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REVIEW

Focus on microvascular invasion in hepatocellular carcinoma: a pathological perspective

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Abstract: Microvascular invasion (MVI) is considered an important pathological prognostic parameter for hepatocellular carcinoma (HCC). Growing evidence has revealed that MVI can be regarded as a stratified index for clinical intervention. However, few studies have focused on how to conduct a standardized assessment of MVI, which renders conclusions of related clinical studies unreliable. This review introduces the definition of MVI, a seven-point baseline sampling protocol for HCC, and MVI risk classification protocol, all of which have been promoted in China. These concepts should be applied in the diagnosis of HCC in the future. Furthermore, recent studies on the molecular mechanism of MVI have been summarized to provide a reference for future research on underlying molecular pathology.

Key words: hepatocellular carcinoma, microvascular invasion, sampling, staging, molecular mechanism

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***Abbreviations*:** MVI, Microvascular invasion; HCC, hepatocellular carcinoma; RFS, recurrence-free survival; OS, overall survival.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related deaths worldwide[1]. Despite the continuous progress in the treatment strategies, the recurrence rate of HCC remains high[2]. Based on the findings of multiple retrospective studies, the presence of microvascular invasion (MVI) is a critical determinant of early recurrence and poor prognosis of HCC[3]. A growing number of studies have reported that adjuvant interventions, such as transcatheter arterial chemoembolization[4], antiviral therapy[5], and targeted drugs[6], could effectively reduce the recurrence rate in HCC patients with MVI. However, the methodology sections of these studies have failed to systematically elaborate the diagnosis and evaluation of MVI. Differential MVI evaluation will inevitably lead to a bias in the detection rate and risk assessment, which results in unreliable conclusions from these studies. Accordingly, it is urgent to promote the implementation of standardized MVI assessments. In the present review, we examine recent research on the clinical pathology of MVI, focusing on how to standardize the diagnosis and evaluation of MVI. Furthermore, the rapid development of genomics and deep sequencing can help better understand the occurrence and progression of HCC at the molecular level[7,8]. In recent years, some studies have focused on the molecular mechanism of MVI, which is of great significance in terms of future accurate diagnosis and drug development. Therefore, we summarized the related content in this study. Based on these studies, we discussed future directions for the management of HCC, during which application of the MVI status may allow for more nuanced and stratified treatment algorithms that could help avoid futile interventions and improve prognosis.

2. Definition and diagnosis of MVI

Notably, MVI is identified as the presence of tumor emboli in the vascular space on microscopy[9]. However, the location and shape of the MVI may have distinct implications. MVI can occur in the branches of the hepatic artery, portal vein, and hepatic vein. MVI in the arteriole may be related to the destruction of tumor blood vessels and the origin of circulating tumor cells[10], while in the portal and hepatic vein, MVI may be the source of intrahepatic metastases and distant metastasis, respectively[11]. Furthermore, microsatellite lesions, which are tumor nests without endothelial wrapping, were detected in HCC slides, and are deemed as MVI that subsequently invaded a new region of the liver[12]. Accordingly, the current pathological concept is far from satisfying the need to define MVI with prognostic value. Considering the differences in the diagnostic ability of pathologists, the Guidelines for the Diagnosis and Treatment of Hepatocellular Carcinoma (2019 Edition) recommend that all classic MVI and satellite lesions be enumerated uniformly during MVI grading (Figure 1)[13]. Moreover, it is worth noting that intratumoral MVI has no significant prognostic value[14]. Therefore, only MVI in the tumor capsule and liver tissue should be considered for diagnosis. Although it is not necessary to distinguish classic MVI from satellite lesions in the MVI grading, it is recommended to describe them separately in pathological reports, possibly employing reticular fiber staining and CD34 immunohistochemical staining for differentiation.

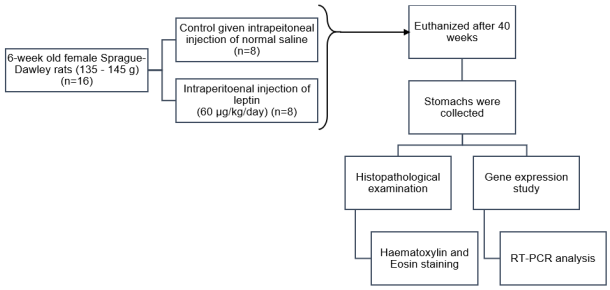

Figure 1. Flowchart of study design.

