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CD105/Ki67 double immunostaining expression in liver metastasis from colon carcinoma

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Abstract

The liver is the most common and critical site for the development of colon cancer metastases. Tumor angiogenesis in liver metastasis from colon carcinoma is a controversial subject. Liver microenvironment, immunophenotypical and morphological particularities of hepatic vessels are only few aspects, which establish difficulties in quantification of tumor vascularisation from liver metastasis. The aim of this work is to study the distribution of CD105 positive vessels and the proliferation rate of endothelial cells from liver metastasis of colon carcinoma based on double immunostaining CD105/Ki67. In liver metastasis from well-differentiated adenocarcinoma we found a high number of CD105+/Ki67- vessels. On the other hand, in liver metastasis from poorly differentiated adenocarcinoma we noticed rare CD105+/Ki67+ vessels. It is hypothesized that neoangiogenesis of liver metastasis is performed through intussusceptive mechanism rather than sprouting and could be supported by the presence of kissing phenomenon, CD105 positive transcapillary pillars and the absence of endothelial cells proliferation in this vessels. We conclude that in liver metastasis principal mechanism of neovascularisation formation is based on intussusception.

Keywords: angiogenesis, intussusceptions, colon carcinoma, liver metastasis.

Introduction

CD105 (endoglin), 180 kD transmembrane glycoprotein, is a receptor for TGF beta 1, molecule which is overexpressed in tumor neoangiogenesis [1]. Endoglin (CD105) is predominantly expressed on activated endothelial cells, which are involved in tumor angiogenesis. Weak and negative expression of CD105 was noticed in vascular endothelium from normal tissue [2, 3]. Also, CD105 is weakly expressed on non-endothelial cells of different histotypes, like activated monocytes, macrophages, fibroblasts, melanocytes and vascular smooth muscle cells [4, 5]. CD105 expression is strong and restricted to capillary endothelial cells in cancer lesions (>73%). In contrast, expression of CD105 was seen in less than 20% of non-cancerous areas in the same organs. CD105 is specifically expressed during tumor angiogenesis of brain, lung, breast, stomach, and colon cancers [6].

Ki67 expression is strictly associated with cell proliferation. During interphase, Ki67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0). Ki67 is an excellent marker to determine the growth fraction of a given cell population [7].

Three different growth patterns (replacement, pushing and desmoplastic) for liver metastasis of colorectal and breast cancers have been described [8, 9]. During replacement growth, the architecture of the liver is preserved and the endothelial cells of sinusoids show low proliferative activity. Pushing and desmoplastic types of growth disturb the liver architecture. In the pushing growth pattern, severely compressed liver parenchyma is present at the surface metastases; a fibrous capsule develops at the tumor-liver parenchyma interface in the desmoplastic growth pattern.

Several different mechanisms of angiogenesis do exist in primary tumors and metastases: capillary sprouting, intussusceptive angiogenesis, vessel incorporation, and glomeruloid body formation [10]. Tumor induced angiogenesis depends on both tumor type and site of tumor growth. The mechanism of metastasis neovascularisation, origin and types of vessels from liver metastasis of colon carcinoma are controversial and insufficiently clarified.

Materials and Methods

In the present paper, we investigated the activation

and proliferation of endothelial cells by using 12 liver biopsies from patients who present metastasis from previous diagnosis colon carcinoma.

Resected liver specimens were fixed in 10% buffered formalin for 48 hours and paraffin embedded. Five micrometers thick serial sections were performed from each paraffin block and sections were mounted on silanized slides.

Sections from each case were stained with routine Hematoxylin and Eosin (HE) method for histopathologic evaluation.

Immunohistochemical study included a double stain method for colocalization of CD105 and Ki67 markers on the same section.

On dewaxed and rehydrated slides, we performed endogenous peroxidase blocking with 3% hydrogen peroxide for 5 minutes followed by pretreatment with Proteinase K for 15 minutes at room temperature. Incubation with CD105 primary antibody, clone SN6h from Dako Cytomation for one hour (dilution 1:10) preceded the first application of Advance working system and visualization with 3,3'-diaminobenzidine as chromogen.

Automated method with PT Link module of heat induced epitope retrieval in citrate buffer pH 6 (Dako Cytomation) was used for 30 minutes to unmask Ki67 epitope.

