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Original Article

Vitamin B1 suppresses liver cancer growth and restores tumor microcirculation

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Abstract: Inadequate perfusion and hypoxia constitute the primary characteristics of tumor vasculature and drive malignant progression. Strategies aiming at alleviating hypoxia and enhancing blood perfusion may potentiate chemotherapy and immunotherapy, thereby restraining tumor growth. Vitamin B1 (VitB1), an essential coenzyme for multiple metabolic enzymes, plays a crucial role in cellular energy metabolism and in maintaining the balance between glycolysis and the tricarboxylic acid cycle. However, whether the tumor-suppressive effects of VitB1 are associated with modulation of tumor microcirculation remains unclear. This study aimed to evaluate the anti-tumor effects of VitB1 and to investigate the potential relationship with microcirculatory changes in liver cancer. VitB1 inhibited tumor-cell proliferation *in vitro*, indicating a potential direct effect on tumor cells. *In vivo*, VitB1 suppressed liver cancer growth in a dose-dependent manner. High- and medium-dose VitB1 treatment increased microvascular blood perfusion and flow velocity assessed using a laser Doppler flowmetry (LDF) system and decreased tumor microvessel density, without affecting tumor microvascular red blood cell concentration. Moreover, high- and medium-dose VitB1 significantly increased the amplitudes of the neurogenic and endothelial oscillatory components of blood perfusion and flow velocity. These findings suggest that the anti-tumor effects of VitB1 may involve the modulation of neurogenic and endothelial activity, which is associated with improved tumor microhemodynamics. Further studies are needed to elucidate the precise mechanisms and the interplay between direct tumor cell effects and microcirculatory changes.

Keywords: Vitamin B1, tumor growth, microhemodynamics, biological oscillators, microcirculation

Introduction

Liver cancer is among the most prevalent and lethal malignancies worldwide, particularly in regions with a high burden of viral hepatitis [1]. It is characterized by aggressive behavior, frequent recurrence, and resistance to conventional therapies [2]. Within the tumor microenvironment (TME), an imbalance between pro-angiogenic and anti-angiogenic factors drives the rapid and disorganized formation of blood vessels [3]. These tumor-associated vessels display pathological features distinct from those of normal vasculature, including disrupted adherens and tight junctions between endothelial cells. Additionally, reduced basement membrane integrity and insufficient coverage by pericytes and smooth muscle cells further compromise vascular stability [4]. This dysregulated angiogenesis results in pronounced structural and functional heterogeneity, manifested by vessel rupture, increased permeability, and inadequate perfusion [5]. These vascular abnormalities contribute to tumor progression through multiple mechanisms: they exacerbate local hypoxia, impair immune surveillance, facilitate tumor cell invasion and limit effective drug delivery [6].

The identification of vascular endothelial growth factor (VEGF) as a crucial mediator of tumor angiogenesis has driven extensive efforts to develop therapies targeting VEGF signaling. Their primary objective is to induce tumor regression by disrupting the tumor blood supply. However, anti-angiogenic strategies based

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on growth factor inhibition have not consistently achieved the anticipated therapeutic benefits, or improved survival outcomes [7]. Consequently, research focus has shifted from broadly suppressing angiogenesis to restoring the structural and functional integrity of aberrant tumor vasculature, generating increasing interest in agents capable of promoting vascular normalization [8]. This normalization strategy seeks to improve the efficacy of chemotherapy and immunotherapy, thereby constraining tumor progression.

Vitamin B1 (VitB1), also known as thiamine, is an essential coenzyme for three key enzymes in glucose metabolism: transketolase, pyruvate dehydrogenase (PDH), and α-ketoglutarate dehydrogenase. These enzymes catalyze the conversion of intracellular glycolytic intermediates through critical steps in the citric acid cycle and the pentose phosphate pathway [9]. VitB1 has garnered increasing attention for its neuroprotective properties [10, 11]. VitB1 preserves neuronal function by regulating sodium channels in neuronal membranes and inhibiting acetylcholinesterase, the enzyme responsible for acetylcholine degradation [12]. VitB1 deficiency disrupts this balance, resulting in impaired signal transmission and neuronal degeneration [13]. Mechanistically, VitB1 exhibits anti-inflammatory effects, mitigates oxidative stress and promotes the expression of antioxidant enzymes. However, the impact of VitB1 on tumor growth, particularly on tumor vasculature, and the underlying mechanisms remain poorly defined [14, 15]. In this study, we investigated the effects of VitB1 on liver cancer growth, with a particular focus on tumor microvascular perfusion in a mouse model.

Materials and methods

Cell cultivation

The human liver cancer cell lines HepG2 and Huh7 were obtained from PriCells Biomedical Technology (Wuhan, China). HepG2 cells were cultured in MEM (NICR) supplemented with 10% fetal bovine serum (FBS; Gibco, UK) and 1× antibiotic-antimycotic solution (penicillin 100 U/ml, streptomycin 100 μg/ml; NICR). All cell lines were authenticated by short tandem repeat (STR) profiling and maintained under standard conditions (37°C, 5% CO_2).

Cell proliferation assay

The assay was performed by experienced researchers. HepG2 cells were seeded into 96-well plates at a density of 2×10^3 cells per well in 50 μL MEM and treated with a range of VitB1 concentrations (0, 500 μg/ml, 1 mg/ml, 2 mg/ml, and 5 mg/ml) for 48 h. Cell proliferation was monitored using an IncuCyte Zoom Live-Cell Imaging System (2015A, Essen Bioscience, Ann Arbor, MI, USA). Proliferation was quantified as relative confluence based on phase-contrast images.

In vivo tumor formation assay

All animal procedures and experimental protocols were conducted in accordance with the 8th edition (2010) of the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Academies Press, Washington, DC) and were approved by the Institutional Animal Care and Use Committee of the Institute of Microcirculation, Chinese Academy of Medical Sciences (approval number: WXH-LL-2022-003). The xenograft model was established by trained personnel. Six-week-old female BALB/c nude mice were obtained from SPF (Beijing) Biotechnology Co., Ltd. (Beijing, China). Prior to cell implantation, mice were acclimated for 5 days under controlled conditions (24 ± 1°C, 55 ± 5% relative humidity) with a 12-hour light/dark cycle, housed at five per cage. During the study, mice had ad libitum access to a standard diet (Rat & Mouse Growth and Reproduction Formula Feed, Beijing Keao Xieli Feed Co., Ltd., Beijing, China) and drinking water.

For tumor induction, 5×10^6 exponentially growing cells were suspended in 100 μL phosphate-buffered saline (PBS) and inoculated into the axillary region of each nude mouse. When tumor volumes reached approximately 50 mm^3, mice were randomly assigned to four experimental groups (n=5 per group): Control (Ctrl, 100 μL PBS, daily peritumoral injections), VitB1 high-dose (High, 125 mg/kg, daily peritumoral injection), VitB1 medium-dose (Medium, 62.5 mg/kg, daily peritumoral injection), and VitB1 low-dose (Low, 12.5 mg/kg, daily peritumoral injection). Body weight and tumor size were monitored every three days throughout the study. After three weeks of treatment, all mice were euthanized. Tumor volume was calculated using the modified ellipsoid formula:

$V = \frac{\text{length} \times \text{width}^2}{2}$, where length represents the longest tumor diameter and width corresponds to the perpendicular shorter axis. In line with established ethical guidelines, mice were euthanized immediately if tumors met the criteria for humane endpoints: (I) Tumor volume ≥2×10^3 mm^3, (II) Tumor diameter >20 mm, or (III) Abrupt or sustained body weight loss exceeding 20% within 72 hours. Due to the soft consistency of the tumors, minor inaccuracies may occur in both subcutaneous measurements and the visual representation of imaging data. At the end of the study, all experimental mice were sacrificed by cervical dislocation.