Standardized sampling and MVI detection

Gross specimen sampling is the cornerstone of all pathological evaluations. However, previous studies on HCC have rarely mentioned the specific sampling scheme utilized. The number and location of samples directly affect the diagnosis of MVI[15]. Hence, the current conclusions regarding the significance of MVI may be biased. There are two standpoints in terms of sampling numbers worldwide. One is to determine the number of blocks based on the maximum diameter of the tumor. In general, if the tumor increases by 1 cm, one additional tissue mass is considered. The other is to fix the number of blocks, usually four tumor tissue blocks. Both viewpoints have related merits. The former has the advantage of providing more detailed archives, but the unnecessary workload is increased. The latter can perform consistency appraisal among patients but may result in a misdiagnosis, such as missed diagnosis of combined HCC and cholangiocarcinoma. As for the sampling site, the current international consensus is that the junction between cancer and paracancerous should be obtained to evaluate MVI as it has been confirmed that intratumoral MVI has no prognostic value[14]. Accordingly, Chinese pathologists proposed a seven-point baseline sampling scheme, which stipulates that at least four tissue specimens should be sampled at the tumor margin in a 1:1 ratio of the tumor and adjacent liver tissues, at the 3, 6, 9, and 12 o'clock positions. Specimens should also be sampled at both

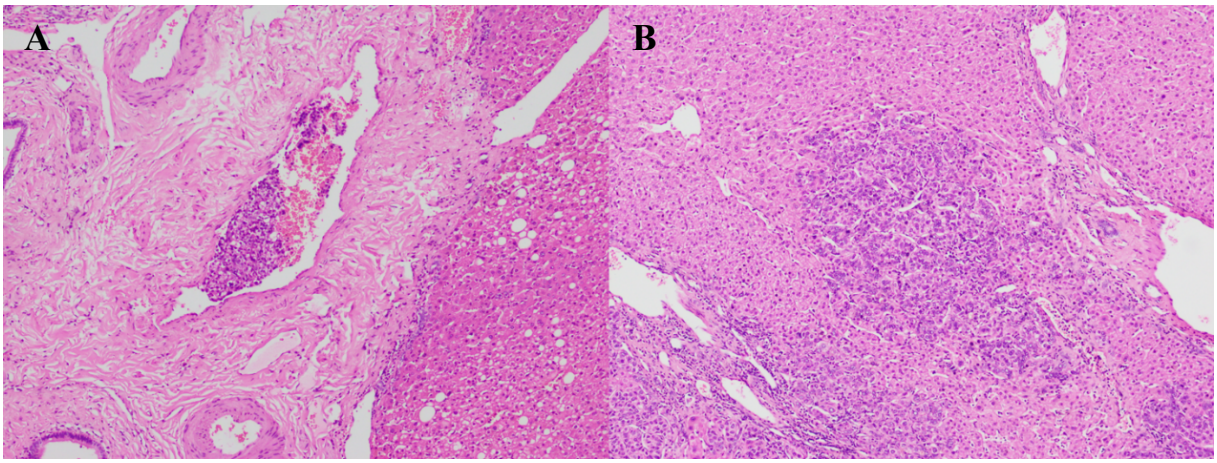

Figure 1. Histopathological morphology (HE section, 100×). A: classical MVI; B: satellite lesions. MVI, microvascular invasion.

