Hepatocellular Carcinoma Microvessel Density Quantitation with Image Analysis: Correlation with Prognosis

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Abstract: Hepatocellular carcinoma (HCC) has a progression considered to be dependent on angiogenesis. Intratumoral microvessel density (MVD) has been associated with metastasis and recurrence risk; however, selection bias, counting errors, and lack of standardized assessment criteria have limited the clinical utility of angiogenesis quantitation. Therefore, we analyzed HCC angiogenesis with image cytometry using different methods and determined the correlation to prognosis. Tissue microarrays with 135 HCCs were CD31 and CD34 immunostained and quantitated with the Dako ACIS III Image Cytometer labeling index (LI) and Aperio Scanscope XT and MVD algorithm. LI and MVD were compared to each other and to pathologic features and prognosis (recurrence free survival). Using median cutoffs of microvessel quantitation, survival curve analysis showed a statistically significant difference between CD31 MVD algorithm measurement and prognosis (low MVD mean survival = 56.6 months and high MVD mean = 26.5 months; Log-Rank P = 0.0076). Survival was not significantly related to CD31 LI, CD34 LI or CD34 MVD. By linear regression, a direct correlation was observed between CD31 and CD34 using MVD (r = 0.45, P <0.0001), between CD31 MVD and CD31 LI (r = 0.55, P < 0.0001), and between CD31 LI and CD34 LI (r = 0.51, P < 0.0001). In addition, there was a weak but statistically significant relationship between CD31 MVD and CD34 LI (r = 0.25, P = 0.0050). Together, this data confirms previous studies linking angiogenesis to disease prognosis and suggests the utility of MVD image analysis algorithms.

Keywords: Hepatocellular carcinoma, microvessel density, immunohistochemistry, prognosis.

BACKGROUND

Primary liver cancer is the sixth most common cancer worldwide. According to the American Cancer Society, newly diagnosed cases of liver cancer during 2006 included 18,510 people in the United States, and over 660,000 worldwide. There were 630,000 resultant deaths estimated worldwide in 2009 [1-3]. Since liver cancer is often diagnosed late, it is considered one of the most common causes of death from cancer. The most common type of primary liver cancer is hepatocellular carcinoma (HCC), which is typically a highly vascular tumor, characterized by high potential of vascular invasion and metastases [4]. There are many risk factors for HCC, including cirrhosis from alcohol abuse, hepatitis B or C virus infection, or iron overload (i.e., hemochromatosis), aflatoxins, Wilson's disease, and, less commonly, autoimmune disease [5-8].

The study of angiogenesis, the physiological process involving the growth of new blood vessels in normal organ physiology development and tissue repair, as well as in a variety of pathological processes, is important in the pathogenesis of neoplasia, particularly HCC. When a tumor becomes vascularized, its growth often accelerates [4]. Circulating levels of various angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF) have been found to be significantly elevated in patients with HCC compared with normal controls or patients with benign chronic liver disease [1].

A correlation has been found with higher levels of endothelial markers and advanced tumor stage, vascular invasion, and early recurrence following resection; all of these factors are considered poor prognostic features in patients with HCC. Several studies describe the neovascularization process and the specificity of the endothelial markers in distinguishing normal sinusoids from sinusoid-like tumor vessels in liver. Endothelial markers that have been studied include CD34 and CD31; and although these markers have limitations, they are considered somewhat sensitive and specific for vascular endothelium [9].

Accumulated evidence has demonstrated that intratumoral microvessel density (MVD) is highlighted by expression of endothelial markers essential for angiogenesis, tumor growth, and metastases. In addition, the microvasculature provides a new target for potential therapeutic agents and in guiding surgical and chemotherapeutic treatments [10-12]. However, although many studies show the significance of this intratumoral MVD in the prediction of recurrence risk and prognosis in patients with HCC [6, 13, 14], its clinical utility is limited whether due to significant selection bias or to errors in counting [1].

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The aim of this paper is to analyze the angiogenesis biomarkers (CD31 and CD34) immunohistochemically by image cytometry using both labeling index (LI) and microvessel density algorithms (MVD), and to study their correlation to prognosis, metastases and to each other in patients with HCC.