Measurement of the tumoral microhemodynamics

Tumor microcirculatory parameters, including blood perfusion, RBC concentration, RBC speed, and blood oxygen saturation, were assessed using a laser Doppler flowmetry (LDF) system (VMS-LDF2, Moor Instruments, Axminster, UK). LDF quantifies microvascular perfusion by measuring the Doppler shift of coherent monochromatic light scattered by moving RBCs. The instrument output represents the product of relative RBC speed and concentration [16]. After a 10-minute acclimation period, mice were anaesthetized via inhalation of 2% (v/v) isoflurane (RWD Life Science Co., Shenzhen, Guangdong, China) mixed with 50% oxygen for induction, followed by 1-2% isoflurane for maintenance, delivered through a small-animal anesthesia system (Matrix VMR; Midmark Corporation, OH, USA). Mice were placed supine on a heating pad, and the skin overlying the tumor was carefully removed to expose the tumor surface. The VP4 probe was then securely positioned on the exposed tissue. Backscattered light collected by the probe was processed through analog and digital signals to generate data on tumor microhemodynamics. LDF signals of microvascular perfusion were analyzed using Moor software (moorVMS-PC 3.1, Moor Instruments).

Laser speckle contrast imaging of tumor microvascular blood flow

Tumor tissue perfusion was monitored using a laser speckle contrast imaging system (moorFLPI-2 blood flow imager, Moor Instruments). Following exposure of the tumor, blood flow was recorded under laser illumination. Acquired images were analyzed using the manufacturer's dedicated software, which automatically quantified flow signals and calculated the average perfusion index within the region of interest.

Wavelet transform spectral analysis (WTSA)

Combining LDF with wavelet transform allows assessment of microcirculatory dynamics associated with physiological processes. In this study, wavelet transform was applied to convert microhemodynamic signals into the time-frequency domain, enabling identification of the contributions of distinct biological oscillators to fluctuations in microcirculation.

The frequency spectrum was divided into multiple sub-bands, each corresponding to a specific biological oscillator: nitric oxide (NO)-independent endothelial, NO-dependent endothelial, neurogenic, myogenic, respiratory, and cardiac oscillators. These sub-bands were assigned to the following frequency ranges: 0.005-0.0095 Hz, 0.0095-0.02 Hz, 0.02-0.05 Hz, 0.05-0.15 Hz, 0.15-2.0 Hz, and 2.0-8.0 Hz, respectively [17, 18].

Using the microhemodynamic spectrum as a reference, the Morlet wavelet was scaled to generate a Gaussian window, which was then shifted across both time and frequency domains. Spectral amplitudes were calculated by averaging the wavelet coefficients [19-21], producing a three-dimensional amplitude spectral scalogram of the wavelet-transformed microhemodynamic data. The scalogram coordinates comprised time (seconds), frequency (Hz), and spectral amplitude (arbitrary units, AU). The amplitudes of the six oscillators were subsequently compared across the four experimental groups. WTSA was performed using moorVMS-PC 3.1 software.

Immunohistochemical (IHC) and hematoxylin-eosin (H&E) staining

For IHC and H&E analyses, 5 μm-thick paraffin-embedded tumor sections were incubated at 60°C for 60 minutes, followed by deparaffinization with xylene and rehydration through a graded ethanol series. H&E staining was performed to evaluate tissue morphology. For IHC, antigen retrieval was performed by microwave heating in 10 mmol/L sodium citrate buffer (pH

6.0) for 20 minutes, and endogenous peroxidase activity was blocked using 3% hydrogen peroxide. Sections were rinsed with PBS and incubated overnight at 4°C with primary antibodies: anti-CD31 (ab281583, 1:2000, Abcam) and anti-Ki67 (ab15580, 5 μg/ml, Abcam). Quantitative analysis was performed using Image J software, with the proportion of positively stained cells assessed in representative tissue sections.

Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical analyses were conducted using GraphPad Prism 10 (GraphPad Software Inc., San Diego, CA, USA). Comparisons between two groups were performed using the unpaired Student's *t*-test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test for multiple comparisons. The tumor growth curves were analyzed using two-way repeated-measures ANOVA, followed by Bonferroni's post-test for multiple comparisons. A *p*-value <0.05 was considered statistically significant. Figures were generated using GraphPad Prism.

Data were presented as mean ± standard error of the mean (SEM). Statistical analyses were conducted using GraphPad Prism 10 (GraphPad Software Inc., San Diego, CA, USA). Comparisons between two groups were performed using the Student's *t*-test, while one-way analysis of variance (ANOVA) was applied for comparisons among multiple groups. A *p*-value <0.05 was considered statistically significant. Figures were generated using GraphPad Prism.

Results

VitB1 suppressed tumor growth in tumor-bearing mice

To evaluate the anti-tumor effects of VitB1, a HepG2 xenograft model in nude mice was established. Both high- and medium-dose VitB1 significantly reduced tumor burden *in vivo*. In the high-dose group, tumor volumes decreased by 38.3%, 46.8%, and 48.4% on days 16, 19, and 22 of treatment, respectively, compared with the control group (**Figure 1A-C**). The cell proliferation marker Ki67 expression was assessed in tumor tissues. VitB1 treatment at all tested doses significantly reduced Ki67 expression, with the greatest reduction observed in the high-dose group (**Figure 1D**, **1E**). H&E staining of liver and kidney tissues demonstrated no overt toxicity across the four groups (**Figure 1F**). Liver architecture was preserved, with clearly defined interlobular bile ducts, veins, and arteries in portal areas, intact hepatic cords along central veins, and no evidence of hepatocyte degeneration, necrosis, sinusoidal dilation, or congestion. Renal structures, including glomeruli, tubules, and interstitium, appeared normal without detectable damage. These observations indicate that the VitB1 doses used were well tolerated and did not induce liver or kidney toxicity.

In vitro proliferation assays further confirmed the anti-tumor activity of VitB1 in human liver cancer cell lines HepG2 and Huh7. Over a 48-hour treatment period, VitB1 inhibited cell proliferation in a dose-dependent manner, with high doses producing the most pronounced effects (**Figure 1G** and **1H**). These results suggest that VitB1 effectively suppresses liver cancer cell growth both *in vitro* and *in vivo*.

Effect of VitB1 on blood perfusion, oxygen saturation and microvessel density (MVD) in a liver cancer mouse model

It is established that vascular normalization improves vascular integrity and perfusion, leading to tissue normoxia, enhanced stromal synthesis of pro-inflammatory chemokines, and increased tumor-infiltrating lymphocytes (TILs). It also alleviates hypoxia within the TME, thereby reducing resistance to chemotherapy and reshaping the immune milieu of tumors [22]. In this study, we further evaluated the effects of VitB1 on blood perfusion, oxygen saturation, and MVD in a liver cancer mouse model. Tumor microvascular perfusion profiles are shown in **Figure 2A** and **2B**. High- and medium-dose VitB1 significantly increased average microcirculatory perfusion, with the greatest enhancement observed in the high-dose group (297.8 PU ± 35.17 PU; PU, perfusion units) compared with controls (100.8 PU ± 12.91 PU). In contrast, low-dose VitB1 had no effect on tumor blood perfusion. Spectral analyses (**Figure 2C**, **2D**) deconvoluted the complex oscillatory dynamics of tumor microcirculation and highlighted the impact of VitB1 on six characteristic frequency bands through two-dimensional spec-

