Vascular endothelial growth factor receptor 2-targeted ultrasound contrast agent selectively accumulates in pancreatic carcinoma in the allograft mouse model: A pilot study using time-intensity curve analysis of EUS imaging

Dear Editor,

Molecular-targeted ultrasound contrast agent (UCA) has improved the *in vivo* etiological analysis of various neoplastic diseases, particularly those associated with angiogenesis. For instance, a vascular endothelial growth factor receptor 2 (VEGFR2)-targeted UCA enabled earlier detection of pancreatic carcinoma (PC) by imaging using a transabdominal ultrasound (TUS) transducer;[1] however, the dynamics of molecular-targeted UCA use in PC tissues are yet to assessed by time-intensity curve (TIC) analysis. Furthermore, whether the tissue enhancement of molecular-targeted UCA can be identified using EUS with a miniaturized and strongly curved transducer remains unconfirmed. In this study, we evaluated the advantages of aVEGFR2-targeted UCA for PC imaging using an EUS transducer and analyzed the TIC parameters *in vivo* using allograft mouse models.

We used 15 allograft mouse models of C57BL/6J (CLEA Japan) with Pan-02 cells (Frederick, MD, USA) inoculated into their backs. Of these, 5 mice received 30 µL of VEGFR2-targeted UCA (VISISTAR®, Targeson), 5 mice received 30 µL of a nontargeted UCA (SONAZOID, Daiichi Sankyo), and the final 5 mice received 30 µL of both agents administered individually and separated by a 1-hour interval. Each mouse was stabilized on their back, and then, convex-type EUS (GF-UCT 260, Olympus Medical Systems, Tokyo, Japan) was applied under water for 10 min with a processor (ProSound F75 Premier,

Hitachi-ALOKA medical, ALOKA), using the tissue harmonic echo-imaging mode with 0.3 of mechanical index and a 1-cm imaging depth. TIC was analyzed using the following parameters: peak intensity (PI, dB), peak duration (PD, second), time to peak from initial rise (TTP, second), and area under the curve (AUC). Finally, tumors were histologically evaluated with hematoxylin and eosin staining and immunohistochemical staining for CD31 and VEGFR2. Microvascular density (MVD) was measured by counting CD31-stained vessels.[2] The Wilcoxon rank-sum test was used for statistical analysis.

The overall results from our 15 animal models showed significantly higher PI, longer PD, and larger AUC with the VEGFR2-targeted UCA than with the nontargeted UCA. This trend of TIC parameters was almost the same in the first 10 mice and second 5 mice, with the exception of AUC. The histology showed no difference in morphology, MVD, or VEGFR2 expression among the animals [Table 1 and Figure 1].

PI is relevant to the number of UCA in a tissue region, while PD relates to the locoregional UCA flow and affinity. Our TIC analysis thus suggested that the VEGFR2-targeted UCA is highly specific for PC tissues by virtue of its affinity for VEGFR2 and that the EUS transducer was equivalent to the TUS transducer for tumor visualization, in line with proposed clinical applications. In conclusion, the VEGFR2-targeted UCA showed selective accumulation into PC tissue during a longer period than nontargeted UCA in *in vivo* allograft mouse models, and tissue enhancement of the molecular-targeted UCA could be confirmed using a EUS transducer. Our study thus highlighted the applicability of this molecular imaging model to the EUS diagnosis of PC.

Table 1. TIC parameters and microvascular density

Experiment	Parameters	VEGFR2-targeted UCA	Non-targeted UCA	P
1st 10 animals	MVD (counts/HPF)	21.8±2.21	25.1±2.6	0.1
	PI (dB)	15.8±1.52	9.8±1.51	0.02*
	PD (s)	208±5.83	87.8±2.29	<0.01*
	AUC	63369.5±17147.9	40521.7±21629.1	0.12
	TTP (min)	5.1±0.35	2.5±0.21	<0.01*
2nd 5 animals	MVD (counts/HPF)	24.8±1.6	24.8±1.6	-
	PI (dB)	17.0±1.4	12.2±0.8	0.015*
	PD (s)	210±42.9	95±27.3	0.036*
	AUC	75778.8±5418.9	53372.1±3861.1	0.09
	TTP (min)	6.08±0.59	3.23±1.31	0.03*
Total		VEGFR2-targeted UCA	Non-targeted UCA	P
	PI (dB)	16.4±0.99	11±0.9	<0.01*
	PD (s)	176.2±12.5	73±7.34	<0.01*
	AUC	69574.2±8726.2	44643±4192.2	<0.01*
	TTP (min)	4.93±0.19	2.33±0.2	<0.01*

Data are presented as means±standard deviation. *$P<0.05$ was considered significant. MVD: Microvascular density, HPF: high power field, VEGFR2: Vascular endothelial growth factor receptor 2, UCA: Ultrasound contrast agent, TIC: Time intensity curve, PI: Peak intensity, PD: Peak duration, AUC: Area under the curve, TTP: Time to peak
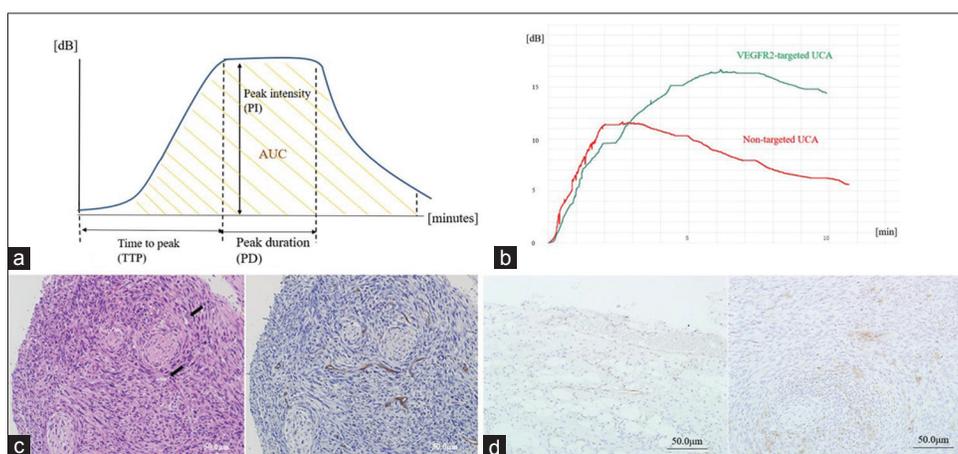
Figure 1. (a) Schema of time-intensity curve. PI: Peak intensity, TTP: Time to peak, PD: Peak duration, AUC: Area under the curve. (b) Overall time-intensity curve results. The time-intensity curve shape differed between groups. (c) Hematoxylin and eosin and CD31 staining. By hematoxylin and eosin staining, pancreatic carcinoma tissue consisted of a few narrow vessels (black arrows) (left). By immunohistochemical staining, more vessels were detected as brown linear structures (right). (d) Vascular endothelial growth factor receptor 2 staining. Brownish spots were detected along the vasculature and around tumor cells both in the peripheral region (left) and the center (right). Both (c and d) represent images taken using a × 200 objective lens

All authors had full access to the data and had final responsibility for the decision to submit for publication. This study has been selected to receive a student travel stipend for presentation at the World Molecular Imaging Congress 2017 in Philadelphia, USA, and was reported at Japan Digestive Disease Week 2017 in Fukuoka, Japan.

Conflicts of interest

There are no conflicts of interest.

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