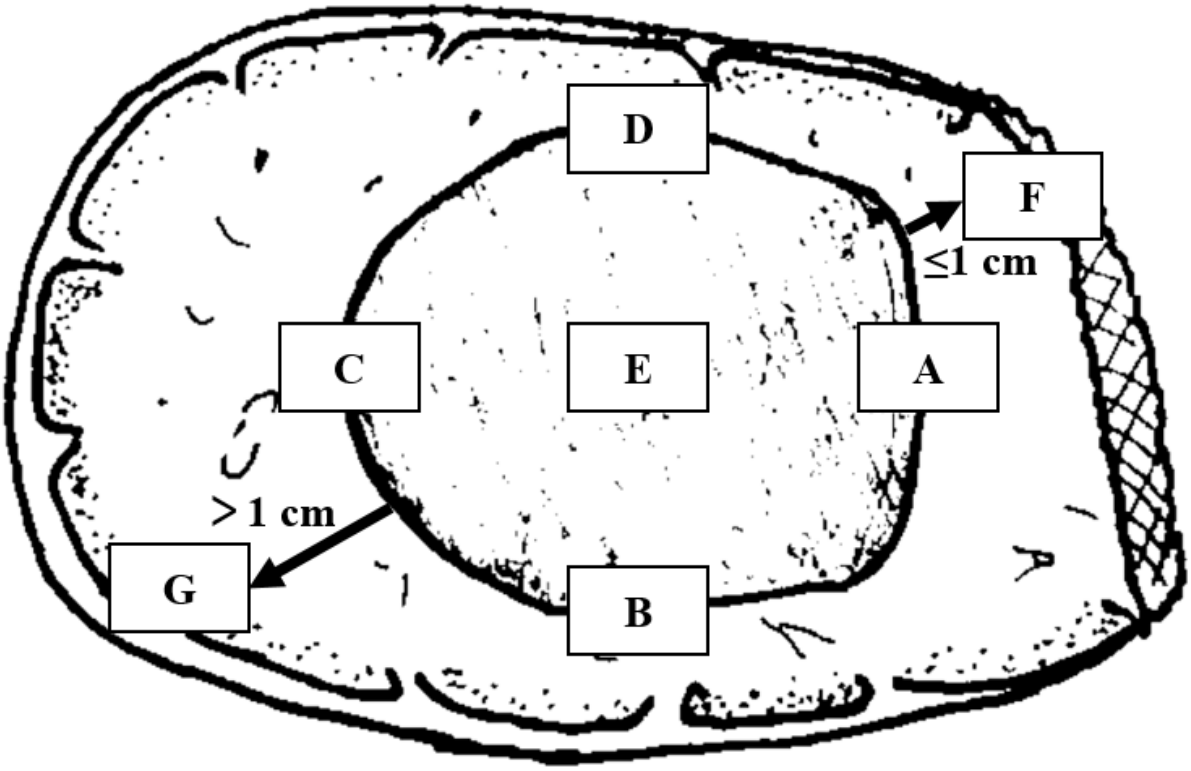

Figure 2. The seven-point baseline sampling protocol. (1) At least four tissue specimens are sampled at the junction of the tumor and adjacent liver tissues at the 3 (A), 6 (B), 9 (C) and 12 (D) o'clock positions; (2) at least one specimen is sampled at the intratumoral zone (E); (3) specimens are sampled from both adjacent peritumoral liver tissues (F, ≤ 1 cm from the tumor capsule) and distant peritumoral liver tissues (G, > 1 cm from the tumor capsule) or the tumor margin.

adjacent (≤ 1 cm from tumor) and distant (> 1 cm from tumor) peritumoral liver tissues or the tumor margins to observe MVI, satellite nodules, dysplastic foci/nodules, and background liver tissues. To perform a molecular pathological examination, another specimen should be sampled at the intratumoral zone, but more specimens should be sampled for tumors harboring different textures or colors (Figure 2)[16]. Based on a recent study, we compared three sampling methods to determine the best strategy to detect MVI. Based on 119 HCC specimens, the MVI detection rate, as determined by the seven-point baseline sampling protocol, was significantly higher than that determined by the three-point sampling method (34.5% *vs*. 47.1%, $P = 0.048$), but was similar to that determined by the thirteen-point sampling method (47.1% *vs*. 51.3%, $P = 0.517$)[17]. Hence, the seven-point baseline sampling protocol is a practical method, presenting the following

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advantages: first, the crucial sampling site is regulated; second, the lower limit of the sampling number is set to suit the working intensity; finally, the evaluation of MVI can be effectively executed through this scheme. However, it should be emphasized that there are limitations in the pathological evaluation of MVI for HCC undergoing narrow margin liver resection, especially for MVI in distant peritumoral liver tissues[18]. Therefore, the 2019 Chinese guidelines of HCC recommend wide margin resection for HCC as far as possible, which not only improves the prognosis but also comprehensively evaluates the MVI[13,19,20]. In summary, we recommend that this sampling scheme should be routinely applied to HCC examination to establish a good foundation for the meta-analysis of future research.