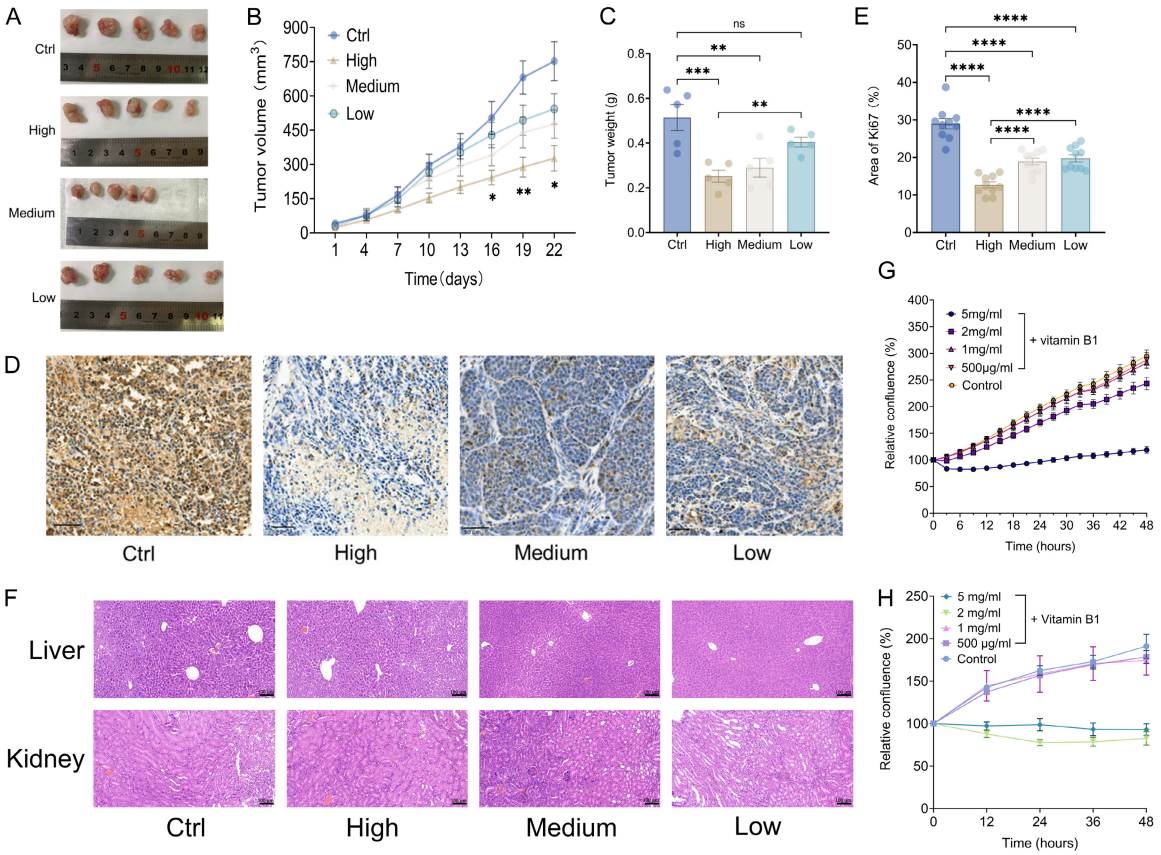

Figure 1. VitB1 inhibits tumor growth *in vivo* and *in vitro*. (A-C) Tumor appearance (A), growth curve (B), and tumor weight (C) in HepG2 subcutaneous tumor-bearing mice during treatment. n=5 per group. ***P*<0.01; ****P*<0.001; (D, E) Representative images of tumor tissue sections with Ki67 immunohistochemical staining (D) and quantitative analysis using ImageJ software (E). Scale bar, 50 μm. n=9 per group, *****P*<0.0001; (F) Representative H&E-stained images of liver and kidney tissue sections; (G, H) Cell proliferation assay showing the effect of different doses of VitB1 on HepG2 (G) and Huh7 (H) cell proliferation.

tral graphs and three-dimensional time-frequency scalograms. High-dose VitB1 significantly increased the amplitudes of neurogenic (388.2 AU ± 56.89 AU vs 116.9 AU ± 12.97 AU; P<0.001), NO-dependent endothelial (333.1 AU ± 59.15 AU vs 90.20 AU ± 8.81 AU, P< 0.01), and NO-independent endothelial oscillators (126.7 AU ± 16.63 AU vs 31.88 AU ± 4.43 AU, P<0.0001) compared to the control group (**Figure 2E**). Medium- and low-dose VitB1 did not significantly affect these three frequency bands. None of the VitB1 doses altered the amplitudes of myogenic, respiratory, or cardiac oscillators. Together, these findings suggest that high-dose VitB1 is associated with enhanced tumor blood perfusion, alongside alterations in neurogenic and endothelial oscillatory activity.

To further illustrate the effect of VitB1 on tumor blood perfusion, we employed the moor-FLPI-2 blood flow image, which can provide intuitive visualization of blood perfusion differences among the four groups. As shown in **Figure 3A** and **3B**, compared with the control group, high-dose VitB1 significantly increased tumor blood perfusion (P<0.001), consistent with the LDF results in **Figure 2**, confirming that high-dose VitB1 increases tumor blood perfusion. Medium- and low-dose VitB1, however, failed to show notable effects. Additionally, we evaluated the effect of VitB1 on tumor MVD. IHC analysis of CD31, a specific marker of microvessels, showed that high- and medium-dose VitB1 obviously reduced MVD in tumor tissues compared with the control group, whereas low-dose VitB1 showed no significant difference (**Figure 3C**, **3D**).

Tissue hemoglobin and oxygen saturation constitute two key physiological parameters reflecting tissue health at the microcirculatory level. Hypoxia, a hallmark of the TME, further

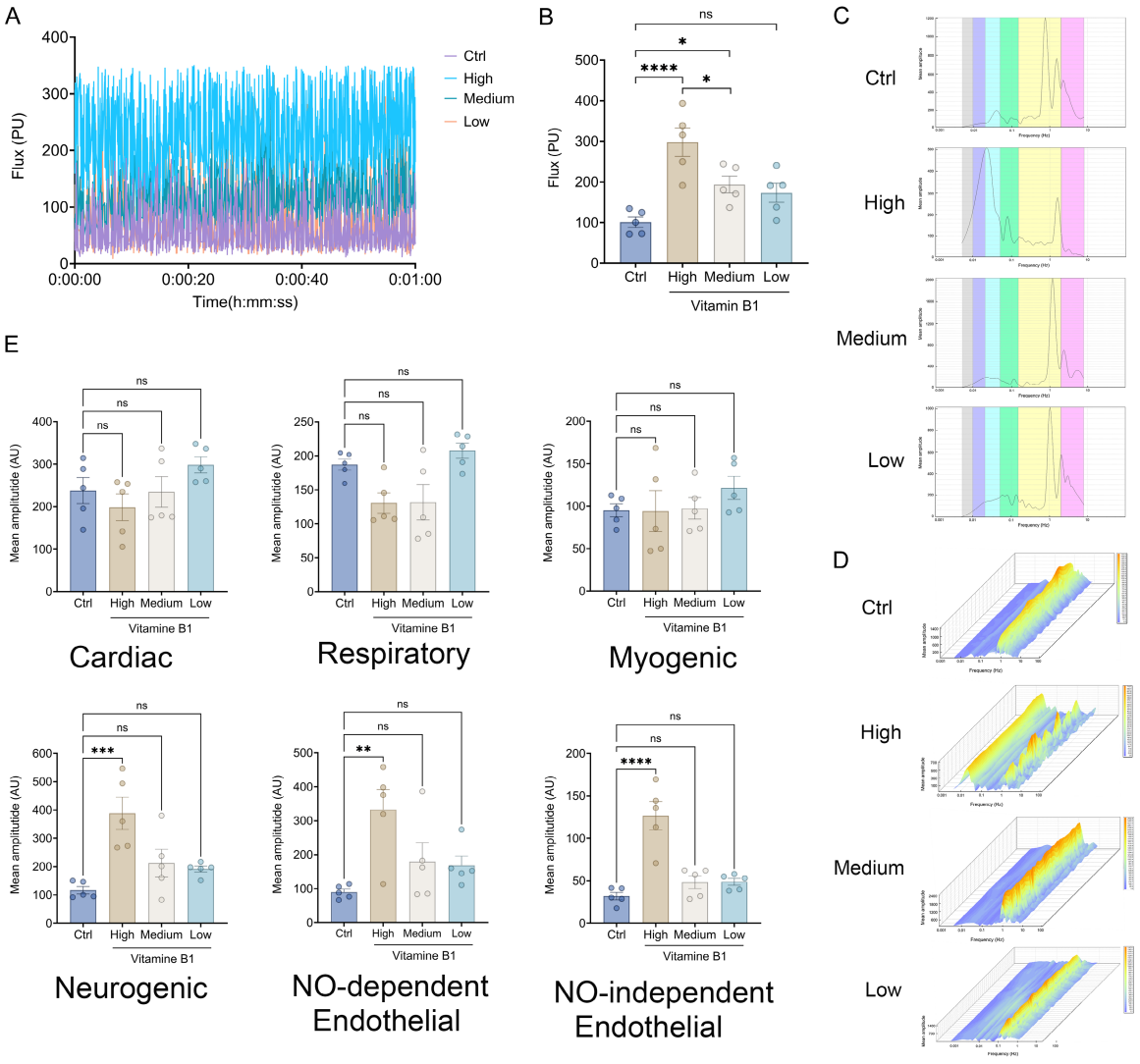

Figure 2. Comparative analysis of tumor microcirculatory blood perfusion and characteristic oscillatory amplitudes in mice treated with different doses of VitB1. (A) Microcirculatory blood perfusion patterns in mice from different groups. PU, perfusion unit; (B) Quantification of blood perfusion in (A); (C, D) Two-dimensional spectrogram (C) and three-dimensional time-frequency spectral scalogram (D) representing characteristic amplitudes of tumor microcirculatory blood perfusions; (E) Quantitative analysis of characteristic oscillatory amplitudes in tumor microcirculatory perfusion. n=5 per group. ns, not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