After 30 minutes incubation with Ki67 ready to use antibody (clone MIB1, Dako), the LSAB+-HRP working system was used, followed by visualization with AEC chromogen for 10 minutes. Counterstain was performed with Lillie's modified Hematoxylin.

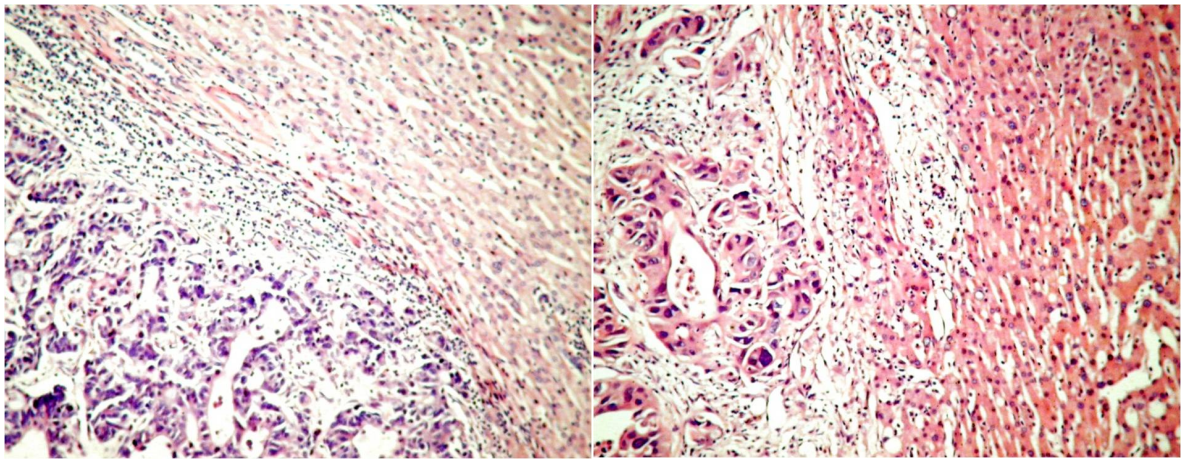
The entire immunohistochemical procedure was performed with DakoCytomation Autostainer.

We quantified vascular structures with lumen, positive for CD105 as a brown staining with cytoplasmic distribution in endothelial cells. Ki67 positive red signals with nuclear distribution were counted in both CD105 endothelial positive cells from activated vessels and tumor cells.

Results

Histopatological evaluation based on routine HE method revealed eight cases of liver metastasis from well-differentiated adenocarcinoma of colon, two cases of liver metastasis from moderately differentiated adenocarcinoma and two cases of liver metastases from poorly differentiated adenocarcinoma.

In liver metastasis from well-differentiated adenocarcinoma (Figure 1a) glands are well formed and show a distinct resemblance to adenomatous epithelium compared with moderately differentiated adenocarcinoma where the glands are less regular. In liver metastasis from poorly differentiated adenocarcinoma cells are arranged in irregular clusters, with little evidence of glandular differentiation (Figure 1b).



(a) **(b)**

Figure 1 – ***(a) Liver metastasis from well-differentiated adenocarcinoma of colon (HE stain, ob. ×10). (b) Liver metastasis from poorly differentiated adenocarcinoma of colon (HE stain, ob. ×10).***

We notice an intense CD105 positive reaction in liver sinusoids from peritumoral area in liver metastasis rise from all three types of adenocarcinoma (Figure 2a).

In liver metastasis from well-differentiated adenocarcinoma, we found a high number of CD105+/Ki67- vessels (Figure 2b).

Concerning liver metastasis from poorly differentiated adenocarcinoma we notice rare CD105+/Ki67+ vessels. There were maximum four vessels for each tumoral area, with low caliber, situate to the periphery of tumoral area. In cases which this type of vessels we found correlation with proliferation rate of tumoral cells and we noticed invasion of proliferative cells in CD105/Ki67 positive vessels.

Sprouting mechanism of endothelial cells is very low and neoangiogenesis of liver metastasis is realized through intussusceptive mechanism. This hypothesis could be supported by presence of kissing phenomenon, characterized by creation of contact zone between opposite capillary walls (Figure 2c) and CD105 positive transcapillary pillars (Figure 2d). In vessels with this peculiarity, we notice the absence of endothelial cells proliferation.

