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Dynamic Evolutionary Changes in Blood Flow Measured by MDCT in a Hepatic VX2 Tumor Implant over an Extended 28-day Growth Period: Time-Density Curve Analysis¹

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Abstract

Rationale and Objectives—The enhancement pattern of malignant tumors has been studied in short-term animal models (7–14 days), but the reported results have been variable and inconsistent. The purpose of this study was to investigate the changing blood flow characteristics of VX2 tumors implanted in rabbit livers with contrast-enhanced multidetector computed tomography (MDCT) to establish a predictable pattern of vascular evolution over an extended 28-day growth period.

Materials and Methods—VX2 carcinoma was implanted in livers of 10 male New Zealand White rabbits. Dynamic CT (2/seconds $\times$ 60 seconds) was conducted on days 7, 14, 21, and 28 after tumor implantation. Enhancement parameters of time-density curve (TDC), time to start (T₀), time to peak (TP), maximum enhancement (ΔH), slope of enhancement (SLe), and washout (SLw) in tumor center, tumor rim, and normal liver were analyzed. Tumor samples corresponding to CT images of one tumor on days 14 and 21 and seven tumors on day 28 were stained with hematoxylin and eosin and anti-CD31 monoclonal antibody. The relationship between enhancement parameters and histology parameters (thickness of tumor border, extent of blood stasis, and luminal vessel density) was analyzed.

Results—Consistent growth, appearance, and vascular changes occurred in 7 of 10 animals over the 4-week observation period. Peripheral rim-like enhancement was noted in CT images. TDC analysis showed that tumor rim enhancement was pronounced and more rapid than normal liver initially but this difference diminished with tumor progression. The SLe, SLw, and ΔH decreased from 10.03 ± 3.25 Hu/second, 0.42 ± 0.25 Hu/sec, and 58.00 ± 25.27 Hu on day 7 to 5.86 ± 2.73 Hu/second, 0.10 ± 0.13 Hu/second, and 37.78 ± 8.89 Hu/second on day 28, respectively. TP increased from 12.71 ± 4.85 seconds on day 7 to 25.57 ± 7.75 seconds on day 28. No significant changes were noted on the TDC parameters in normal liver. The maximum density difference between tumor rim and normal liver ($D_{\text{rim-liver}}$) appeared 10.5 ± 2.1 seconds after contrast injection. The maximum $D_{\text{rim-liver}}$ decreased from 54.33 ± 37.86 Hu on day 7 to 11.16 ± 13.03 Hu on day 28. On histological analysis, viable tumor cells were found in tumor rim with few luminal vessels. The tumor border showed desmoplastic reaction, vascular dilation and proliferation, inflammatory cell infiltration, and blood stasis. These findings were more obvious on day 28 than those on day 14. TP showed significant positive correlations with the extent of blood stasis in

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tumor border and adjacent liver and the maximum thickness of the tumor border ($r = 0.945$ and 0.893 respectively, $P < .05$).

Conclusion—The rabbit VX2 liver tumor is a hypovascular tumor with perilesional enhancement over its lifespan as imaged by MDCT. Consistent changes in the measured vascular parameters correlated with the size/age of the tumor implants. These findings suggest that the accuracy of CT enhancement imaging for VX2 liver tumor detection might be decreased with tumor development.

Keywords

Multidetector computed tomography; liver tumor; animal model; time-density curve analysis

With the increasing speed of imaging technology, dynamic contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) have been widely used in the characterization of solid tumors, particularly for tumors of the liver, kidney, and breast (1-7). Among these studies, analyzing the enhancement pattern of the liver tumor was used as a vital approach to differentiate malignant from benign lesions (8,9), evaluate tumor vascularity (10), and response to treatment (11,12). However, the degree of the tumor enhancement was varied. This variation was reported to be due to the differences of tumor vasculature and permeability (13), the extent of peritumoral inflammatory reactions (14), the existence of arteriportal shunts (15), and the circulation time of contrast in the systemic and portal systems (16).