influences tumor blood perfusion and MVD. Tumor vessels, characterized by abnormal morphology and marked dysfunction in both barrier and transport properties, are a major contributor to hypoxia within the TME. Strategies aimed at alleviating hypoxia and improving blood perfusion have been shown to enhance the therapeutic efficacy of anticancer drugs. In this study, we evaluated tumor microvascular oxygenation - including relative tissue hemoglobin concentrations (oxyHb, deoxyHb, and totalHb) and oxygen saturation - across the four experimental groups using the moorVMS-OXY integrated with the LDF system. As shown in **Figure 3E**, both high- and medium-dose VitB1 significantly increased blood oxygen saturation (55.36% ± 2.44% and 49.44% ± 2.83% *vs* 31.42% ± 4.58%, P<0.01) and oxyhemoglobin content (108.3 AU ± 10.06 AU, and 102.7 AU ± 14.90 AU *vs* 50.34 AU ± 4.69 AU both P<0.05), reduced deoxyhemoglobin levels (62.14 AU ± 5.74 AU *vs* 106.1 AU ± 6.80 AU, P<0.01 for high-dose; 69.06 AU ± 10.19 AU, P<0.05 for medium-dose), and had no effect on total hemoglobin content (P>0.05) compared with controls. Low-dose VitB1 had no impact on blood oxygen saturation, oxyhemoglobin, or total hemoglobin (P>0.05), only slightly decrea-

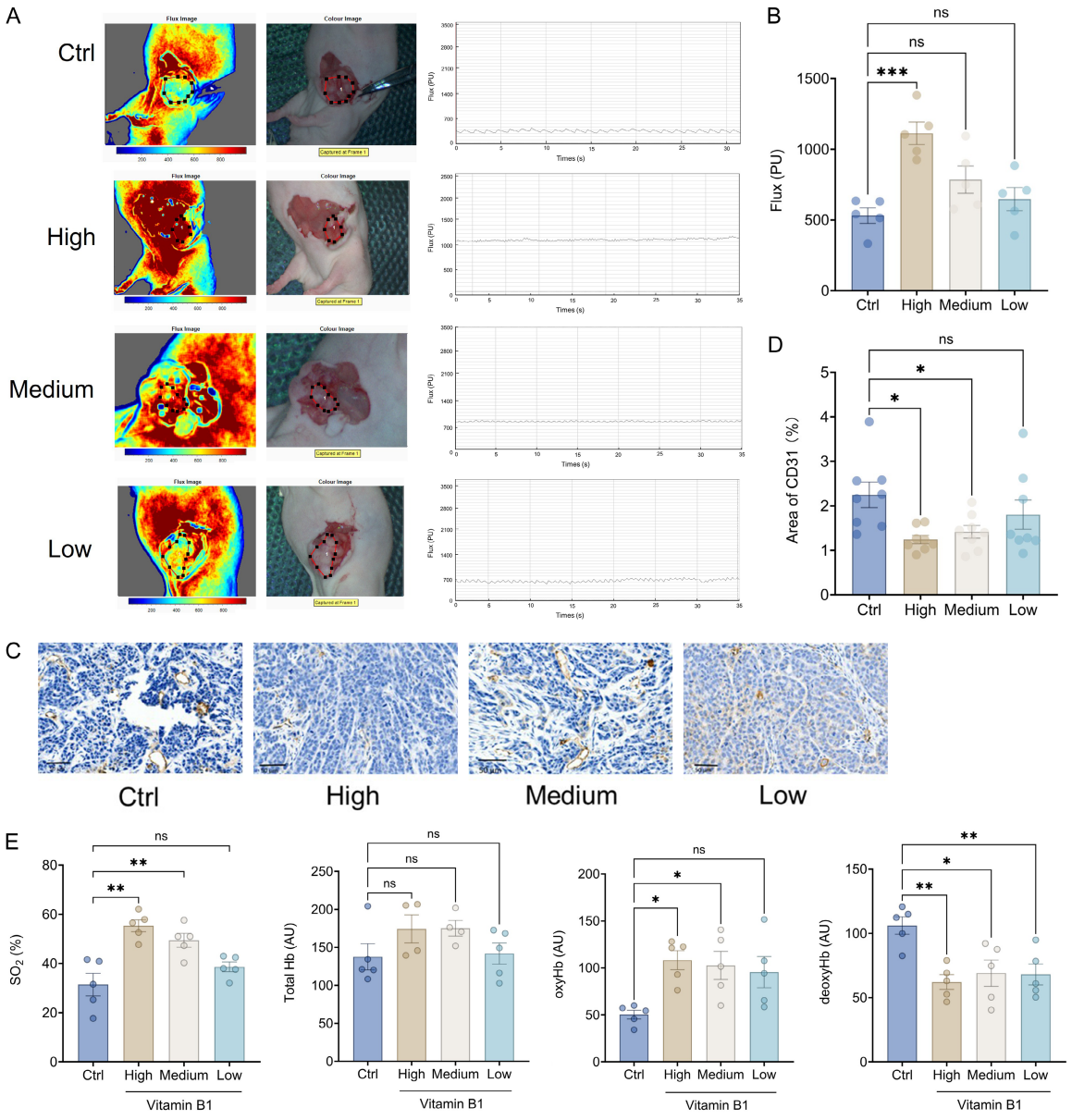

Figure 3. Visualization of tumor blood perfusion and comparative analysis of tumor oxygen saturation and MVD in mice treated with different doses of VitB1. (A) Visualization of tumor blood perfusion (left) and real-time monitoring using the moorFLPI-2 blood flow imager; (B) Quantification of blood perfusion in (A); (C) IHC staining for CD31 in tumor tissues. Scale bar = 500 μm; (D) Quantitative analysis of CD31-positive area percentage; (E) Comparative analysis of tumor microcirculatory SO_2 measured using the moorVMS-OXY system integrated with LDF. ns, not significant; *P<0.05; **P<0.01; ***P<0.001. MVD, microvessel density.

sing deoxyhemoglobin levels. These results indicate that both medium- and high-dose VitB1 effectively alleviate hypoxia in the TME.