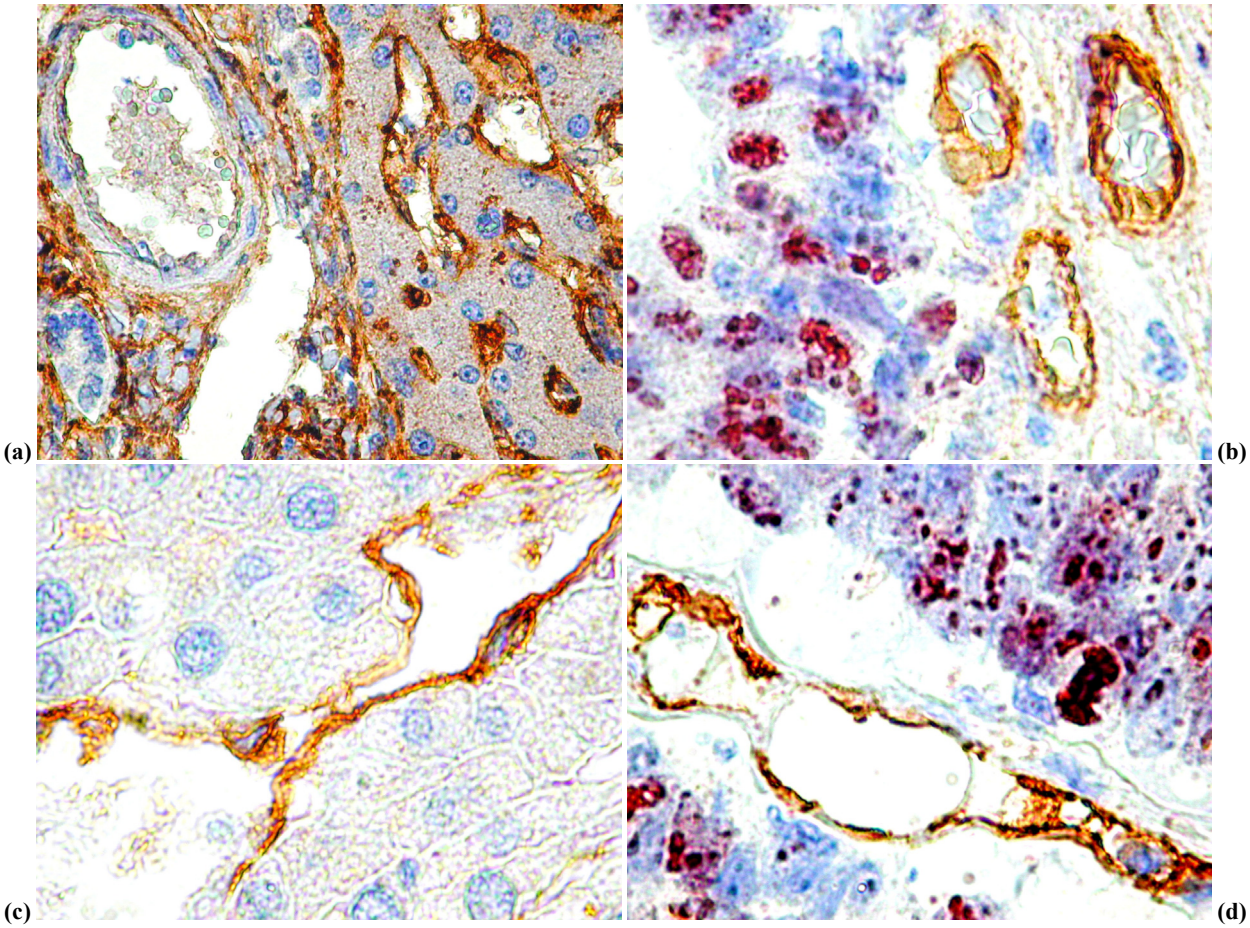


Figure 2 – ***(a) CD105 intense expression in liver sinusoids from peritumoral area, double immunostaining CD105/Ki67, ob. ×40. (b) CD105+/Ki67- vessels in liver metastasis from well differentiated adenocarcinoma, double immunostaining CD105/Ki67, ob. ×40. (c) Kissing phenomenon, double immunostaining CD105/Ki67, ob. ×100. (d) CD105 positive transcapillary pillars, double immunostaining CD105/Ki67, ob. ×100.***