Numerous studies have shown that tumor growth and angiogenesis are complex processes that involve the expression of many growth factors, nutrient and oxygen delivery, metabolic waste drainage, and immunoreactions of the host (17,18). With tumor development, the effect of these factors likely changes. Previous studies reported that serum VEGF levels were positively correlated with the stage of ovarian cancer (19,20). Au et al (21) found that the mRNA levels of glucose transporter 1 and glucose transporter 3 of ascites tumor cells increased progressively in the tumor during development. Stewart et al (22) explored the relationship between glucose metabolism and growth of VX2 liver tumors using fluorine 18 fluorodeoxyglucose positron emission tomography/CT scan and found that the glucose utilization of tumors increased with tumor growth. We hypothesize that tumor perfusion will change with the stage or development of the liver tumor.

In the current study, we used dynamic CT to study the development of tumor vasculature over time in an experimental animal liver tumor model. In a group of 10 animals, we employed 64-slice high-resolution multidetector CT to quantify the growth and necrosis of a tumor and the associated evolution of its CT contrast enhancement pattern over a 28-day period. This uninterrupted evaluation provides new insights into the tumor anatomic and vascular evolution over an extended period. Characterization and quantification of these dynamic temporal changes provides new insights into the fundamental knowledge of tumor growth. These findings correlate with and clarify numerous clinical and animal model findings reported by others and add to the utility of functional imaging perfusion studies for clinical and research purposes.

MATERIALS AND METHODS

Overall Experiment Design

VX2 tumor pieces were implanted in the liver of 10 male New Zealand White rabbits. CT perfusion scans were performed on days 7, 14, 21, and 28 after tumor implantation. Time-density curve (TDC) parameters (T_0 , time to peak, maximum enhancement, slope of

enhancement, and slope of washout) in tumor core, tumor rim, and normal liver were analyzed. The tumor samples corresponding to CT images were stained with hematoxylin and eosin (H&E) and anti-CD31 monoclonal antibody. The differences of TDC parameters among different time points and regions of interest were compared and correlated to histopathological analysis.

VX2 Tumor Model

All animal procedures were approved by the Institutional Animal Care and Use Committee at our institute and followed all applicable guidelines. For this study, we introduced the VX2 carcinoma into livers of 10 male New Zealand White rabbits 2.9–3.1 kg (Covance, Princeton, NJ) as described previously (23). Briefly, frozen VX2 tumor pieces were rapidly thawed and washed with Hank's buffered salt solution. Rabbits were anesthetized using a combination of ketamine (35 mg/kg) and xylazine (5 mg/kg). The abdomen was shaved, cleaned, and opened just below the sternum. The middle lobe of liver was exposed. A piece of tumor (approximately 1 mm³) was then inserted into the liver parenchyma and the incisions were closed. During recovery, all animals received Buprenex (0.5 mg/kg) for pain management for 2 days.

Dynamic CT

CT scans were performed with the Somaton 64 CT scanner (Siemens Medical System). During each exam, the animal was anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg). An initial, unenhanced baseline scan was carried out. Next, a 3-mL bolus of contrast (Optiray, 240 mg/mL) was administered through a 21-g catheter placed in the marginal ear vein. The dynamic scan protocol, which imaged 12 consecutive slices every 0.5 second for 1 minute was executed using the following parameters: axial scan, 360° scan angle, 2.4 mm slice thickness, field of view 195 mm, 120 KV, 150 mAs/slice.

TDC Analysis

Sequential CT images in the position of tumor maximum diameter were loaded to Dyn Eva software (Siemens Medical System). Any images with obvious movement were deleted to reduce motion artifacts. Regions of interest in the tumor core, tumor rim, normal liver, and aorta were drawn by hand based on the maximum enhanced tumor image. The tumor rim was defined as a donut shape of the enhancing peripheral zone of tumor. The center less or nonenhanced zone was defined as the tumor core. The absolute time-density measurements within each region of interest were exported to Microsoft Excel and smoothed with an adjacent-averaging method using Origin 8.0 (OriginLab Corporation, MA).