Effect of VitB1 on tumor microvascular blood flow speed

Relative tumor microvascular blood flow velocity, an indicator of microvascular autoregulatory activity, was assessed in this study. Under physiological conditions in mice, the ratio of RBCs to other blood cells (leukocytes and platelets) is approximately 30-80:1 [23, 24]. Therefore, RBC speed and concentration closely approximate whole-blood flow and cellular density. Compared with the control group (21.68 AU ± 2.38 AU), mice treated with medium- and high-dose VitB1 exhibited significantly increased microvascular blood flow velocities

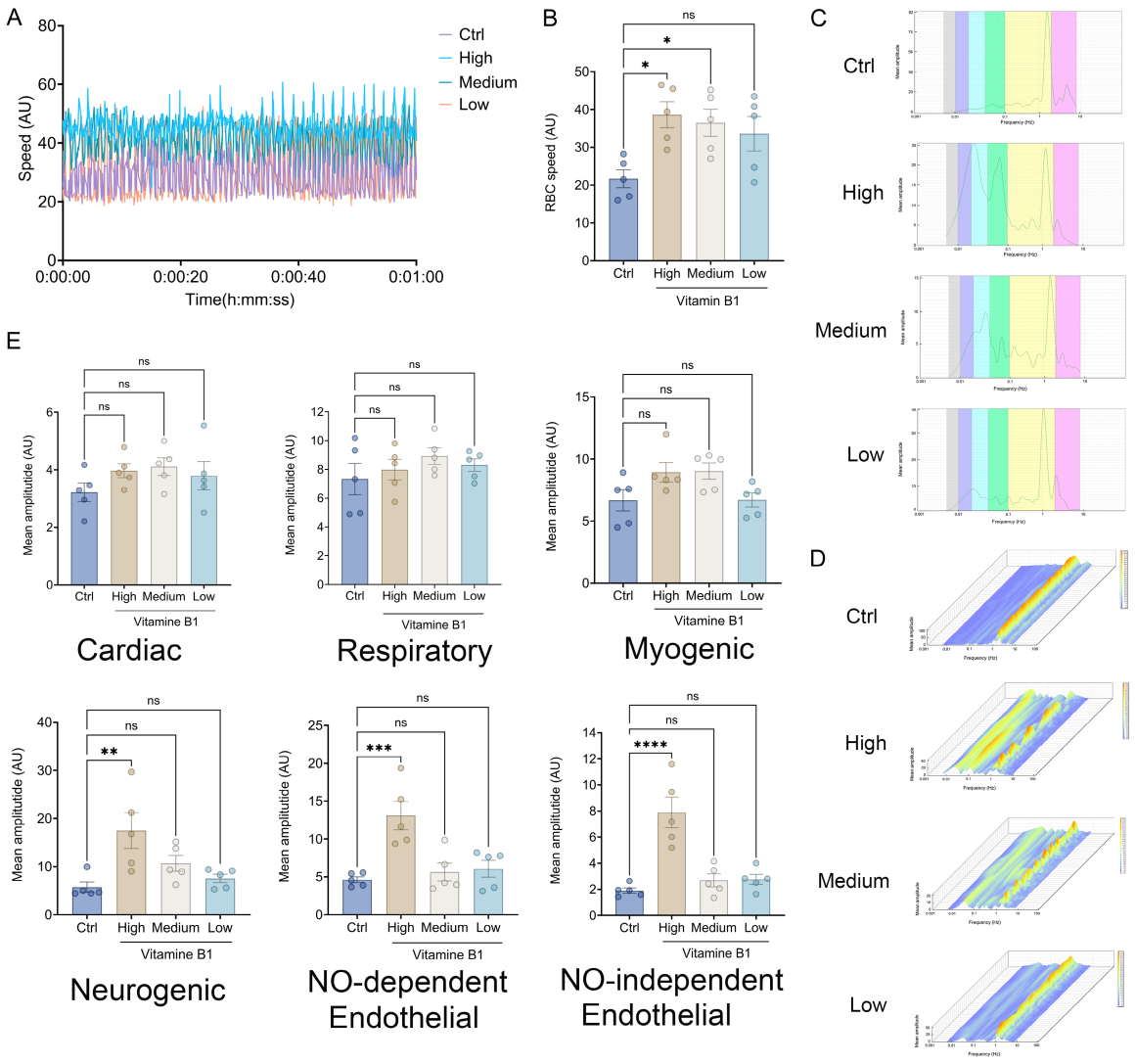

Figure 4. Comparative analysis of tumor microcirculatory blood velocity and characteristic oscillatory amplitudes in mice treated with different doses of VitB1. (A) Microcirculatory blood velocity patterns in mice from different groups; (B) Quantification of blood velocity in (A); (C, D) Two-dimensional spectrogram (C) and three-dimensional time-frequency spectral scalogram (D) representing characteristic amplitudes of tumor microcirculatory blood velocity; (E) Quantitative analysis of characteristic oscillatory amplitudes in tumor microcirculatory blood velocity. n=5 per group. ns, not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

(38.64 AU ± 3.42 AU, and 36.48 AU ± 3.58 AU, both P<0.05; **Figure 4A**, **4B**), whereas low-dose VitB1 showed no significant effect relative to controls.

Wavelet transform analysis revealed distinct patterns of peak velocities within the tumor blood velocity spectra across experimental groups. Although the largest peaks were primarily observed in the respiratory frequency range in all groups (**Figure 4C**), secondary peaks within the neurogenic and myogenic ranges were more prominent across all VitB1-treated mice, particularly in the high- and medium-dose groups. Three-dimensional time-frequency mapping further illustrated temporal fluctuations of these velocity signals, showing more regular and coordinated variations in mice receiving medium- and high-dose VitB1 (**Figure 4D**).

Quantitative analysis of the wavelet-transformed data revealed that mice in the high-dose group exhibited significantly greater amplitudes in the neurogenic (17.49 AU ± 3.74 AU *vs* 5.708 AU ± 1.07 AU, P<0.01), NO-dependent endothe-

