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A Correlative Study between CT Perfusion Parameters and Angiogenesis in Rabbit VX2 Liver Tumors

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Abstract

Objective: The purpose of this study was to evaluate the correlation between CT perfusion parameters and the hypoxia-inducible factor-1 alpha (HIF-1α), vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2) and microvessel density (MVD) marked by CD34 molecular of rabbit VX2 liver tumors and to investigate the value of CT perfusion imaging in evaluating tumor angiogenesis. Material and methods: Twenty-four cases of rabbit VX2 liver tumor were performed by CT perfusion scanning. Hepatic artery perfusion (HAP), portal vein perfusion (PVP), total hepatic blood flow (THBF) and hepatic perfusion index (HPI) were measured by perfusion software. HIF-1α, VEGF and MMP-2 expression and MVD were detected in the 24 rabbit VX2 liver tumor tissue samples using immunohistochemical method. The correlation between the HIF-1α, VEGF, MMP-2 expression and MVD and CT perfusion parameters were analyzed. Results: Correlation analysis revealed that the expression of HIF-1α, MMP-2, MVD were positively related to the HAP, THBF, HPI ($p < 0.01$), but no relations with PVP ($p > 0.05$); and correlation analysis revealed that the expression of VEGF was positively related to the HAP, HPI ($p < 0.01$), but no relations with PVP and THBF ($p > 0.05$). There was a positive relationship between the expression of HIF-1α, VEGF, MMP-2 and MVD ($p < 0.01$). Conclusions: CT perfusion imaging can reflect the blood perfusion of the rabbit VX2 liver tumors and evaluate the information of angiogenesis about tumors.

Keywords

CT Perfusion Imaging, VX2 Liver Tumor, Vascular Endothelial Growth Factor, Hypoxia-Inducible Factor-1 Alpha, CD34 Molecule, Microvessel Density

1. Introduction

The occurrence, development, and metastasis of malignant tumors are closely corre-

lated with tumor angiogenesis [1] [2]. Tumor angiogenesis is regulated by various angiogenic factors. Hypoxia-inducible factor-1 alpha (HIF-1*α*) is the key regulator of angiogenesis in hypoxia, which directly and indirectly affects angiogenesis by influencing the expression of other angiogenic growth factors, and plays a vital role in the evolution of malignant tumors [3]-[5]. Vascular endothelial growth factor (VEGF) is a key factor in early angiogenesis that greatly increases vascular permeability and can promote capillary formation [5] [6]. Matrix metalloproteinase-2 (MMP-2) enhances tumor cell invasion and metastasis into surrounding tissue by promoting tumor angiogenesis and extracellular matrix degradation [7].

The study of angiogenesis in tumor tissue is extremely valuable for determining the biological characteristics of tumors and evaluating treatment efficacy, prognosis, and other factors. Microvessel density (MVD) measurement is the gold standard for quantitative analysis of tumor angiogenesis, and MVD is an important indicator that reflects the biological behavior of malignant tumors [8] [9]. However, MVD measurement requires pathological specimens, which can only be obtained via invasive examination, and has strict requirements for tissue sampling [10]. Computed tomography (CT) perfusion imaging is a new functional imaging technique that can quantitatively measure blood perfusion in tissues and organs and thereby reflect these tissues' and organs' microcirculation characteristics [11]-[17].

In this study, CT perfusion imaging was used to quantitatively determine hepatic carcinoma blood perfusion parameters of rabbit VX2 liver tumors. We also conducted immunohistochemical staining on rabbit VX2 liver tumors tissue to measure HIF-1*α*, VEGF, and MMP-2 expression levels and MVD. In addition, we analyzed correlations between hepatic carcinoma blood perfusion parameters and HIF-1*α*, VEGF, MMP-2, and MVD levels, with the objective of exploring the value of CT perfusion imaging in evaluating tumor angiogenesis.

2. Methods

The experimental protocol used in this study was in accordance with animal welfare guidelines and approved by our Ethics Committee. We used 24 New Zealand white rabbits with an average age of 3 months and a weight of 2.4 ± 0.2 kg. The tumor block embedding method was used to produce the rabbit VX2 liver tumors model. Two weeks after transplantation, the experimental rabbits were anesthetized, and a Toshiba Aquilion 16-slice spiral CT scanner was used to perform CT perfusion imaging. The contrast agent was iohexol, which was administered at a dose of 6 ml via ear vein injection at a rate of 2 ml/s. The following CT scanning parameters were employed: a voltage of 120 kV, a current of 60 mA, a 512 × 512 matrix, a thickness of 1 mm, and an interlayer spacing of 1 mm. Hepatic artery perfusion (HAP), portal vein perfusion (PVP), total hepatic blood flow (THBF) and hepatic perfusion index (HPI) were calculated by perfusion software.