Discussion

Identification of newly formed vessels is still one of the most controversial problems of the angiogenesis study because of the lack of specific markers for tumor vessels. Saad RS *et al.* (2004) [3] showed that endoglin is expressed with high sensitivity intra and peritumoral, but blood vessels from non-neoplastic tissues do not express, or present a weakly expression of CD105. This is supported by previous studies, which showed that endoglin was specifically expressed in proliferating blood vessels, while CD34 was positive in all blood vessels [1, 11–13]. This suggesting that endoglin is expressed preferentially in the vessels involved in neoangiogenesis. CD105 is expressed predominantly in angiogenic endothelial cells and is overexpressed in hypoxic conditions. CD105 is highly expressed on activated endothelial cells and is involved in vascular remodeling and development. It was demonstrated that CD105 is a marker of angiogenesis better than CD34 in malignant melanoma, breast cancers and colorectal carcinomas [14].

CD105 was negative in vascular endothelial cells from normal liver sections, but was intensely positive in hepatocellular carcinoma [15]. In normal liver, CD105 was highly expressed in both sinusoidal and portal endothelial cells of the non-cancerous liver tissue [6].

The use of markers with high specificity for activated tumor vessels combined with proliferative marker could partially resolve the problem of newly formed blood vessels identification. Double immunostaining with CD105 and Ki67 represent an alternative, which is performed in a limitates number of tumor types until now. Using CD105/Ki67 double immunostaining, Grisanti S *et al.* (2003) [16] noticed that endoglin expression in endothelial cells of choroidal neovascularization membranes is increased, but rarely associated with a concomitant expression of the proliferation marker Ki67.

Double stain for CD105/Ki67 applied for benign and malignant lesions of the uterine cervix showed that these two markers had divergent expression on endothelial cells from associated tumor blood vessels dependent of lesion type and proliferation status of tumor cells. Divergent expression of CD105/Ki67 was correlated with histopathology of the uterine cervix lesions and tumor proliferative status. Lack of endoglin expression in vessels from invasive carcinoma together with a higher rate of endothelial proliferation in these vessels demonstrates that activation of blood vessels linked to CD105 expression is an early event in pre-malignant lesions of the uterine cervix while endothelial proliferation is often associated with cervical invasive malignant lesions [17]. In liver metastasis from well-

differentiated adenocarcinoma we noticed presence of numerous CD105+/Ki67- vessels. CD105+/Ki67+ vessels were found in liver metastasis from poorly differentiated adenocarcinoma.

Paku S *et al.* (2005) [10] demonstrated that vascularisation of liver metastasis is a heterogeneous process, depending on the degree of tumor differentiation or localization of the metastases within the liver. Concerning this aspect, despite low number of cases, we noticed minor differences between liver metastasis from well and poorly differentiated adenocarcinoma also. In an experimental model of liver metastasis of Lewis, lung carcinoma identified two types of angiogenesis: sinusoidal type, with tortuous vessels without basement membrane and portal type with high microvascular density and basal membrane positive reaction [18]. The first type was the most frequent; tumor cells are located among the hepatocytes and sinusoidal endothelial cells. In metastatic liver of colic cancer in rats has been reported presence of sinusoidal endothelial cells at the periphery of metastastatic area and invading tumor with increasing lesion diameter [19] These observations suggest that the sinusoidal endothelial cells are precursors of tumor vessels.

Djonov V *et al.* (2000) [20] demonstrated that intussusceptive angiogenesis does not require intense local proliferation of endothelial cells. Central process of this phenomenon is transcapillary pillar formation. We also found this appearance in liver metastasis – the existence of high number CD105 positive transcapillary pillar and absence of endothelial proliferation in vessels with this phenomenon.

Conclusions

Our data suggest that in liver metastases of colon carcinoma occurs activation of peripheral tumor vessels, which had a low proliferation index. Reduced proliferation and numerous vessels with intraluminal pillars highlighted on immunohistochemical stains suggest that the main mechanism of neovascularisation formation in liver metastases is intussusception rather than sprouting type.

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