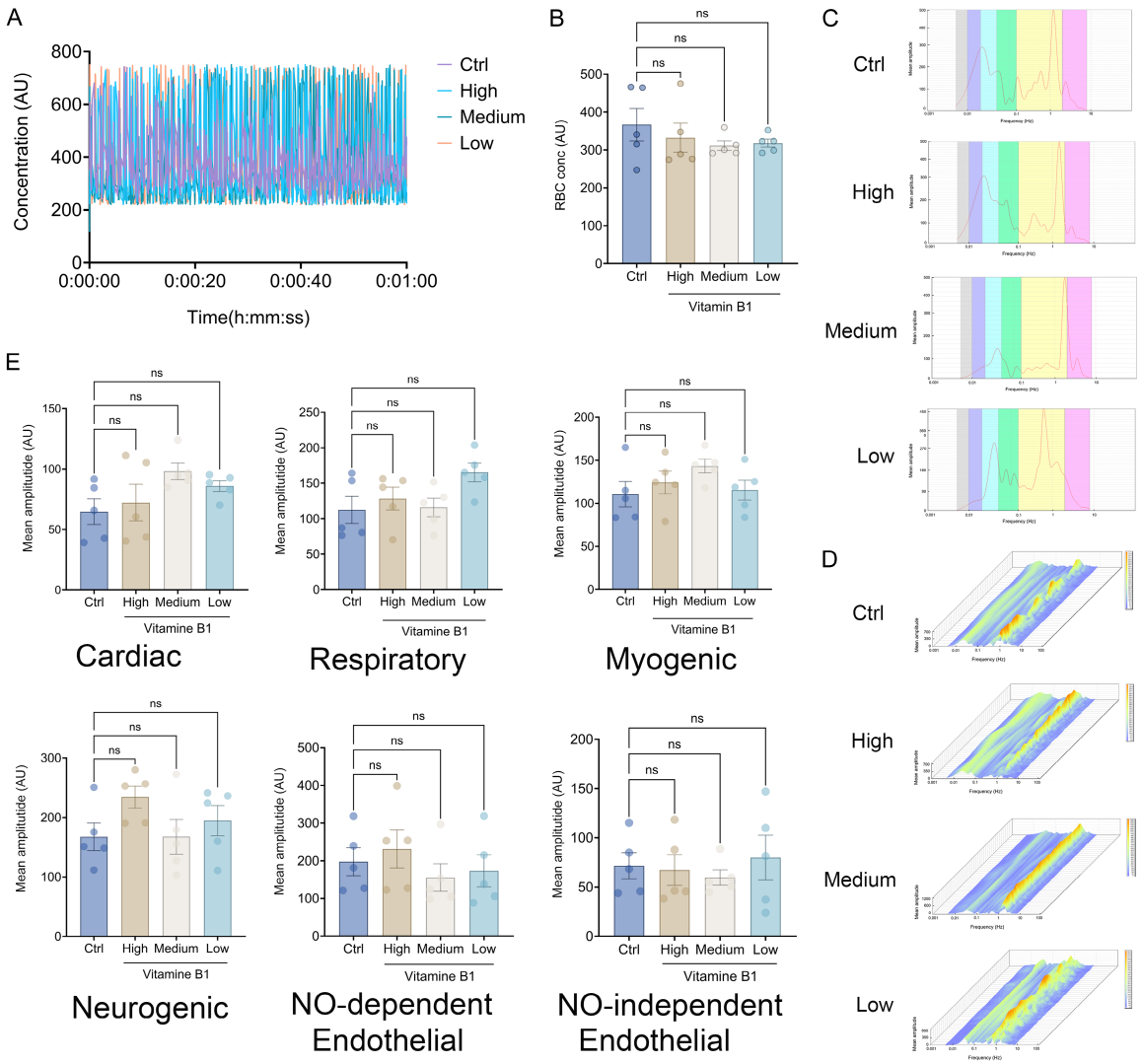

Figure 5. Comparative analysis of tumor microcirculatory RBC concentration and characteristic oscillatory amplitudes in mice treated with different doses of VitB1. (A) Microcirculatory RBC concentration patterns in mice from different groups; (B) Quantification of microcirculatory RBC concentration in (A); (C, D) Two-dimensional spectrogram (C) and three-dimensional time-frequency spectral scalogram (D) representing characteristic amplitudes of tumor microcirculatory RBC concentration; (E) Quantitative analysis of characteristic oscillatory amplitudes in tumor microcirculatory RBC concentration. n=5 per group. ns, not significant.

lial (13.09 AU ± 1.87 AU *vs* 4.61 AU ± 0.39 AU, P<0.001), and NO-independent endothelial spectra (7.89 AU ± 1.17 AU vs 1.9 AU ± 0.21 AU, P<0.001) compared with the control group (**Figure 4E**). Medium- and low-dose VitB1 had no significant effects on these three frequency bands (**Figure 4E**). Furthermore, none of the VitB1 doses affected the amplitudes of the myogenic, respiratory, or cardiac spectra. Collectively, these results indicate that high-dose VitB1 enhances tumor microvascular blood flow velocities primarily through modulating neurogenic and endothelial activity.

Effect of VitB1 on tumor microcirculatory RBC concentration

Tumor microvascular hematocrit, a key indicator of microhemodynamic function, reflects the proportion of RBCs in the bloodstream and provides important information regarding tissue oxygenation and nutrient delivery. No significant differences in mean RBC concentration were observed among the four experimental groups (**Figure 5A**, **5B**).

Wavelet transform analysis revealed a consistent distribution pattern of peak velocities with-

in the tumor RBC concentration spectra across all groups. Two-dimensional spectrograms and three-dimensional time-frequency representations of RBC concentration signals demonstrated similar patterns in all four groups (**Figure 5C**, **5D**), showing the main peak primarily in the respiratory frequency range. Quantitative analysis of six characteristic frequency bands showed no significant differences between the control and VitB1-treated groups (**Figure 5E**), indicating that VitB1 did not affect tumor microvascular RBC concentration.

Discussion

Unlike many cancers that have shown improvements in survival over recent decades, the prognosis of liver cancer has remained largely unchanged prior to the advent of immunotherapy. Most patients are diagnosed at advanced stages, when surgical interventions (resection, transplantation) or locoregional therapies (embolization, ablation) are either infeasible or ineffective. However, over two-thirds of cases present with advanced disease and limited curative options. Furthermore, chemotherapy and radiotherapy are often ineffective or contraindicated in patients with cirrhosis. These limitations highlight the urgent need to elucidate mechanisms of tumor metastasis and to develop novel therapeutic strategies with high efficacy and safety. VitB1, an essential, rate-limiting cofactor for numerous enzymes involved in carbohydrate metabolism and energy production, has received relatively little attention regarding its effects on tumor growth and progression. In this study, we demonstrated that high, medium, and low doses of VitB1 effectively inhibited tumor growth both *in vitro* and *in vivo*. These findings were inconsistent with some previous reports. For example, Comín-Anduix et al. reported that thiamine supplementation sufficient to reverse pre-existing deficiency can promote tumor proliferation [15]. Exogenous thiamine partially, but significantly, rescues proliferation defects caused by knockdown of oncogenic miR-155 in human breast cancer cell lines [25]. Chang et al. observed that ultra-processed foods fortified with vitamins and minerals may potentially increase cancer susceptibility [26]. Collectively, these studies suggest that VitB1 can exert both tumor-promoting and tumor-suppressive effects, which are influenced by genomic and non-genomic factors [14, 15]. The elevated metabolic demands in neoplastic tissues may lead to VitB1 deficiency. A recent single-center prospective cohort study reported a high prevalence of significant VitB1 deficiency among cancer patients [27]. More importantly, researchers caution that moderate thiamine supplementation intended to correct deficiency in oncology patients could facilitate tumor growth in some cases. Conversely, other studies have found no association between thiamine intake and an increased risk of common cancers in women, including breast, endometrial, ovarian, colorectal, and lung cancers [28]. Taken together, these divergent findings underscore the need for further investigation into the roles and underlying mechanisms of VitB1 in the initiation and progression of diverse malignancies.

Malfunctional tumor vasculature frequently leads to structural and functional abnormalities, including increased vascular permeability and inadequate blood perfusion. Poorly perfused tumor vessels impair the delivery of oxygen and nutrients to neoplastic tissues, exacerbating hypoxia and promoting tumor dissemination. Numerous studies have demonstrated that novel agents, particularly natural compounds, can modulate tumor angiogenesis while simultaneously inhibiting tumor growth across various cancer types [29]. For instance, lncRNA ZNF667-AS1 suppresses proliferation, invasion, and angiogenesis in gastric cancer [30]. *Astragali Radix-Curcumae Rhizoma* normalizes tumor blood vessels, thereby reducing tumor growth and metastasis in colon cancer [31]. *Panax notoginseng* saponins promote tumor vascular normalization by improving the immune microenvironment in breast cancer, ultimately inhibiting proliferation and metastasis [32]. Consistent with these findings, our results showed that high- and medium-dose VitB1 markedly enhanced tumor microvascular perfusion and increased oxygen saturation in tumor-bearing mice, indicating that VitB1 may alleviate hypoxia in the TME.

Wavelet transform analysis was employed to decompose continuous blood flow signals, including perfusion, RBC speed, and RBC concentration, enabling a detailed investigation of vasomotion within tumor microvasculature. Tumors are thought to undergo a progressive reduction in the complexity of their physiologi-

cal functions, resulting from impaired regulatory elements and/or disrupted interactions among these components, which diminishes their ability to respond to stress stimuli. Our results demonstrated that high-dose VitB1 positively modulated tumor microvascular blood flow, as evidenced by enhanced amplitudes of blood perfusion and velocity oscillations within the neurogenic, NO-dependent, and NO-independent endothelial frequency ranges. These findings suggest that high-dose VitB1 may confer therapeutic benefits by restoring neurogenic and endothelial activity in tumor microvasculature.

The nervous system is increasingly recognized as a critical regulator of cancer, influencing oncogenesis, malignant proliferation, and metastatic dissemination [33]. Preclinical studies across multiple malignancies have consistently shown that nervous system activity not only modulates cancer initiation but also profoundly impacts tumor progression and metastasis [33, 34]. Within the TME, neurons and glial cells communicate directly with malignant cells via paracrine factors and, in certain contexts, through neuron-to-cancer cell synapses. Indirect interactions also occur remotely, mediated by circulating signals and through the regulation of immune cell trafficking and function. Owing to its neuroprotective properties, VitB1 has been reported to attenuate ethanol-induced cerebral blood flow dysregulation and neuronal damage by reducing reactive oxygen species, thereby alleviating headache-related symptoms [35]. Similarly, Mansoor Husn et al. demonstrated that VitB1 markedly mitigates neuroinflammation and improves cognitive performance in a traumatic brain injury mouse model [36]. Consistent with these observations, our wavelet transform analysis of tumor blood flow signals revealed that high-dose VitB1 increased the contribution of neurogenic factors to tumor microhemodynamics. However, the precise mechanisms by which VitB1 modulates neurogenic regulation, particularly within tumors, remain to be elucidated in further study.