Several parameters were defined for the TDCs of tumor rim, tumor core, and normal liver. Time to start (T0) was defined as the time delay relative to the aorta. Time to peak (TP) was defined as the time needed to reach the peak. The maximum enhancement (ΔH) was calculated by the peak CT value of the curve minus the baseline CT value. If the upslope of the curve composed of two phases (fast enhancement phase and slow enhancement phase), TP1, $\Delta H1$ and TP2, $\Delta H2$ were used to describe the TP and ΔH of the two phases, respectively. The slope of enhancement was defined as the maximum slope of the rise. The data from the washout phase was fitted linearly, and the slope was defined as the slope of washout (SLw). A representative TDC in tumor rim and the analysis parameters are shown in Figure 1. The difference between the density in tumor rim and in normal liver ($D_{\text{rim-liver}}$) was calculated and plotted to time. The baseline, peak, and time to peak (TP) of the difference of rim-liver were evaluated.

Tumor Growth Monitoring and Tumor Size Determination

Tumor growth was monitored with CT. The tumor presence was confirmed on contrast-enhanced images, which showed an enhanced nodule in the arterial phase. Total tumor size was determined by measuring the diameter of the largest tumor dimension (including the hypodense hypovascular or necrotic core and the enhanced outer rim) and calculating the area based on this measurement. The area of the necrotic core was calculated in a similar manner, but using the measured diameter of the unenhanced or less enhanced (on day 7) inner core only. The viable tumor rim area was calculated by subtraction of the necrotic core area from the total area.

Histology and Immunohistochemistry

Immediately after the last perfusion scan, rabbits were euthanized using an approved method. The tumors and surrounding liver tissue were dissected and fixed in 10% formalin. Consecutive 5- μ m sections were sectioned and mounted on glass slides. Sections were stained with H&E. All tissue processing was carried out by the Tissue Procurement and Histology Core Facility. The JC/70 monoclonal antibody (Dako, Glostrup, Denmark) against CD31 was used for microvessel staining using the LSAB method (Dako LSAB kit; Glostrup, Denmark). Negative controls were prepared by phosphate-buffered saline substitution for the primary antibodies staining, and known positive controls were included in each staining run.

The histopathologic evaluation included assessment of tumor necrosis, location of luminal vessels in the tumor and border, changes of tumor borders including peritumoral desmoplastic reaction, lymphocytic infiltration, vascular proliferation, stasis of border vessels and adjacent liver sinuses, compression of hepatic parenchyma, and atrophy of the hepatic cords. Tumor border was defined as the desmoplastic reaction zone. In each tumor sample, the histologic images of 5–10 regions of tumor border were taken at a magnification of $\times 25$, and $\times 100$, respectively. The thickness of tumor border was measured on the images at a magnification of $\times 25$. The mean and median of these 5–10 regions were then recorded. The extent of the blood stasis of the vessels in the tumor border vessels and adjacent liver sinuses was evaluated in a semiquantitative fashion by using the following scales: +++ indicated prominent; ++, moderate; +, mild; and –, none.

Data Analysis

All data are presented as mean standard error unless otherwise noted. The values of perfusion parameters were compared using one-way analysis of variance. The Pearson and Spearman correlation tests were performed to determine the strength of the relationships between enhancement parameters and tumor border thickness or stasis grade. All statistical analyses were performed using Minitab 15.1.0.0. A P value $< .05$ was considered statistically significant.

RESULTS

Progression of Tumor Growth

Consistent tumor development and growth was observed in 7/10 rabbits during the 4-week observation period. The other three animals were not completely evaluated. Two animals died from complications of anesthesia and one animal showed no tumor progression. These animals were excluded from the final dataset. A typical VX2 tumor 28 days after implantation is shown in Figure 2. Central necrosis occupied the majority of the tumor volume with the outer viable capsule having an approximate thickness of only 2 mm and a tumor diameter of >2 cm.

Sequential growth of the VX2 tumor and the progression of necrosis are shown in Table 1 and Figure 3. The rate of viable tumor growth for the first 2 weeks exceeded that of necrosis. However, between weeks 2 and 3, the tumor experienced major necrosis, which is contrasted by the steady progressive growth of the overall tumor. In the seven active tumors, the mean diameter and area of the largest cross section increased from 0.83 cm and 0.55 cm² at day 7 to 2.05 cm and 3.11 cm² at day 28, respectively. The necrotic core increased at a rate faster than the viable tumor rim during week 3 and week 4. The size of necrosis ranged from 0.13 cm² to 2.02 cm², whereas the viable outer rim increased from 0.42 cm² on day 7 to 1.09 cm² on day 28, respectively.