DESIGN

Study Group

The study group was composed of HCC patients diagnosed at Emory University Hospital between 2000 and 2010 with tissue available in previously constructed tissue microarrays (TMAs). Permission to use the TMAs and to review pathology reports and patient charts was obtained from the Institutional Review Board of Emory University. Criteria for inclusion were: occurrence of HCC with or without coexisting cirrhosis.

Immunohistochemical Study

TMAs were constructed using two 1.0 mm tissue cores from each cancer. TMAs were stained for CD31 (monoclonal JC70A clone, 1:80 titration, Dako Corporation, Carpinteria, CA) and CD34 (monoclonal QBEND clone, 1:320 titration, Dako).

Image Cytometry for Angiogenesis and Mean Vessel Density (MVD)

After immunostaining for CD31 and CD34, TMAs were analyzed by image cytometry using both labeling

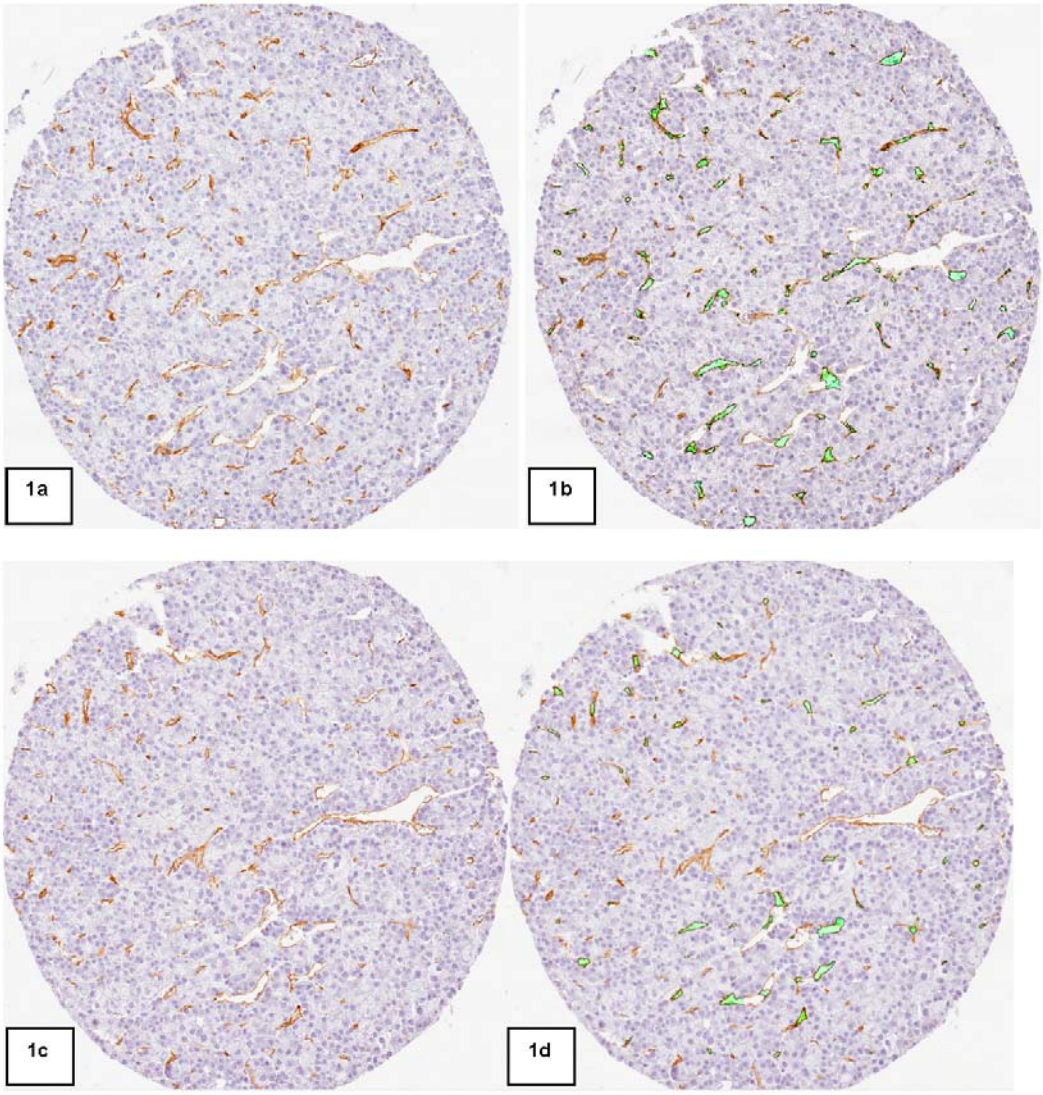

Figure 1: a) Hepatocellular carcinoma tissue microarray stained with CD31 immunohistochemistry. **b**) Markup image generated by the microvessel density algorithm highlighting the microvessels counted in green. **c**) Hepatocellular carcinoma tissue microarray stained with CD34 immunohistochemistry. **d**) Markup image generated by the microvessel density algorithm highlighting the microvessels counted in green.