After CT scanning had been completed, all of the 24 animals were sacrificed by anesthesia, and their liver tumor tissues were obtained. The Elivision method was used for immunohistochemical staining to examine and determine HIF-1*α*, VEGF, and MMP-2 expression and MVD. All antibody reagents, including mouse anti-rabbit

HIF-1*α* monoclonal antibodies, mouse anti-rabbit VEGF monoclonal antibodies, mouse anti-rabbit MMP-2 monoclonal antibodies, and mouse anti-rabbit CD34 monoclonal antibodies, were purchased from Orbigen (6827 Nancy Ridge Drive, San Diego, CA 92121, United States). The expression levels of each factor were analyzed according to the methods reported in the literature [18]-[24].

3. Statistical Analysis

SPSS 20.0 software was used to analyze the experimental data. Correlations between CT perfusion parameters and HIF-1*α*, VEGF, MMP-2, and MVD levels were analyzed using the Pearson method. Differences were regarded as statistically significant if $P < 0.05$.

4. Results

The tumors were nearly round and nodular, with cut surfaces appearing gray. Light microscopy indicated that tumor cells were distributed in a nest-like manner, with irregular morphology and a lack of organization; nuclei were large and deeply stained. CT scanning demonstrated that tumors had slightly reduced density, whereas enhanced scans revealed ring enhancement.

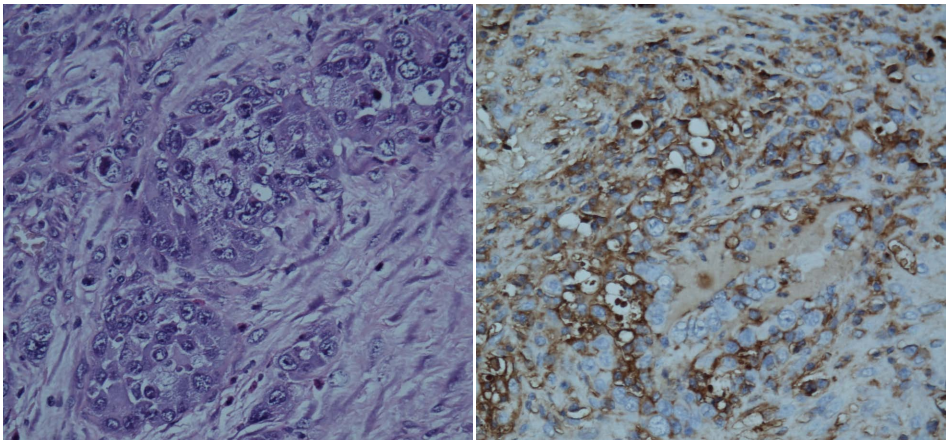
Correlation analysis revealed that the expression of HIF-1*α*, MMP-2, MVD were positively related to the HAP, THBF, HPI ($p < 0.01$), but no relations with PVP ($p > 0.05$); and correlation analysis revealed that the expression of VEGF was positively related to the HAP, HPI ($p < 0.01$), but no relations with PVP and THBF ($p > 0.05$). There was a positive relationship between the expression of HIF-1*α*, VEGF, MMP-2 and MVD ($p < 0.01$) (**Table 1** and **Figure 1**).

5. Discussion

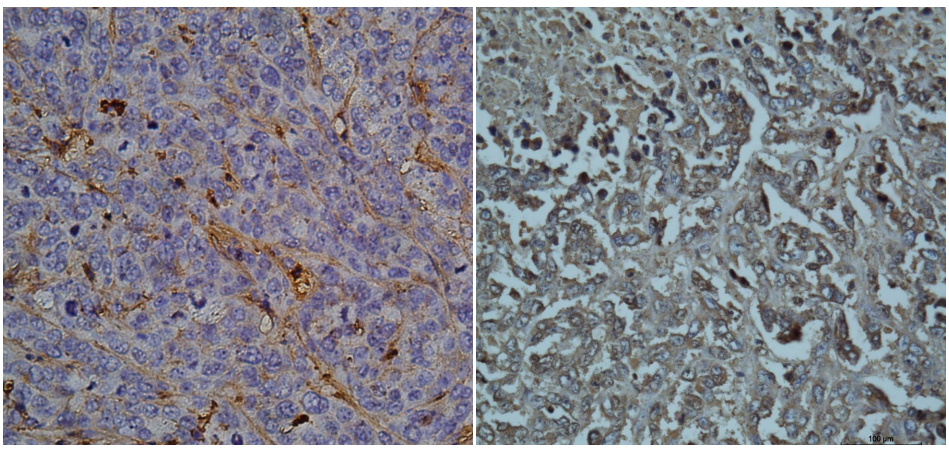
Shope *et al.* described the induction of rabbit VX2 liver tumors. These papillomas are induced on rabbit skin using a virus and the VX2 squamous carcinoma cell line [25]. Due to the lack of antibodies against such tumors in rabbits, these squamous carcinomas

Table 1. The correlations between CT perfusion parameters and hypoxia-inducible factor-1 alpha, vascular endothelial growth factor, matrix metalloproteinase-2, and microvessel density in rabbit VX2 liver tumors.