TDC Analysis

Lesions showed a rim-like enhancement during the arterial phase of contrast-enhanced CT images. The CT images of a representative VX2 liver tumor at the four observed time points are shown in Figure 4. TDC parameters in the tumor center, tumor rim, and normal liver are listed in Table 2. No significant changes were noted in the enhancement parameters of normal liver. The tumor rim enhancement was rapid and more intense than that of normal liver. T0 and TP1 in the tumor rim were significantly shorter than those in normal liver at every time point. With tumor growth, the tumor enhancement onset was delayed. TP2 increased from 12.71 ± 4.85 seconds on day 7 to 25.57 ± 7.75 seconds on day 28. The calculated slope of enhancement and the maximal enhancement (ΔH_2) decreased from 10.03 ± 3.25 Hu/second and 58.00 ± 25.27 Hu on day 7 to 5.86 ± 2.73 Hu/second and 37.78 ± 8.89 Hu on day 28. On days 7 and 14, the calculated slopes of washout in tumor rim were similar to that in normal liver (-0.42 ± 0.25 Hu/second vs. -0.44 ± 0.25 Hu/second on day 7 and -0.41 ± 0.25 Hu/second vs. -0.49 ± 0.19 Hu/second on day 14). However, the speed of contrast washout in tumor rim was much slower than that in normal liver on days 21 and 28 (-0.10 ± 0.11 Hu/second vs. -0.51 ± 0.32 Hu/second on day 21 and -0.10 ± 0.13 Hu/second vs. -0.44 ± 0.11 Hu/second on day 28, respectively).

The patterns of contrast enhancement of the tumor rim are shown in Figure 5. Enhancement was classified into four categories: I, slow enhancement (two steps in enhancement phase) and persistent enhancement in the latter phase ($SLw > 0$ Hu/second); II, slow enhancement and slow washout (-0.15 Hu/second $< SLw < 0$ Hu/second); III, slow enhancement and fast washout ($SLw < -0.15$ Hu/second); and IV, fast enhancement and fast washout (no TP2). The distributions of the tumor rim enhancement in the four categories are summarized in Table 3. On days 7 and 14, fast washout patterns (types III and IV) accounted for 7/7 and 6/7 tumors, respectively. However, 5/7 and 4/7 tumors showed slow washout (type II) or persistent enhancement (type I) on days 21 and 28, respectively.

TDCs of tumor rim, normal liver, and the difference between tumor rim and normal liver of a representative VX2 liver tumor after contrast administration are shown in Figure 6. The baseline CT density of tumor rim was 12.28 ± 4.81 Hu lower than that in normal liver. The $D_{rim-liver}$ reached the maximum at 10.52 ± 2.06 seconds after contrast injection. With tumor development, the maximum $D_{rim-liver}$ decreased from 54.33 ± 37.86 Hu on day 7 to 11.16 ± 13.03 Hu on day 28 (Table 2). The evolution of the rim-liver curve of a representative animal is shown in Figure 7.

Histology Findings

The gross view and corresponding H&E and CD31 stained images of representative VX2 liver tumor on day 14 and day 28 are shown in Figure 8. At histopathologic examination, viable tumor cells were found in the tumor rim. Liquefactive necrosis was noted in the tumor center. Only a few CD31 positive cells, lacking lumen formation, were noted in the viable tumor rim. The tumor border showed a moderate to prominent desmoplastic reaction,

vascular proliferation, and inflammatory cell infiltration. Blood stasis was noted in the tumor border vessels and adjacent liver sinuses, which showed dilated vessels and liver sinusoids engorged with red blood cells. The thickness of the tumor border ranged from 0.36 mm to 1.30 mm. Compressed hepatic parenchyma and atrophy of hepatic cords were seen in the adjacent liver. These findings were most prominent on day 28.