MVI grading and clinical prognosis

Another important concern is how to quantitatively evaluate MVI. Previous studies mostly classified MVI based on its presence or absence, which is irrational. Several scholars have reported that some parameters of MVI indicate distinct prognoses. Roayaie et al. collected clinicopathological data of 131 HCC patients with MVI and observed that whether MVI invaded the blood vessel wall and the distance between MVI and tumor capsule is closely related to recurrence-free survival (RFS) and overall survival (OS). Therefore, patients with MVI were divided into three grades based on these two risk factors. The median OS of patients without risk factors and with one or two risk factors was 72.1 months, 36.9 months, and 8.2 months, respectively (P < 0.001), and the corresponding median recurrence interval was 27.1 months, 19.0 months, and 7.1 months, respectively (P < 0.028)[21]. Fujita et al. reported that patients presenting > two MVI (5-year OS rate: 84.2% *vs*. 57.7%, P = 0.0046; 5-year disease-free survival rate: 31.3% *vs*. 19.5%, P = 0.0053) and > 50 tumor cells per MVI (5-year disease-free survival rate 32.6% *vs*. 17.2%, P = 0.0006) revealed a significantly worse prognosis than those without MVI and with one risk factor[22]. Sumie et al. determined that the number of MVI (none, 1-5, > 5) was an independent risk factor for RFS and disease-specific survival time in patients with HCC after undergoing hepatectomy[23]. Furthermore, some scholars have explored the significance of MVI grading in patients with HCC undergoing liver transplantation, and observed that a combination of > 50 tumor cells per MVI and multiple MVI indicate a poor prognosis (RFS: HR 3.374; 95%CI 1.123-10.131; P = 0.030)[24]. Zhao H et al. divided MVI into three grades based on the number of MVI, the number of tumor cells, and the location of MVI, and observed a good distinction between RFS and OS based on these three parameters[25]. Furthermore, Feng et al. reported that the morphology of MVI can be divided into free, adherent, invasive, and punctured types. Combined with the morphology and number of MVI, the patients with HCC were divided into no, low-, medium-, and high-risk groups after undergoing radical hepatectomy, which can better distinguish the recurrence interval and OS[26]. These conclusions encourage the use of MVI staging classification during HCC diagnosis. Considering the difficulty in evaluating various parameters of MVI, Chinese pathology guidelines recommend risk stratification based on the number and location of MVI. The criteria were as follows: M0, no MVI; M1 (low-risk), MVI < 5 and ≤ 1 cm away from the adjacent liver tissues; M2 (high-risk): MVI of > 5 or > 1 cm away from the adjacent liver tissues (Table 1). Our recent study revealed that, based on 2573 patients with HCC, the 3-year recurrence rates in M0, M1, and M2 MVI groups were 62.5%, 71.6%, and 86.1%, respectively (P < 0.001), and the corresponding 3-year OS rates were 94.1%, 87.5%, and 67.0%, respectively (P < 0.001). M1 grade was associated with early recurrence, while M2 grade was associated with both early and late recurrence, which confirmed that distinguishing M1 from M2 is of great clinical value[17]. Furthermore, some studies have confirmed that this classification can greatly distribute the prognosis of different subpopulations of HCC [27,28]. This MVI grading standard has been strongly linked with the seven-point baseline sampling scheme and is practical for pathologists, which is recommended in the routine diagnosis of HCC.

Table 1. MVI risk classification protocol.

MVI grading	Criteria
M0	No MVI
M1	1-5 MVIs and at ≤1 cm away from adjacent liver tissue
M2	>5 MVIs, or any MVI at >1 cm away from adjacent liver tissue

Note: MVI, microvascular invasion.

Molecular pathology of MVI

As MVI is a microscopic tumor tissue, it is difficult to perform direct molecular pathological studies. Therefore, most current research on the mechanism of MVI focuses on HCC tumor tissues in the presence of MVI. With advancing research, scholars continue to identify molecules that are highly related to the occurrence of MVI. For example, Huang et al. detected that Sox12 upregulation induced by FoxQ1 promotes HCC invasion and metastasis by transactivating Twist1 and FGFBP1 expression[29]. Zhang et al. observed that PRMT1 promoted HCC metastasis *in vitro* and *in vivo* by activating the STAT3 signaling pathway [30]. Krishnan et al. identified that MYC upregulates

fibronectin expression, which promotes HCC invasiveness, reporting that fibronectin is a promising non-invasive proteomic biomarker of vascular invasion in HCC[31]. Qi et al. revealed that S100P overexpression in HCC highly correlates with MVI formation, and hence, S100P could be employed as a potential therapeutic target for HCC metastasis[32]. However, the clinical value of these molecules remains extremely low, and their clinical significance needs to be further verified by large-scale multicenter clinical trials. We believe that these studies have, to some extent, blurred differences between the main tumor and MVI, which indicates these studies reflect the "molecular mechanism of HCC with invasive biological behavior" Therefore, future investigations on the molecular mechanism of MVI should be based on the tumor tissue of MVI isolated by microdissection. This type of tissue is the true MVI tissue, and its biological information is bound to differ from that of other tumor locations.

Conclusions

Standardization of the MVI diagnosis is necessary to promote research cooperation in HCC. We believe that both standard sampling and MVI risk classification schemes are major prerequisites of relevant studies. Once disputes regarding the pathological diagnosis of MVI are settled, academic exchange among investigators assessing MVI would be practicable. Furthermore, the molecular mechanism of MVI remains the focus of pathological research, and there is an urgent need for drug intervention targets to provide a feasible scheme for preoperative cytoreductive and postoperative treatment of MVI. With a deepening understanding of MVI, better management of patients with HCC can be realized to improve survival.

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