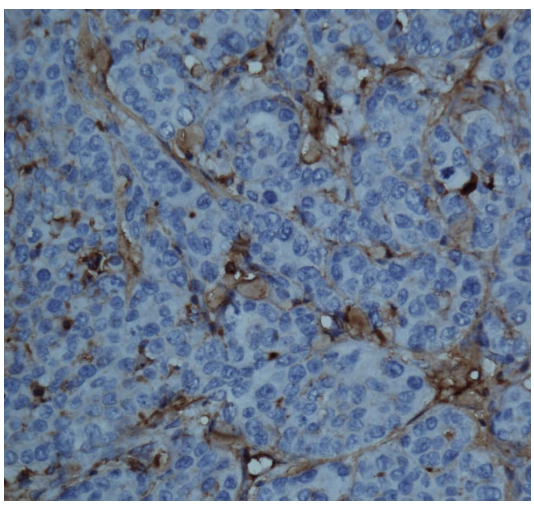
Parameters	Hypoxia-inducible factor-1 alpha		Vascular endothelial growth factor		Matrix metalloproteinase-2		Microvessel density	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Hepatic artery perfusion	0.963	0.000	0.916	0.000	0.918	0.000	0.972	0.000
Portal vein perfusion	0.043	0.840	−0.199	0.350	0.045	0.830	0.085	0.690
Total hepatic blood flow	0.575	0.000	0.358	0.090	0.550	0.000	0.613	0.000
Hepatic perfusion index	0.645	0.000	0.794	0.000	0.616	0.000	0.607	0.000



(a) (b)



(c) (d)



(e)

Figure 1. Microphotographs of the immunohistochemical detection of hypoxia-inducible factor-1 alpha (HIF-1 α), vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2), and microvessel density (MVD) in CD34-positive rabbit VX2 liver tumors. (a) HE staining (×200), (b) HIF-1 α expression (×200), (c) VEGF expression (×200), (d) MMP-2 expression (×200), and (e) the MVD (×200).

can easily grow in rabbit bodies [26]. There are various inoculation and implantation methods for rabbit VX2 liver tumors; these approaches exhibit somewhat different success rates and overall performance [27] [28]. In this study, we utilized tumor block embedding by abdominal celiotomy, which resulted in the formation of a single lesion in the liver. The resulting tumor could stably grow, and the implantation success rate was relatively high.

Hypoxia is a common feature in many types of solid tumors and is considered the major driving force for tumor angiogenesis [29]. HIF-1 is the key factor to regulate angiogenesis. HIF-1 is composed of a constitutively expressed HIF-1β subunit and an oxygen-regulated HIF-1α subunit. Under hypoxic conditions, HIF-1α is stabilized and enters the nucleus, to form a dimer with HIF-1β, where it induces the expression of its target genes [30]. Among these genes, VEGF is a key player in blood vessel formation [31] [32].

MMP-2 is related to invasion and metastasis for various tumors; the relevant mechanisms may involve extracellular matrix collagen degradation, the promotion of tumor invasion and metastasis, and tumor angiogenesis. MMP-2 also interacts with other factors, such as VEGF. A subset of the angiogenesis-promoting activity of VEGF is mediated by MMP-2 and results from the interaction between these two factors [33].

The study results demonstrate that in tumor tissues, HIF-1α, VEGF, and MMP-2 levels significantly correlated with MVD and perfusion parameters. The results indicated that high expression of HIF-1α, VEGF, and MMP-2 stimulates tumor angiogenesis, causing an increase in MVD and thereby allowing for enhanced tumor perfusion.

This research had the following limitations. First, after VX2 tumor cells survive implantation, they may exhibit inconsistent increases in tumor angiogenesis and blood perfusion at different times; however, in this experiment, blood perfusion and angiogenesis for hepatic carcinomas were only examined two weeks after successful implantation, with no analysis of tumors during other growth periods. Second, CT perfusion imaging will increase contrast material volume. Another limitation is that perfusion CT study will increase radiation exposure. Further research is needed to reduce the total radiation dose.

6. Conclusion

In summary, our results indicated that CT perfusion imaging can quantitatively measure the blood perfusion of tissue, which can be used to evaluate tumor angiogenesis.

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