Correlation between Enhancement Parameters and Histology

Spearman rank tests were used to evaluate the correlation between enhancement parameters and histology parameters. TP2 in the tumor rim showed a positive correlation with the extent of blood stasis in the tumor border and the maximum thickness of the tumor border ($r = 0.945$ and 0.893 , respectively, $P < .05$). The maximum thickness of the tumor border showed a positive correlation ($r = 0.945$) with the extent of blood stasis ($P < .05$). The enhancement parameters in the tumor rim showed no correlation with tumor size.

DISCUSSION

The VX2 liver tumor model has been widely used for research on liver tumor imaging methods and interventional therapy (22,24-27). Angiography and CT or MRI contrast enhancement studies have shown that the VX2 tumor is vascularized by the hepatic artery and exhibits early “ring” enhancement and rapid washout, which resembles human hepatocellular carcinoma in terms of hemodynamics. The vascular characteristics of the VX2 liver tumor model have been reported inconsistently. Some reports regard the VX2 as a hypervascular tumor (25,28-30), whereas others consider it hypovascular (31,32). However, the dedicated radiologic-pathologic correlation of the VX2 liver tumor has not been reported. In the present study, an early “ring” enhancement of the VX2 liver tumor was noted in dynamic CT imaging as shown in previous literature (31). Our histology examination showed that few luminal vessels were found in the tumor region, even in the viable tumor rim. On the contrary, luminal vessels were located in the desmoplastic reaction zone or tumor capsule. Based on these facts, we propose that the VX2 tumor model is hypovascular, and the enhancement seen in imaging studies is primarily peritumoral or perilesional and not viable tumor enhancement. However, it should be noted that this study is not an in depth histological study, and it is not possible to conclude from the data presented whether the VX2 tumor model is indeed “hypovascular” or “hypervascular.” A further intensive histological study is needed for definitive classification.

Perilesional rim enhancement of lesions in early-phase CT or MRI dynamic images has been recognized as one of the characteristic findings of some malignant solid tumors, such as metastatic liver tumors (3,14,33-35) and breast cancer (36). The hepatic metastasis most commonly associated with perilesional rim enhancement has been from colorectal carcinoma. These tumors normally appear as hypovascular lesions with central necrosis. The rim enhancement of hepatic metastases on arterial phase images was initially thought to arise from the viable tumor rim (9). However, recent imaging-histology correlation studies have shown that rim enhancement mainly represents the perilesional area (14,34).

Tumor CT or MRI contrast perilesional enhancement patterns have been evaluated by several investigators and regarded as an important criterion in differentiating malignant and benign tumors. However, a large variation of enhancement patterns has been reported by these studies. Semelka et al (14) reported on MRIs of seven patients with histopathologically proven hepatic metastases. On early (arterial) phase images, prominent, moderate and no perilesional enhancement were showed in three, one, and three patients, respectively. A large sample study of perilesional enhancement of hepatic metastases on dynamic MRI was reported by Yu et al (3). In a total of 134 metastatic liver lesions, 87 lesions (65%) showed hyperintense rim enhancement, 30 lesions showed hypovascular tumor without rim

enhancement, 17 lesions showed diffuse iso-signal intensity enhancement, and 3 lesions showed diffuse hyper-signal intensity enhancement. The pattern of perilesional washout was treated as a criterion in malignant liver tumor diagnosis as well. Mahfouz et al (37) reported that 24.5% of malignant liver tumors (12/49) had a “peripheral washout” sign on the dynamic enhanced MRIs, which appeared as a hypointense rim on the 10-minute images after contrast administration. The “peripheral washout” sign didn’t show in the other 75.5% of malignant tumors and all of the benign tumors.

Previous literature reported that the peritumoral inflammatory reaction was the main cause of the peritumoral enhancement in CT or MRI. Histology showed that the tumor boundary presented with peritumoral desmoplastic reaction, peritumoral inflammatory cell infiltration such as eosinophils and lymphocytes, and vascular proliferation correlated best with perilesional enhancement (14) and sinusoidal congestion (38). Blood stasis in adjacent liver sinusoids was also reported by Kan et al (39), who observed the blood supply to hepatic metastasis in a rat model using an in vivo microscope. They found that tumor vasculature communicated with the portal venules and hepatic sinusoids that surrounded the tumors and the hepatic artery supplied the tumor through arteriportal communications. Tumor cells and inflammatory cells tended to adhere to the endothelial lining in the hepatic sinusoids at the tumor border, causing stagnation or occlusion of blood flow at the periphery of the tumor.