index (LI) and an intratumoral microvessel density (MVD) algorithm. For the LI, slides were scanned and quantitated with the Dako Automated Cellular Imaging System (ACIS®) III. For MVD, stained TMA slides were scanned with an Aperio Scanscope XT (Aperio Technologies, Inc., Vista, CA) and quantitated with the Aperio MVD. Examples of the MVD quantitation are shown in Figure **1**. LI and MVD for each antibody were compared to each other and to pathologic parameters including tumor size, grade, T-stage, vascular invasion, focality and mitotic rate and to prognosis parameters including metastases, recurrence and survival period by months from the first date of diagnosis.

Statistics

Data collection and preliminary data analysis was performed in Microsoft Excel (Microsoft Corporation, Redmond, WA), and further statistical analysis was performed using SAS JMP software Version 10.0 (SAS Institute, Inc.; Cary, NC). Where appropriate, data from groups were compared using a Student's t test or Pearson's χ^2-test with a P value < 0.05 considered to be statistically significant.

RESULTS

The study group was composed of 135 HCC patients with demographics for the study cohort shown in Table **1**. Criteria for inclusion were: occurrence of HCC with or without coexisting cirrhosis. There were 100 males and 35 females. The median age was 58.7 years. The different causes of chronic liver disease were: chronic hepatitis C virus (HCV) infection (n = 37), chronic hepatitis B virus (HBV) infection (n = 14), hepatitis A (n = 1), alcoholic cirrhosis (n = 13), cryptogenic cirrhosis (n = 50), sclerosing cholangitis (n = 6 cases), autoimmune hepatitis (n= 9), and non-alcoholic steatohepatitis (n= 5).

A comparison between measurements of the microvasculature and various tumoral features (Table **2**) revealed a significant relationship between tumor focality and mitoses/ 10 high power fields. CD31 MVD, CD31 LI, and CD34 LI were significantly related to tumor focality; and CD31 LI was significantly related to mitotic rate/10 high power fields. Other relationships with tumor size, grade, T-stage, vascular invasion, metastases, focality and mitotic rate were not significant.

Table 1: Demographics

Variable	Parameter
Number of cases (#)	135 (male: 100, female: 35)
Age (Mean +/- Standard Deviation)	58.7 +/-12.5
Size of largest tumor (Mean +/- Standard Deviation)	5.3 cm +/- 3.8
T stage (#)	T1 = 52 T2 = 58 T3 = 25
N stage (# of Nodal metastases)	(+)= 6, (-)= 129
M stage (# of Metastases)	(+)=25, (-)=110
Outcome (# of months)	
Alive no disease (Mean +/- Standard Deviation)	71 cases (26.4+/-29.9)
Alive with disease (Mean +/- Standard Deviation)	32 cases (24.6+/-22.3)
Dead no disease (Mean +/- Standard Deviation)	9 cases (20.3+/-24)
Dead with disease (Mean +/- Standard Deviation)	23 cases (2.2+/- 3.0)
Different causes of chronic liver disease:	
▪Chronic hepatitis C virus (HCV) infection	37 cases
▪Chronic hepatitis B virus (HBV) infection	14 cases
▪Hepatitis A	1 case
▪Alcoholic cirrhosis	13 cases
▪Cryptogenic cirrhosis	50 cases
▪Sclerosing cholangitis	6 cases
▪Autoimmune hepatitis	9 cases
▪Non-alcoholic steatohepatitis	5 cases

Table 2: Correlation Between Measurement Methods and Tumoral Features

P values	CD31 MVD	CD34 MVD	CD31 LI	CD34 LI
Size	NS	NS	NS	NS
Grade	NS	NS	NS	NS
T-Stage	NS	NS	NS	NS
Vascular invasion	NS	NS	NS	NS
Metastases	NS	NS	NS	NS
Focality	0.0011	NS	0.02	NS
Mitoses/ 10 HPF	NS	NS	0.02	NS

NS: Non-significant, MVD: Microvessel Density, LI: Labeling Index, HPF: High power field.