As a key component of vascular structural abnormalities, endothelial disarray contributes to aberrant intra-tumoral perfusion and establishes a vicious cycle characterized by local hypoxia, impaired permeability, extracellular matrix degradation, and cancer cell dissemination [37]. The role of endothelial cells in cancer is increasingly recognized as extending beyond their structural function as the lining of tumor-perfusing vessels to acting as a stromal paracrine regulatory element. Dysfunctionally activated endothelial cells can amplify pro-inflammatory signaling within the TME, thereby enhancing cancer cell invasiveness [38]. Previous studies have shown that VitB1 improves endothelium-dependent vasodilatation under hyperglycemic conditions [39], and benfotiamine, a highly bioavailable thiamine prodrug, mitigates smoking-induced vascular dysfunction in healthy smokers [40]. In the present study, high-dose VitB1 significantly increased endothelial oscillators in tumor microvascular blood flow, suggesting potential restoration of endothelial function within the tumor microvasculature. However, the precise mechanism by which VitB1 regulates endothelial cell function to improve tumor microcirculation, as well as whether this process involves neural regulation, remains to be further investigated.

Given that VitB1 participates in numerous essential biochemical processes, including the pentose phosphate pathway and the tricarboxylic acid (TCA) cycle, both central to carbohydrate metabolism, its deficiency has been reported to induce hyperphosphorylation of PDH via overexpression of pyruvate dehydrogenase kinases (PDKs), thereby promoting aerobic glycolysis to compensate for energy production in the TCA cycle [41]. Therefore, the inhibitory effects of VitB1 on liver cancer observed in the present study may be linked to its regulation of aerobic glycolysis. Future studies will focus on elucidating the underlying molecular mechanisms, particularly the impact of VitB1 on aerobic glycolytic pathways.

Several limitations of this study should be acknowledged. Firstly, the sample size of this *in vivo* study warrant careful consideration. The experiment included four groups with five nude mice per group, which limited our ability to detect subtle inter-group differences in tumor volume, tumor weight, and associated protein expression. The constraints on sample size were primarily due to two factors: (1) The Animal Ethics Committee strictly regulates the use of immunodeficient nude mice, given the ethical considerations associated with immunocompromised animal models; (2) Subcuta-

neous tumor formation in nude mice exhibits high individual variability, with a risk of accidental death or tumor formation failure during the experiment. Expanding the sample size beyond ethical limits would not only would violate these regulations but also compromise experimental feasibility. It should be emphasized that the findings of this study are preliminary and exploratory, demonstrating only the potential inhibitory effect of the intervention on subcutaneous tumor growth in nude mice. These results cannot be directly extrapolated to clinical settings or interpreted as definitive conclusions. Future studies should optimize tumor cell inoculation methods to reduce individual variability, obtain ethical approval to expand sample size, and further validate the stability and reproducibility of the experimental outcomes.

Secondly, while this approach effectively demonstrates the biological activity of high-dose VitB1 in modulating the tumor microcirculation, the subcutaneous model does not fully recapitulate the complex anatomical and physiological microenvironments of spontaneous human tumors. Furthermore, the peritumoral injection route is a localized intervention that differs significantly from the systemic administration (e.g., intravenous or oral) typically required in clinical oncology practice. However, it is important to note that this experimental design was primarily chosen as a proof-of-concept strategy. By administering the drug directly around the tumor, we aimed to ensure a high local concentration to robustly validate the therapeutic mechanism - specifically the improvement of tumor microcirculation - in this initial study. Looking ahead, future investigations should focus on evaluating the efficacy of VitB1 via systemic delivery routes. Additionally, utilizing orthotopic tumor models or genetically engineered mouse models would provide a more clinically relevant context for assessing the true translational potential of this therapeutic strategy.

Thirdly, in this study, we observed that VitB1 treatment led to increased tumor perfusion and oxygenation alongside a reduction in CD31-positive MVD. While these findings suggest improved vascular function, we acknowledge that additional markers are required to fully characterize vascular normalization. Due to the constraints of the current study, we did not perform direct assessments of vessel maturity (e.g., pericyte coverage using α-SMA or NG2 staining) or vessel integrity (e.g., basement membrane components or permeability assays). These are important aspects that we plan to investigate in our future research.

In addition, it is important to acknowledge that the current investigation primarily establishes a correlative relationship between the improvement of microcirculation and the efficacy of VitB1. While our data strongly suggest that enhanced perfusion contributes to the therapeutic outcome, we have not yet provided direct experimental evidence to confirm that these vascular alterations are strictly indispensable for the mechanism of action. However, we propose that the observed the improved amplitude of blood perfusion oscillation - specifically a significant increase in the amplitude of neurogenic and endothelial oscillators - are likely causes of improved oxygen and nutrient delivery in tumor microcirculation. To move beyond correlation to causation, further studies should explore the mechanism by which VitB1 improves tumor blood perfusion through modulation of neural and endothelial function, and consequently affects tumor growth.

Conclusion

In conclusion, our findings demonstrate that VitB1 administration is associated with the suppression of liver cancer growth, alongside the amelioration of pathological changes in tumor microcirculation and alterations in neurogenic and endothelial oscillatory components within tumor blood perfusion signals. While these physiological findings suggest a potential link between microcirculatory dynamics and tumor growth inhibition, they represent indirect correlates rather than direct evidence of specific neuronal or endothelial mechanisms. Further studies employing direct measurements of neuronal and endothelial function are warranted to validate these observations and to elucidate the precise mechanisms through which VitB1 may exert its anti-tumor effects.

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Disclosure of conflict of interest

None.

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References

[1] Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J and Finn RS. Hepatocellular carcinoma. Nat Rev Dis Primers 2021; 7: 6.

[2] Li X, Ramadori P, Pfister D, Seehawer M, Zender L and Heikenwalder M. The immunological and metabolic landscape in primary and metastatic liver cancer. Nat Rev Cancer 2021; 21: 541-557.

[3] Viallard C and Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis 2017; 20: 409-426.

[4] Zeng Y, Zhang S, Li S, Song G, Meng T, Yuan H and Hu F. Normalizing tumor blood vessels to improve chemotherapy and inhibit breast cancer metastasis by multifunctional nanoparticles. Mol Pharm 2023; 20: 5078-5089.

[5] Cartier A, Leigh T, Liu CH and Hla T. Endothelial sphingosine 1-phosphate receptors promote vascular normalization and antitumor therapy. Proc Natl Acad Sci U S A 2020; 117: 3157-3166.

[6] Liang Q, Zhou L, Li Y, Liu J and Liu Y. Nano drug delivery system reconstruct tumour vasculature for the tumour vascular normalisation. J Drug Target 2022; 30: 119-130.

[7] Lugano R, Ramachandran M and Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cell Mol Life Sci 2020; 77: 1745-1770.

[8] Qian C, Zhou Y, Zhang T, Dong G, Song M, Tang Y, Wei Z, Yu S, Shen Q, Chen W, Choi JP, Yan J, Zhong C, Wan L, Li J, Wang A, Lu Y and Zhao Y. Targeting PKM2 signaling cascade with salvianic acid A normalizes tumor blood vessels to facilitate chemotherapeutic drug delivery. Acta Pharm Sin B 2024; 14: 2077-2096.

[9] Kazmierczak-Baranska J, Halczuk K and Karwowski BT. Thiamine (Vitamin B1)-an essential health regulator. Nutrients 2025; 17: 2206.

[10] Li EY, Zhao PJ, Jian J, Yin BQ, Sun ZY, Xu CX, Tang YC and Wu H. Vitamin B1 and B12 mitigates neuron apoptosis in cerebral palsy by augmenting BDNF expression through MALAT1/miR-1 axis. Cell Cycle 2019; 18: 2849-2859.

[11] Peterson CT, Rodionov DA, Osterman AL and Peterson SN. B vitamins and their role in immune regulation and cancer. Nutrients 2020; 12: 3380.