The causes of the diversity of the enhancement patterns of metastatic liver tumors were also explored by radiologicpathologic correlation. Semelka et al (14) reported that an excellent correlation was found between the extent of enhancement and the thickness of the tumor border. Yu et al (3) evaluated the perilesional enhancement on dynamic MRI of 134 hepatic metastases and found that the lesions with a higher vascular component showed a lesser degree of perilesional enhancement and vice versa. Tumor size was reported as a factor of the enhancement pattern of a hepatic tumor by Burgener et al (40). In the rat tumor model, a significantly greater contrast accumulation was found at 1 and 5 minutes in the smaller tumors when compared to the larger lesions. In human tumors, a greater initial contrast enhancement and a faster washout was found in smaller lesions, a finding which was explained as a relative greater capillary surface area in small tumors, allowing a faster contrast equilibration between intravascular and interstitial space. However, due to the limitations of CT scanners in 1980s, the tumors were imaged with a scan time of 4.8 seconds before and in regular intervals (5 or 10 minutes) up to 30 or 40 minutes after intravenous contrast administration.

For the current study, implantation of tumor pieces was used to inoculate VX2 tumor into the liver. This single implant produced a single clearly definable lesion well suited for consistent repetitive imaging. The growth properties could not be affected by competitive interference for nutrient supply or waste elimination if other coexisting tumors were present. We opted not to use the alternate method of tumor cell injection into the portal vein (26,41) because of its drawbacks which include uncontrollable number and distribution of lesions, overlap of tumor nutritional supply or waste disposal, and early high mortality preventing long-term study.

In this study, the enhancement pattern of VX2 tumors in rabbit livers was evaluated using a rapid dynamic CT scan. The liver was scanned every 0.5 seconds for 1 minute. The enhancement pattern represents primarily the first-pass dynamics of contrast agent, which is influenced by the quantity and size of luminal vessels and blood flow velocity. We found that the enhancement pattern of the tumor changed during its life cycle. The peritumoral enhancement in the early development stage (days 7 and 14) was more intense and faster and the washout was faster compared to tumors in later stage (days 21 and 28). However, changes in enhancement parameters in normal liver were negligible. These results agree

with a study reported by Stewart et al (22), which demonstrated that with tumor growth the mean hepatic blood flow in VX2 liver tumor decreased. This indicates that the stage of tumor development is a factor of its enhancement pattern.

The liver has a dual blood supply from the hepatic artery and portal vein. It has been reported that hepatic blood flow is changed in different stages of liver diseases, and hepatic arterial blood flow is more relevant to liver function and disease than total hepatic blood flow (22,42). In this study, the maximum enhancement in the tumor rim occurred in the early enhancement phase, which represents hepatic artery perfusion. Our histological analysis showed that with VX2 liver tumor growth, necrosis developed in the tumor center, the tumor border became thicker, and a greater extent of blood stasis in the tumor rim and adjacent liver sinuses was noted. TP2 significantly positively correlated with the extent of blood stasis and the thickness of the tumor border, which means that with tumor development, the enhancement of the tumor rim becomes slower from the increasing extent of blood stasis. The maximum thickness of the tumor border correlates with the extent of the host desmoplastic reaction. A positive correlation was noted between the maximum thickness of the tumor border and the extent of blood stasis in this study. We speculate that the increasing extent of blood stasis and tumor-induced inflammatory process may be caused by the accumulation of tumor waste products causing increased interstitial edema/pressure, the compression of thin walled vessels, and invasion of tumor draining veins. Supportive evidence is that a negative correlation was found between TP2 in the tumor rim and the extent of blood stasis.