Using median cutoffs of MVD and LI to compare to survival rates, there was a significant correlation between the CD31 MVD algorithm and prognosis. Patients with low MVD had a longer recurrence free survival time (mean = 56.5 months) than those with higher MVD (mean = 26.5 months) with Log-Rank *P* = 0.0076 (Figure **2**). There were no statistically significant correlations between CD31 LI, CD34 LI, and CD34 MVD when they were compared with survival rate in patients with HCC (Log-Rank *P* >0.05).

By linear regression, a direct correlation was observed between CD31 and CD34 MVD (r=0.45, P <0.0001), between CD31 MVD and CD31 LI (r = 0.55, P < 0.0001), and between CD31 LI and CD34 LI (r = 0.51, P < 0.0001). In addition, there was a weak but statistically significant relationship between CD31 MVD and CD34 LI (r = 0.25, P = 0.0050) (Figure **3**). Other comparisons between measurement methods were not statistically significant.

DISCUSSION/CONCLUSIONS

Angiogenesis, the formation of new blood vessels from pre-existing vascular beds, supplies the tumor cells with adequate nutrients and oxygen, enabling them to grow, proliferate, and metastasize. These newly formed vessels are structurally and functionally abnormal with characteristics that have an impact on both diagnostic and therapeutic strategies for malignant tumors [11, 12]. In HCC, through a process referred to as capillarization, the sinusoidal arterial blood supply is replaced by capillaries with loss of endothelium and formation of variable amounts of basement membrane [11, 12].

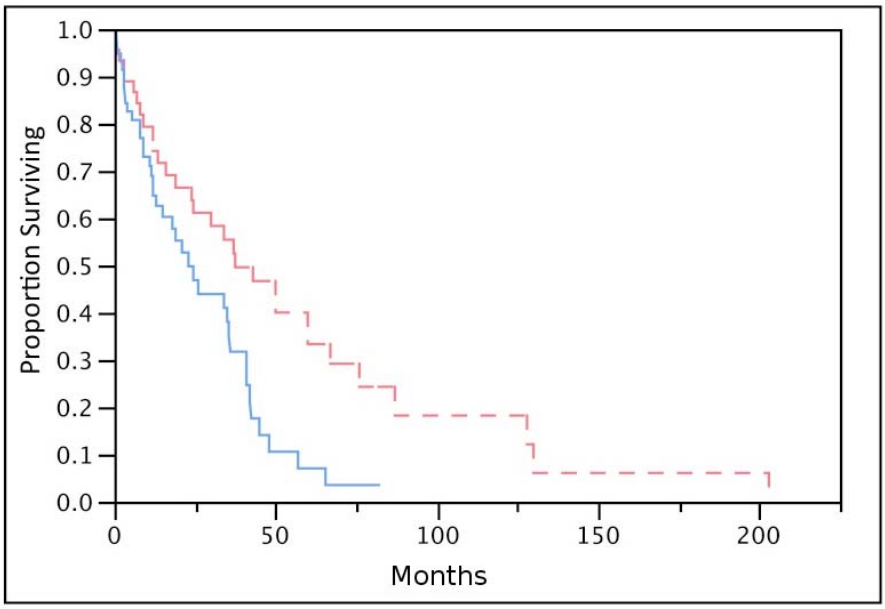

Figure 2: Using median cutoffs of microvessel quantitation, survival curve analysis showed a significant relationship between CD31 MVD algorithm measurements and prognosis (- -: Low MVD with a mean recurrence free survival survival of 56.5 months and —: High MVD with a mean recurrence free survival of 26.5 months, Log-Rank P = 0.0076).