[12] Saberi R, Mirazi N, Amirahmadi S, Darbandi ZK, Vafaee F, Rajabian A and Hosseini M. Ameliorative effects of thiamin on learning behavior and memory dysfunction in a rat model of hypothyroidism: implication of oxidative stress and acetylcholinesterase. Metab Brain Dis 2023; 38: 2603-2613.

[13] Mochizuki H. Arsenic neurotoxicity in humans. Int J Mol Sci 2019; 20: 3418.

[14] Niu C, Qiu W, Li X, Li H, Zhou J and Zhu H. Transketolase serves as a biomarker for poor prognosis in human lung adenocarcinoma. J Cancer 2022; 13: 2584-2593.

[15] Comin-Anduix B, Boren J, Martinez S, Moro C, Centelles JJ, Trebukhina R, Petushok N, Lee WN, Boros LG and Cascante M. The effect of thiamine supplementation on tumour proliferation. A metabolic control analysis study. Eur J Biochem 2001; 268: 4177-4182.

[16] Mizeva I, Frick P and Podtaev S. Relationship of oscillating and average components of laser Doppler flowmetry signal. J Biomed Opt 2016; 21: 85002.

[17] Astashev ME, Serov DA and Tankanag AV. Anesthesia effects on the low frequency blood flow oscillations in mouse skin. Skin Res Technol 2019; 25: 40-46.

[18] Monteiro Rodrigues L, Nazaré Silva H, Ferreira H and Gadeau AP. Characterizing vascular dysfunction in genetically modified mice through the hyperoxia model. Int J Mol Sci 2019; 20: 2178.

[19] Aleksandrin VV, Ivanov AV, Virus ED, Bulgakova PO and Kubatiev AA. Application of wavelet analysis to detect dysfunction in cerebral blood flow autoregulation during experimental hyperhomocysteinaemia. Lasers Med Sci 2018; 33: 1327-1333.

[20] Lancaster G, Stefanovska A, Pesce M, Marco Vezzoni G, Loggini B, Pingitore R, Ghiara F, Barachini P, Cervadoro G, Romanelli M and Rossi M. Dynamic markers based on blood perfusion fluctuations for selecting skin melanocytic lesions for biopsy. Sci Rep 2015; 5: 12825.

[21] Smirni S, MacDonald MP, Robertson CP, McNamara PM, O'Gorman S, Leahy MJ and Khan F. Application of cmOCT and continuous wavelet transform analysis to the assessment of skin microcirculation dynamics. J Biomed Opt 2018; 23: 1-13.

[22] Wang K, Chen Q, Liu N, Zhang J and Pan X. Recent advances in, and challenges of, anti-

angiogenesis agents for tumor chemotherapy based on vascular normalization. Drug Discov Today 2021; 26: 2743-2753.
[23] Patel S, Patel S, Kotadiya A, Patel S, Shrimali B, Tank M, Patel T, Trivedi H, Kshirsagar S and Jain M. Comparative analysis of the effect of sex and age on the hematological and biochemical profile of BALB/c and C57BL/6 inbred mice. J Am Assoc Lab Anim Sci 2025; 64: 132-145.
[24] Fernandes DP, Pimentel MML, Santos FAD, Praxedes ÉA, Brito PD, Lima MA, Lelis ICNG, Macedo MF and Bezerra MB. Hematological and biochemical profile of BALB/c nude and C57BL/6 SCID female mice after ovarian xenograft. An Acad Bras Cienc 2018; 90: 3941-3948.
[25] Kim S, Rhee JK, Yoo HJ, Lee HJ, Lee EJ, Lee JW, Yu JH, Son BH, Gong G, Kim SB, Singh SR, Ahn SH and Chang S. Bioinformatic and metabolomic analysis reveals miR-155 regulates thiamine level in breast cancer. Cancer Lett 2015; 357: 488-497.
[26] Chang K, Gunter MJ, Rauber F, Levy RB, Huybrechts I, Kliemann N, Millett C and Vamos EP. Ultra-processed food consumption, cancer risk and cancer mortality: a large-scale prospective analysis within the UK Biobank. EClinicalMedicine 2023; 56: 101840.
[27] Boopathy D, Grahf D, Ross J, Hawatian K, Rammal JA, Alaimo K and Miller JB. Thiamine deficiency is common and underrecognized in emergency department oncology patients. J Clin Med 2025; 14: 257.
[28] Kabat GC, Miller AB, Jain M and Rohan TE. Dietary intake of selected B vitamins in relation to risk of major cancers in women. Br J Cancer 2008; 99: 816-821.
[29] Yan Y, Yao L, Sun H, Pang S, Kong X, Zhao S and Xu S. Effects of wogonoside on invasion and migration of lung cancer A549 cells and angiogenesis in xenograft tumors of nude mice. J Thorac Dis 2020; 12: 1552-1560.
[30] Yu C, Chen W, Cai Y, Du M, Zong D, Qian L, Jiang X and Zhu H. The lncRNA ZNF667-AS1 inhibits propagation, invasion, and angiogenesis of gastric cancer by silencing the expression of N-cadherin and VEGFA. J Oncol 2022; 2022: 3579547.
[31] Liang Y, Su T, Zhu S, Sun R, Qin J, Yue Z, Wang X, Liang Z, Tan X, Bian Y, Zhao F, Tang D and Yin G. Astragali Radix-Curcumae Rhizoma normalizes tumor blood vessels by HIF-1alpha to antitumor metastasis in colon cancer. Phytomedicine 2025; 140: 156562.
[32] Xia L, Liu X, Mao W, Guo Y, Huang J, Hu Y, Jin L, Liu X, Fu H, Du Y and Shou Q. Panax notoginseng saponins normalises tumour blood vessels by inhibiting EphA2 gene expression to modulate the tumour microenvironment of breast cancer. Phytomedicine 2023; 114: 154787.
[33] Chen P, Wang W, Liu R, Lyu J, Zhang L, Li B, Qiu B, Tian A, Jiang W, Ying H, Jing R, Wang Q, Zhu K, Bai R, Zeng L, Duan S and Liu C. Olfactory sensory experience regulates gliomagenesis via neuronal IGF1. Nature 2022; 606: 550-556.
[34] Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ and Frenette PS. Autonomic nerve development contributes to prostate cancer progression. Science 2013; 341: 1236361.
[35] Cao Q, Gao Y, Li Z, Xiao Y, Shen C, Xiong Y, Xu S, Chua Y, Li Y, Yang J, Cao X and Meng L. The protective role of vitamin B1 in alcohol related headaches induced by disturbance of blood flow and direct cytotoxicity to hippocampal neuron. Nutr Neurosci 2025; 28: 1632-1649.
[36] Husn M, Amin Z, Ali Y, Kanwal L, Sabir K, Shah SA and Shah SF. Neuroprotective effects of vitamin B1 on memory impairment and suppression of pro-inflammatory cytokines in traumatic brain injury. Metab Brain Dis 2023; 38: 2175-2184.
[37] Franses JW, Baker AB, Chitalia VC and Edelman ER. Stromal endothelial cells directly influence cancer progression. Sci Transl Med 2011; 3: 66ra65.
[38] Franses JW, Drosu NC, Gibson WJ, Chitalia VC and Edelman ER. Dysfunctional endothelial cells directly stimulate cancer inflammation and metastasis. Int J Cancer 2013; 133: 1334-1344.
[39] Arora S, Lidor A, Abularrage CJ, Weiswasser JM, Nylen E, Kellicut D and Sidawy AN. Thiamine (vitamin B1) improves endothelium-dependent vasodilatation in the presence of hyperglycemia. Ann Vasc Surg 2006; 20: 653-658.
[40] Stirban A, Nandrean S, Kirana S, Götting C, Veresiu IA and Tschoepe D. Benfotiamine counteracts smoking-induced vascular dysfunction in healthy smokers. Int J Vasc Med 2012; 2012: 968761.
[41] Liu X, Montissol S, Uber A, Ganley S, Grossestreuer AV, Berg K, Heydrick S and Donnino MW. The effects of thiamine on breast cancer cells. Molecules 2018; 23: 1464.