the study was extended to include a second route of injection, namely via the retrobulbar sinus. Imaging of the same HCC nodule after contrast agent administration via the two injection routes provided similar results, indicating that the tested intravenous routes were equally effective[4].

Conclusion

In this work we developed a CEUS method involving the innovative ultrasound agent, PolySon L, to non-invasively detect liver nodules in a mouse model of HCC. The results demonstrated that Polyson L is highly suited for the detection of hepatic lesions and enables dynamic visualization of the characteristic vascular phases, typical of HCC. Therefore, the method provides researchers with a reliable tool for the preclinical detection and monitoring of HCC and may be particularly useful in evaluation of the efficacy of new therapies.

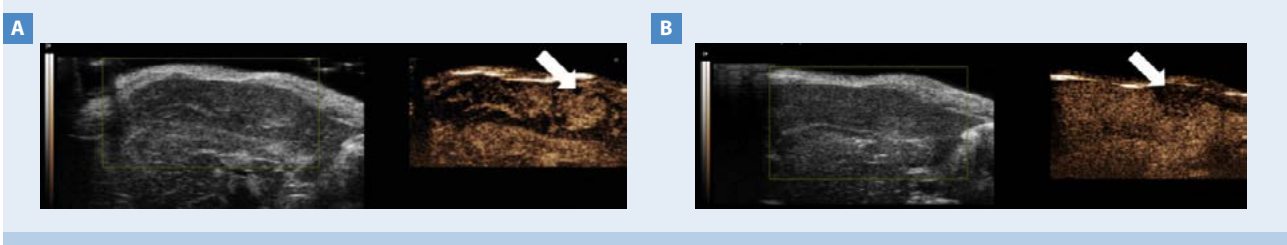
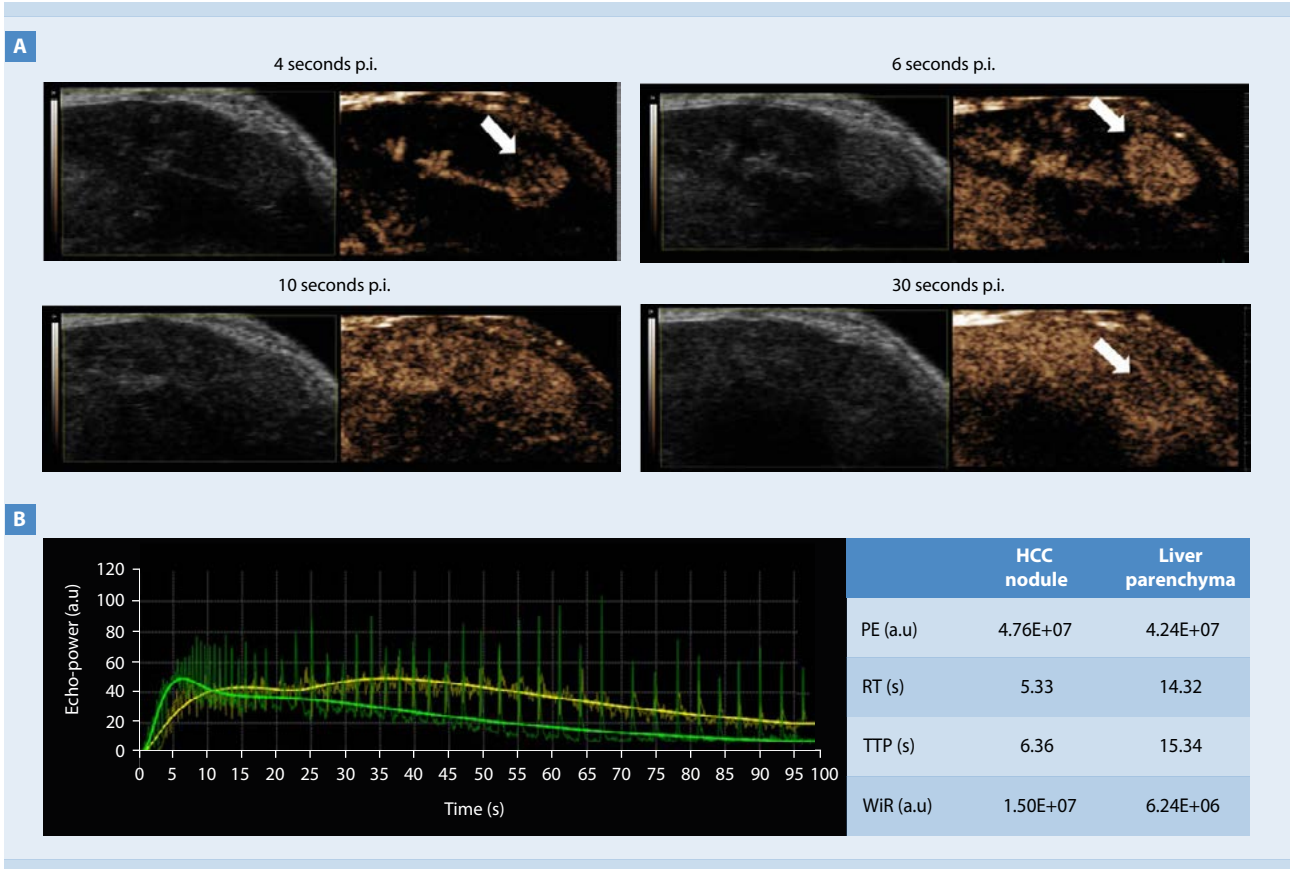


Figure 2: Side-by-side displays of B-mode (left) and CEUS (right) images of the left liver lobe of an HBV-transgenic mouse after injection of PolySon L.
A. During the wash-in phase, the lesion shows hyperenhancement due to the blood supply from feeding vessels. **B.** During the wash-out phase, the lesion appears hypoechoic compared to surrounding liver parenchyma. The white arrows on the CEUS images point to the liver lesion.



	HCC nodule	Liver parenchyma
PE (a.u)	4.76E+07	4.24E+07
RT (s)	5.33	14.32
TTP (s)	6.36	15.34
WiR (a.u)	1.50E+07	6.24E+06

Figure 3: Visualization of the typical wash-in/wash-out pattern of HCC nodule enhancement over a period of 100 s post injection (p.i.) of PolySon L. **A.** Side-by-side displays of B-mode (left) and CEUS (right) images at selected timepoints showing rapid hyperenhancement in the wash-in phase, followed by hypoenhancement in the wash-out phase. The white arrows on the CEUS images point to the liver lesion. **B.** Time-course of the ultrasound signal intensity in the HCC nodule (green) and surrounding liver parenchyma (yellow), and corresponding kinetic parameters (peak enhancement, PE; rise time, RT; time to peak; TTP and wash-in rate; WiR) proving the presence of moderately differentiated HCC.

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