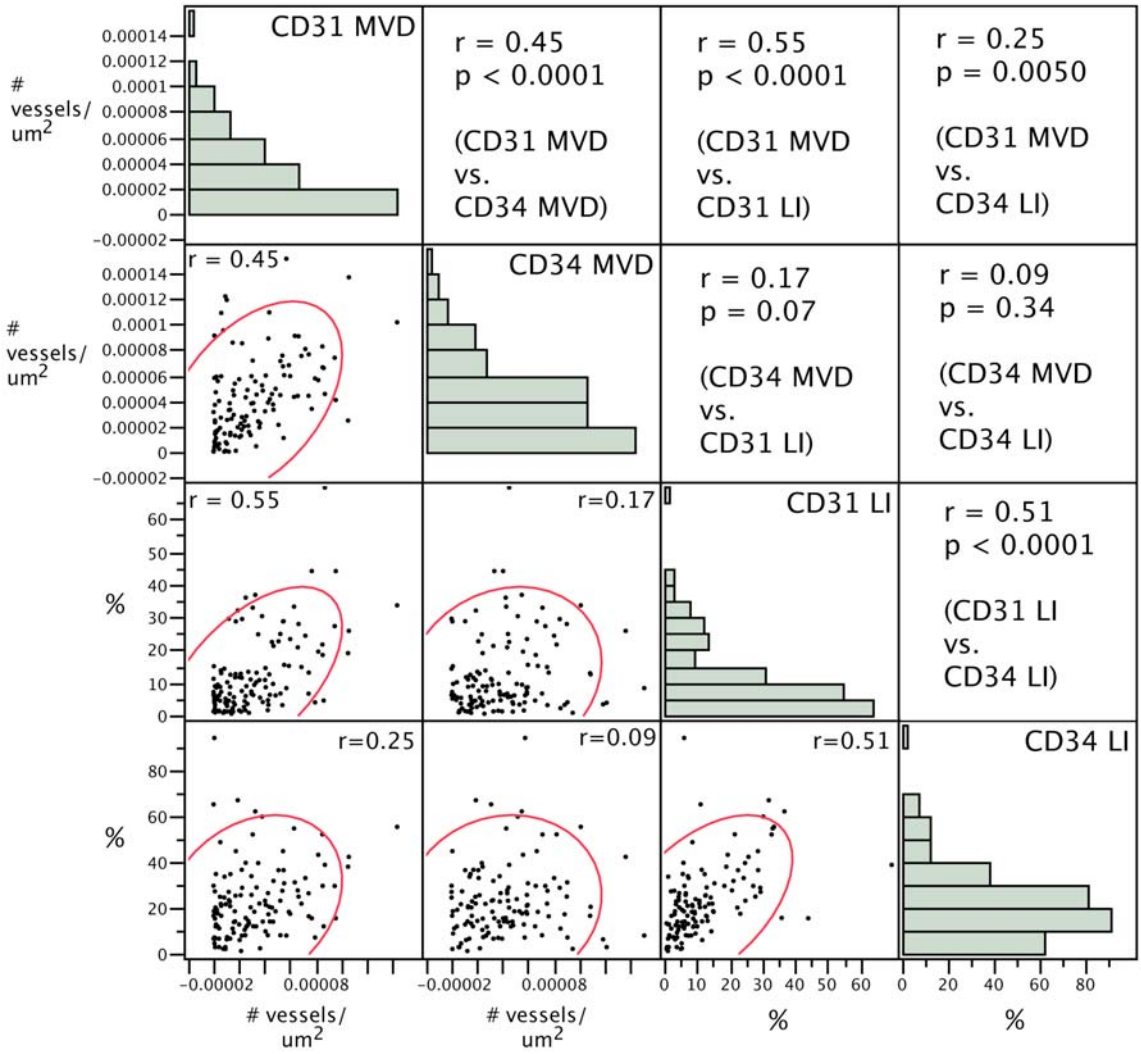

Figure 3: Linear correlation between microvessel density (MVD) measurement methods. Histograms depict the proportion of cases at each level of microvessel density (MVD) and % labeling index (LI). For each linear regression, correlation r values are depicted; and their corresponding p values are depicted in the upper right portion of the graph. Ellipses surround 95% of the cases in each regression.

Endothelial cells are typically positive for CD31 and CD34; therefore, CD31 and CD34 can be used as markers to measure tumor MVD, which is considered one of the most important factors related to rapid growth and metastasis in patients with HCC [9, 15, 16]. Previous similar studies showed this correlation but also showed significant bias errors in counting and lack of standardized assessment, limiting their clinical utility [11, 12].

