Quantitative CEUS analysis of orthotopic pancreatic adenocarcinoma in C57BL/6 mouse model: preliminary results

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Authors: Y. Dong, Y. Jiang, W.-P. Wang, J. Y. Cao, D. Fu; Shanghai/CN
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Aims and objectives

Pancreatic adenocarcinoma is the most common primary malignant tumor of pancreas, representing about 80% of all pancreatic tumors with high mortality rate [1-3]. However, in the early stage of pancreatic adenocarcinoma, clinical or imaging signs are often not typical or nonspecific. Therefore, early and accurate diagnosis and differentiation of pancreatic adenocarcinoma remains challenging in clinical situations [4-5]. In recent years, with the advanced of real-time contrast-enhanced ultrasonography (CEUS) and quantitative software, it is possible to quantitatively estimate the tissue enhancement and describe blood flow by quantifying the contrast enhancement [6-7]. The aim of this study was to investigate the perfusion features of orthotopic exocrine pancreatic adenocarcinoma (OPAs) in C57BL/6 mouse model with CEUS, and to evaluate the value of time intensity curve (TICs) and quantitative indexes in early diagnosis of OPAs.
Methods and materials

In this animal care and use committee-approved study, study was carried out in accordance with the guidelines issued by the National Institutes of Health for care of laboratory animals. The models of OPAs were established in C57BL/6 mice (18-22 g, 6-8 weeks, female, n=40). The murine pancreatic adenocarcinoma cell line Pan02 was purchased from the Frederick National Laboratory for Cancer Research (New York, United States). Pan02 cells suspended (1×10^6 viable cells) in ice-cold matrigel (Sannois, United States) were injected into the parenchyma of mice pancreatic tails. Postoperative animal status and wound healing were monitored every day for one week after operation. Rates of tumors’ formation and the successfully establishment of OPA were confirmed pathologically. CEUS quantitative evaluation of tumor perfusion was performed by intravenous bolus injection of 0.05 ml SonoVue® (Bracco S.P.A., Milan, Italy) every week after injecting of Pan02 cells. All CEUS procedures were performed by a sonographer, with 10 years of experience. Contrast-enhanced perfusion imaging was carried out on a Philips iU22 (Bothell, WA, USA) equipped with Qlab software (Version 9.0; Philips Medical Systems, Bothell, WA, USA). Time-intensity curves (TIC) of OPAs in C57BL/6 mice and quantitative indexes were obtained. A L9-3 liner transducer was used and the mechanical index (MI) was set at 0.07.

The Fisher’s exact probability test was performed for comparison, and \( P \) values < 0.05 were considered to be significant.
Results

A visible tumor appeared one week after injection and death occurred 4 week after injection. Immunohistochemistry and pathological results confirmed that two to three weeks after Pan02 cells injection, the formation rates of OPAs were 100%, with epithelial origin and exocrine marker positive. With the progression of tumors' formation, OPAs showed delayed and decreased enhancement in TICs. Earliest significant changes happened in 3 weeks after Pan02 cells injection. Derived peak intensity (DPI) and time to peak (TTP) of OPAs were changed from 10.01±2.12 to 7.58±4.66 dB and 7.53±1.04 to 11.64±4.02 sec, respectively (P<0.05).
Conclusion

In our current study, genetically engineered animal models of pancreatic cancer were successfully established [8-9]. It is suitable for the study of certain microcirculation perfusion in tumorigenesis and tumor progression [10-11] (Fig. 1). The OPAs cancer models established in the pancreatic tail of mice mimicked the pathological behavior of the human pancreatic carcinoma (Fig. 2). Further HE staining of tumor sections revealed poorly-differentiated oval-shaped tumor cells were arranged in a weave pattern (Fig. 3). We considered the rapid progress and poor survival of OPAs in C57BL/6 mice markedly simulated of human pancreatic cancer. After a bolus injection of contrast agent, the shape of the TIC observed in the pancreatic microcirculation resembles a highly skewed Gaussian curve and indicator-dilution type. The gamma-variate function represented a suitable curve fit approximation [12-13]. Quantitative perfusion parameters including: the slope rate of ascending curve (A), the slope rate of descending curve (#), area under curve (AUC), derived peak intensity (DPI), time to peak (TTP) were measured by using Qlab software to estimate microcirculation blood flow [14-15]. In our study, CEUS and quantitative analysis of OPAs in C57BL/6 mice showed real time contrast perfusion changes in the very early stage of pancreatic adenocarcinoma. Also, further TICs and quantitative analysis showed earliest significant changes happened after operation on DPI and TTP (Fig. 4). The importance of measuring pancreatic tumor microvascular perfusion in pancreatic carcinoma is well accepted [16-18]. Nowadays, ultrasound is still the first imaging examination performed in patients suspected of pancreatic cancer [19-20]. So, we made our initial conclusions that, CEUS can display the perfusion changes of OPAs in the early period. Quantitative analysis would be a promising objective and effective imaging evaluation method.
Fig. 1: Adherence, proliferation and expansion of Pan02 cells suspended in Matrigel and cultured ex vivo: (a) 0 day; (b) 1 day; (c) 2 days; (d) 4 days. References: Department of Pancreatic Surgery, Huashan Hospital, Fudan University, Yong-jian Jiang/China

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**Fig. 2:** Tumor conformation. Pan 02 cells were injected into the tail of pancreas of C57BL/6 mice. Half of the mice were sacrificed at two weeks (A) and the remaining at three weeks (B) after injection respectively. Tumor conformation (indicated by green arrow) at the injection site was thoroughly examined and marked. References: Department of Pancreatic Surgery, Huashan Hospital, Fudan University, Yong-jian Jiang/China

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Fig. 3: Fig. 3: Crosssection and HE staining of orthotopic pancreatic cancer in C57BL/6 mice: (a) full view of pancreatic tail cancer (×40); (b) full view of pancreatic head cancer (×40); (c) local view indicated the duct-shape structure within the tumor tissue (×200). References: Department of Pancreatic Surgery, Huashan Hospital, Fudan University, Yong-jian Jiang/China

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Fig. 4: CEUS and quantitative analysis of orthotopic pancreatic cancer in C57BL/6 mice: (A) Real time contrast perfusion changes of pancreatic tail cancer; (B) Quantitative analysis and TICs. References: Department of Ultrasound, Zhongshan Hospital, Fudan University- Wen-Ping Wang /China

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References