Another important finding of this study is that the difference between tumor and normal liver in contrast-enhanced CT images decreased with tumor growth. The difference between tumor and normal liver is a key criterion for tumor detection on both unenhanced and enhanced CT images. Plain CT scans have low efficacy in the detection of liver tumors. Contrast agent is commonly used to enhance the density difference between tumor and adjacent normal liver. Our study showed that 10 seconds after contrast injection is the best time window for VX2 liver tumor detection. The decrease in the maximum difference between the tumor rim and normal liver after contrast from day 7 to day 28 indicates that the efficacy of enhanced CT scan for tumor detection decreases with tumor growth. This finding may be explained by the decreasing and slower enhancement of the tumor rim during development.

The current study has several limitations. Motion artifacts and limited spatial resolution of clinical CT could influence the accuracy of time density values in the tumor rim. Intermitting breathing motion using tracheal intubation and mechanical ventilation can be used to avoid motion artifacts during CT scanning. This technique was not used in this study because of a fast cine scan (2 frames/second) lasting 60 seconds and multiple CT scans needed in one animal at four observation time points. Instead, we decreased the motion artifacts by deleting the motion images. To decrease the noise of TDC, an adjacent-averaging smoothing filter was used. These measures enhanced the accuracy of our analysis.

The evolution of VX2 tumor vessels was only superficially evaluated by H&E and anti-CD31 stains. A technique that evaluates the vasculature in three dimensions may provide additional insight. In contrast, the histology evaluation of the tumor was performed only on days 14 and 28, and did not include all four time points corresponding to the CT scans. Thus, the histology results cannot reveal the dynamic change of tumor angiogenesis. In addition, the sample size was small in this study. Although a correlation between tumor perfusion parameters and histology parameters was noted, additional studies may provide a stronger correlation and more reliable correlative results.

In conclusion, the VX2 liver tumor model is more comparable to human hepatic metastases (a hypovascular tumor with perilesional enhancement in CT or MRI) than to primary hepatocellular cancer. The decrease in hepatic artery perilesional enhancement with tumor growth indicates that tumor development or age may influence the enhancement patterns of liver metastases. The decrease in the maximum $D_{\text{rim-liver}}$ suggests that the accuracy of the CT contrast differential decreases as tumors enlarge. However, the impact on VX2 detection is likely unchanged because of the associated larger size of the growing hypovascular center.

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Figure 1. Time-density curves (TDC) of aorta and tumor rim of a typical VX2 liver tumor model after contrast administration and the definition of TDC parameters in tumor rim.

Figure 2.
Gross specimen of a representative VX2 liver tumor on day 28.

Figure 3.
Change in VX2 tumor size as determined by computed tomography.

Figure 4.
Contrast-enhanced computed tomography images of a typical VX2 liver tumor on days 7 (a), 14 (b), 21 (c), and 28 (d).

Figure 5.
Four categories of perilesional enhancement patterns of VX2 liver tumor.

Figure 6. Time-density curves of tumor rim, normal liver, and the difference between tumor rim and normal liver of a typical VX2 liver tumor model after contrast administration.

Figure 7.
Changes of the difference between VX2 liver tumor rim and normal liver (rim-liver) on days 7, 14, 21, and 28.

Figure 8.

The gross view (**a, b**) and corresponding hematoxylin and eosin (**c, d**) and CD31 (**e, f**) stained images of representative VX2 liver tumor on day 14 and day 28. On day 14, a thin inflammatory reaction border surrounds tumor, with mild lymphocyte infiltration and blood stasis in adjacent liver sinus (**c**). On day 28, the tumor inflammatory reaction border becomes much thicker, with prominent lymphocyte infiltration and blood stasis (**d**). CD-31 immunohistochemistry stain indicates that luminal vessels locate in tumor border (**e, f**). T, tumor; L, liver. (**c, d, e, f**: original magnification, 200×).