Our study, using linear regression, showed a significant correlation between LI and MVD algorithm measurements of CD31 and CD34 stains. Only CD31 MVD correlated with prognosis and survival rate in patients with HCC. Patients with hypervascular tumors had a higher risk of recurrence and lower survival rates than those with hypovascular tumors [17-22]. There was no correlation between CD31 LI, CD34 LI, or CD34 MVD and survival rate in patients with HCC. These findings suggest that CD31 MVD is superior to CD34 LI, CD34 MVD, and CD34 LI as a prognostic factor in HCC.

This data confirms previous studies linking angiogenesis to HCC prognosis, specifically the importance of angiogenesis marker CD31 MVD in studying the parameter of survival rate. Angiogenesis is important not only for the rapid growth observed in primary HCC but also for local invasion and metastasis [9, 16]. For this reason, it will be useful to investigate the MVD at the interface between tumor and normal tissue since stromal invasion at the periphery of HCC can be very important in distinguishing dysplastic nodules from fully-developed HCC [23, 24]. Our TMA

approach is limited in investigating this interaction since the TMA samples are typically taken from the central portion of the tumor; therefore, it is possible that MVD assessment can be refined even further in future studies looking at complete sections of the tumor/normal interface. Furthermore, it is possible that the CD31 MVD prognostic predictive ability could improve in the future on clinical resection samples with this tumor/normal interface.

In addition, endothelial markers can be used as candidates for new HCC targeted therapies [25, 26] of significant impact because there is not an existing effective systemic treatment for HCC. Focusing on CD31 receptor-related pathways and other endothelial markers in HCC could potentially be an effective approach to decrease rapid growth and metastasis and for treatment of the primary tumor.

It should be noted that CD31 and CD34 are not perfectly specific for the endothelium. Although CD31 is typically considered a highly sensitive and specific endothelial marker, other markers may be more sensitive in certain situations. For example D2-40 may be more sensitive for vascular invasion but is typically considered a lymphatic marker [27-29]. CD31 immunohistochemistry typically stains hepatic sinusoids [30] but is also positive in platelets and megakaryocytes [31], in macrophages [32], in hematolymphoid cells and plasma cells [33], and in soft tissue fibroblasts and brown fat [34]. CD34 also stains a variety of cells, including most notably a variety of hematopoietic progenitor cells [35] and numerous types of soft tissue cells, including fibroblasts [36]; however CD34 does not usually stain most normal hepatic sinusoids [30]. Therefore, it is possible that prognosis can be predicted with more accuracy in future studies employing antibodies that are more specific for the endothelium. Alternatively, prognostic predictive power may be improved by multiparameter staining able to simultaneously stain for either CD31 or CD34 and other cell types such as inflammatory cells. Multicolor immunohistochemistry or quantum dot staining may be useful in this regard. However, it will take time to develop these staining methods; and the finding of prognostic prediction through CD31 quantitation in this study will hopefully encourage these future studies.

Angiogenesis is already used as a target for novel prognostic and therapeutic approaches in many HCC clinical trials and holds the promise of providing an effective treatment with a significant improvement in the overall diagnosis and outcome for HCC patients [37]. The assessment of CD31 MVD by image cytometry in resection specimens and diagnostic needle biopsies of HCC may prove to be both a future prognostic and predictive marker. Measurements of MVD may also serve a role in theranostics, guiding clinicians in the cases that need further therapy.

CONFLICT OF INTEREST AND SOURCE OF FUNDING

None.

REFERENCES

[1] Park JO, Yeh MM. Clinical significance and implication of neoangiogenesis in hepatocellular carcinoma. J Gastroenterol Hepatol. 2011; 26(5): 792-3. http://dx.doi.org/10.1111/j.1440-1746.2011.06681.x

[2] Kakinoki K, Nakamoto Y, Kagaya T, Tsuchiyama T, Sakai Y, Nakahama T, *et al*. Prevention of intrahepatic metastasis of liver cancer by suicide gene therapy and chemokine ligand 2/monocyte chemoattractant protein-1 delivery in mice. J Gene Med. 2010; 12(12): 1002-13. http://dx.doi.org/10.1002/jgm.1528

[3] El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med. 1999; 340(10): 745-50. http://dx.doi.org/10.1056/NEJM199903113401001

[4] Chen ZY, Wei W, Guo ZX, Lin JR, Shi M, Guo RP. Morphologic classification of microvessels in hepatocellular carcinoma is associated with the prognosis after resection. J Gastroenterol Hepatol. 2011; 26(5): 866-74. http://dx.doi.org/10.1111/j.1440-1746.2010.06511.x

[5] Izumi N, Asahina Y, Noguchi O, Uchihara M, Kanazawa N, Itakura J, *et al*. Risk factors for distant recurrence of hepatocellular carcinoma in the liver after complete coagulation by microwave or radiofrequency ablation. Cancer. 2001; 91(5): 949-56. http://dx.doi.org/10.1002/1097-0142(20010301)91:5<949::AID-CNCR1084>3.0.CO;2-H

[6] Frachon S, Gouysse G, Dumortier J, Couvelard A, Nejjari M, Mion F, *et al*. Endothelial cell marker expression in dysplastic lesions of the liver: an immunohistochemical study. J Hepatol. 2001; 34(6): 850-7. http://dx.doi.org/10.1016/S0168-8278(01)00049-6

[7] Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006; 45(4): 529-38. http://dx.doi.org/10.1016/j.jhep.2006.05.013

[8] Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology 2004; 127(5 Suppl 1): S87-96. http://dx.doi.org/10.1053/j.gastro.2004.09.020

[9] Tanigawa N, Lu C, Mitsui T, Miura S. Quantitation of sinusoid-like vessels in hepatocellular carcinoma: its clinical and prognostic significance. Hepatology 1997; 26(5): 1216-23.

[10] Pang RW, Poon RT. Clinical implications of angiogenesis in cancers. Vasc Health Risk Manag 2006; 2(2): 97-108. http://dx.doi.org/10.2147/vhrm.2006.2.2.97

[11] Poon RT, Ng IO, Lau C, Yu WC, Yang ZF, Fan ST, *et al*. Tumor microvessel density as a predictor of recurrence after resection of hepatocellular carcinoma: a prospective study. J Clin Oncol 2002; 20(7): 1775-85. http://dx.doi.org/10.1200/JCO.2002.07.089

[12] Weidner N. Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol 1995; 147(1): 9-19.

[13] Mitsuhashi N, Shimizu H, Ohtsuka M, Wakabayashi Y, Ito H, Kimura F, *et al*. Angiopoietins and Tie-2 expression in angiogenesis and proliferation of human hepatocellular carcinoma. Hepatology 2003; 37(5): 1105-13. http://dx.doi.org/10.1053/jhep.2003.50204

[14] Mineo TC, Ambrogi V, Baldi A, Rabitti C, Bollero P, Vincenzi B, *et al*. Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. J Clin Pathol 2004; 57(6): 591-7. http://dx.doi.org/10.1136/jcp.2003.013508

[15] Sun HC, Tang ZY, Li XM, Zhou YN, Sun BR, Ma ZC. Microvessel density of hepatocellular carcinoma: its relationship with prognosis. J Cancer Res Clin Oncol 1999; 125(7): 419-26. http://dx.doi.org/10.1007/s004320050296

[16] Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002; 29(6 Suppl 16): 15-8. http://dx.doi.org/10.1016/S0093-7754(02)70065-1

[17] Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. Vasc Health Risk Manag 2006; 2(3): 213-9. http://dx.doi.org/10.2147/vhrm.2006.2.3.213

[18] Weidner N. Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors. Breast Cancer Res Treat 1995; 36(2): 169-80. http://dx.doi.org/10.1007/BF00666038

[19] Yao Y, Kubota T, Takeuchi H, Sato K. Prognostic significance of microvessel density determined by an anti-CD105/endoglin monoclonal antibody in astrocytic tumors: comparison with an anti-CD31 monoclonal antibody. Neuropathology 2005; 25(3): 201-6. http://dx.doi.org/10.1111/j.1440-1789.2005.00632.x

[20] Sumpio BE, Yun S, Cordova AC, Haga M, Zhang J, Koh Y, *et al*. MAPKs (ERK1/2, p38) and AKT can be phosphorylated by shear stress independently of platelet endothelial cell adhesion molecule-1 (CD31) in vascular endothelial cells. J Biol Chem 2005; 280(12): 11185-91. http://dx.doi.org/10.1074/jbc.M414631200