Table 1
Change in VX2 Tumor Size as Determined by Computed Tomography

Tumor Age (Days)	7	14	21	28
Tumor diameter (cm)	0.83 ± 0.27	1.41 ± 0.41	1.77 ± 0.53	2.05 ± 0.75
Tumor area (cm ²)	0.55 ± 0.31	1.50 ± 0.72	2.55 ± 1.70	3.11 ± 1.99
Necrosis area (cm ²)	0.13 ± 0.11	0.63 ± 0.39	1.56 ± 1.32	2.02 ± 1.63
Tumor rim area (cm ²)	0.42 ± 0.22	0.88 ± 0.36	0.98 ± 0.39	1.09 ± 0.42

Table 2
Time-density Curve Parameters Changes in Different ROIs of VX2 Liver Tumor

Tumor Age (Days)	7	14	21	28
Tumor center				
T0 (seconds)	1.50 ± 0.76	2.54 ± 2.64	9.43 ± 10.80	8.93 ± 6.11
TP1 (seconds)	4.93 ± 1.95	16.18 ± 13.90	10.3 ± 5.56	14.71 ± 6.48
TP2 (seconds)	4.93 ± 1.95	16.18 ± 13.90	13.80 ± 8.33	14.71 ± 6.48
ΔH1 (Hu)	43.63 ± 20.82	9.43 ± 7.60	5.28 ± 3.17	1.68 ± 1.90
ΔH2 (Hu)	43.63 ± 20.82	9.43 ± 7.60	5.33 ± 3.24	1.68 ± 1.90
SLe (Hu/second)	11.09 ± 5.81	1.47 ± 1.91	0.71 ± 0.77	0.19 ± 0.23
SLw (Hu/second)	-0.11 ± 0.21	-0.09 ± 0.15	0.04 ± 0.06	0.01 ± 0.02
Tumor rim				
T0 (seconds)	1.57 ± 0.61	1.14 ± 0.85	1.46 ± 0.82	1.79 ± 0.70
TP1 (seconds)	5.21 ± 1.65	5.79 ± 1.32	5.61 ± 1.64	6.21 ± 3.01
TP2 (seconds)	12.71 ± 4.85	13.79 ± 6.94	17.39 ± 6.14	25.57 ± 7.75*
ΔH1 (Hu)	50.18 ± 18.99	48.62 ± 14.09	32.83 ± 13.29	29.45 ± 12.69*
ΔH2 (Hu)	58.00 ± 25.27	56.92 ± 12.24	41.50 ± 13.56	37.78 ± 8.89
SLe (Hu/second)	10.03 ± 3.25	10.25 ± 3.93	7.21 ± 2.86	5.86 ± 2.73*
SLw (Hu/second)	-0.42 ± 0.25	-0.41 ± 0.25	-0.10 ± 0.11	-0.10 ± 0.13*
Normal liver				
T0 (seconds)	3.71 ± 1.55	3.86 ± 2.90	4.36 ± 2.82	4.50 ± 2.69
TP1 (seconds)	16.79 ± 6.51	21.86 ± 10.42	26.64 ± 11.76	19.71 ± 10.37
TP2 (seconds)	16.79 ± 6.51	24.79 ± 7.76	26.64 ± 11.76	23.50 ± 7.75
ΔH1 (Hu)	49.65 ± 14.80	45.52 ± 11.41	49.67 ± 13.28	48.67 ± 25.15
ΔH2 (Hu)	49.65 ± 14.80	48.26 ± 10.81	49.67 ± 13.28	56.29 ± 19.58
SLe (Hu/second)	2.94 ± 1.26	3.67 ± 2.38	2.42 ± 1.17	3.88 ± 3.06
SLw (Hu/second)	-0.44 ± 0.25	-0.49 ± 0.19	-0.51 ± 0.32	-0.44 ± 0.11
Rim-liver				
Baseline (Hu)	-9.12 ± 1.70	-14.9 ± 4.62	-11.82 ± 6.03	-13.66 ± 4.72
TP (seconds)	10.79 ± 1.75	11.36 ± 2.27	9.64 ± 1.80	10.29 ± 2.40
Max. (Hu)	54.33 ± 37.86	37.06 ± 11.98	16.45 ± 12.45	11.16 ± 13.03*

SLe: slope of enhancement; SLw: slope of washout.

* $P < .05$, analysis of variance.

Table 3
The Distribution of Enhancement Patterns in VX2 Liver Tumor Rim

Day	Type			
	I	II	III	IV
7	0	0	6	1
14	0	1	5	1
21	1	4	2	0
28	2	2	3	0