[21] Messerini L, Novelli L, Comin CE. Microvessel density and clinicopathological characteristics in hepatitis C virus and hepatitis B virus related hepatocellular carcinoma. J Clin Pathol 2004; 57(8): 867-71. http://dx.doi.org/10.1136/jcp.2003.015784

[22] Kiss J, Timar J, Somlai B, Gilde K, Fejos Z, Gaudi I, *et al*. Association of microvessel density with infiltrating cells in human cutaneous malignant melanoma. Pathol Oncol Res 2007; 13(1): 21-31. http://dx.doi.org/10.1007/BF02893437

[23] Desmet VJ. East-West pathology agreement on precancerous liver lesions and early hepatocellular carcinoma. Hepatology 2009; 49(2): 355-7. http://dx.doi.org/10.1002/hep.22681

[24] Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology 2009; 49(2): 658-64. http://dx.doi.org/10.1002/hep.22709

[25] Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008; 48(4): 1312-27. http://dx.doi.org/10.1002/hep.22506

[26] Thomas MB, Abbruzzese JL. Opportunities for targeted therapies in hepatocellular carcinoma. J Clin Oncol 2005; 23(31): 8093-108. http://dx.doi.org/10.1200/JCO.2004.00.1537

[27] Saad RS, Lindner JL, Liu Y, Silverman JF. Lymphatic vessel density as prognostic marker in esophageal adenocarcinoma. Am J Clin Pathol 2009; 131(1): 92-8. http://dx.doi.org/10.1309/AJCPKWUQSIPVG90H

[28] Mohammed RA, Martin SG, Gill MS, Green AR, Paish EC, Ellis IO. Improved methods of detection of lymphovascular invasion demonstrate that it is the predominant method of vascular invasion in breast cancer and has important clinical consequences. Am J Surg Pathol 2007; 31(12): 1825-33. http://dx.doi.org/10.1097/PAS.0b013e31806841f6

[29] El-Gohary YM, Metwally G, Saad RS, Robinson MJ, Mesko T, Poppiti RJ. Prognostic significance of intratumoral and peritumoral lymphatic density and blood vessel density in invasive breast carcinomas. Am J Clin Pathol 2008; 129(4): 578-86. http://dx.doi.org/10.1309/2HGNJ1GU57JMBJAQ

[30] Pusztaszeri MP, Seelentag W, Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society 2006; 54(4): 385-95. http://dx.doi.org/10.1369/jhc.4A6514.2005

[31] Hoda SA, Resetkova E, Yusuf Y, Cahan A, Rosen PP. Megakaryocytes mimicking metastatic breast carcinoma. Archiv Pathol Lab Med 2002; 126(5): 618-20.

[32] McKenney JK, Weiss SW, Folpe AL. CD31 expression in intratumoral macrophages: a potential diagnostic pitfall. Am J Surg Pathol 2001; 25(9): 1167-73. http://dx.doi.org/10.1097/00000478-200109000-00007

[33] Govender D, Harilal P, Dada M, Chetty R. CD31 (JC70) expression in plasma cells: an immunohistochemical analysis of reactive and neoplastic plasma cells. J Clin Pathol 1997; 50(6): 490-3. http://dx.doi.org/10.1136/jcp.50.6.490

[34] Rosso R, Lucioni M. Normal and neoplastic cells of brown adipose tissue express the adhesion molecule CD31. Archiv Pathol Lab Med 2006; 130(4): 480-2.

[35] Hassanein NM, Alcancia F, Perkinson KR, Buckley PJ, Lagoo AS. Distinct expression patterns of CD123 and CD34 on normal bone marrow B-cell precursors ("hematogones") and B lymphoblastic leukemia blasts. Am J Clin Pathol 2009; 132(4): 573-80. http://dx.doi.org/10.1309/AJCPO4DS0GTLSOEI

[36] Hirose T, Tani T, Shimada T, Ishizawa K, Shimada S, Sano T. Immunohistochemical demonstration of EMA/Glut1-positive perineurial cells and CD34-positive fibroblastic cells in peripheral nerve sheath tumors. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2003; 16(4): 293-8. http://dx.doi.org/10.1097/01.MP.0000062654.83617.B7

[37] Bishayee A, Darvesh AS. Angiogenesis in hepatocellular carcinoma: a potential target for chemoprevention and therapy. Curr Cancer Drug Targets 2012; 12(9): 1095-118.

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