新潟県厚生連医誌　第9巻　1号　13～17　1999

Original Article

Immunohistochemical identification of lymphovascular invasion with antibodies against endothelium-specific antigens －von Wiellebrand's factor (vWF, factor VIII), CD31, and CD34－

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Background

Lymphovascular invasion was one of the most important histological prognostic factors in cancer. The endothelium was immunohistochemically confirmed with antibodies against endothelium-specific antigens: von Willebrand's factor (vWF, factor VIII), CD31, and CD34, but each of which could not differentiate venous vessels from lymphatic ones. It remains to be determined which reagent is the most reliable.

Methods

Several cases of well differentiated adenocarcinoma of both breast and colon were analyzed immunohistochemically with reagents against vWF, CD31, and CD34 (Dako, Denmark). Each routinely processed section contained both normal tissues and carcinoma foci, including primary organs and regional lymph nodes.

Results

The positive staining was confirmed diffusely on cytoplasmic membrane of endothelium. The vWF stain was the most intense of all and the CD31 positivity was the most weak. The intensity of CD34 staining was medium. In cancerous foci several vessels were negative even with vWF reagent. Because CD34 reagent also stained the intervening immature fibers, the circularly arranged reactants were sometimes regarded as vessels. It was, furthermore, frequently difficult to differentiate the lymphovascular involvement from the intraductal tumor involvement with either periductal or periadnexal positivity for CD34.

Conclusions

vWF reagent very certainly stains the endothelial surface and it is very useful to identify the lymphovascular invasion clinicopathologically.

Key words: lymphovascular invasion, immunohistochemistry, von Wiellebrand's factor (vWF, factor VIII), CD31, CD34

Introduction

Lymphovascular invasion was one of the most reliable factors in prognosis of cancer. Lymphovascular vessels consisted of lymphatic vessels and venous vessels. Recently cancer become regarded as systemic disease even in early stage and micrometastasis has been surveyed through PCR-RFLP (polymerase-chain reaction-retraction fragment length polymorphism). Lymphovascular invasion by cancer cells was routinely judged only with Hematoxylin-Eosin (HE) stained sections. Lymphovascular lumen was identified by their free space. Venous vessels contained erythrocytes but

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lymphatic vessels lacked them. The criteria of lymphovascular invasion was the combination of both intraluminal free space and corresponding casted tumor cells, which might be regarded as the artefact and produced by the tisssue contraction difference in the process of tissue fixation and embedding. This included the probability of false judgement in lymphovascular invasion, which resulted in decreasing the relationship among lymphovascular invasion, lymphatic nodal metastasis, and prognosis of cancer.

For ruling out the ambiguous venous vascular invasion in HE stain, every our cancerous specimens have histochemically been studied to identify the vascular invasion with Elastika-van Gieson method (EVG), which stains vascular elastic laminae black and makes their vascular invasion reliable. (Fig. 1) (1) This additional staining was very troublesome in routine clinicopathologic works, but this step should not be abbreviated for the decrement of false negative cases because we could find more vascular-positive cases after the application of EVG stain. In carcinoma of breast there were many EVG-positive elastic-fibered aggregates mistaken for venous vascular invasion as well: e.g. reactive elastosis with compression aggregate, periductal elastosis with ductal cancer involvement, periadnexal elastosis with adnexal cancer involvement, and so on. (Fig. 1) This required other confirming techniques of venous invasion in carcinoma admixed with either tubules or adnexae. New identifying method for lymphatic vessels was also needed because the lymphatic invasion was frequently too small to confirm.

The vessels were internally lined with endothelium. The endothelium was immunohistochemically confirmed with antibodies against endothelium-specific antigens: von Willebrand's factor (vWF, factor VIII), CD31, and CD34, each of which could not differentiate venous vessels from lymphatic ones. In this article we studied these stainabilities to identify the most reliable reagent for the confirmation of lymphovascular invasion in routine clinicopathological works.

Materials and Methods

Specimens

132 cases of well differentiated adenocarcinoma of both breast and colon were analyzed. These sections contained both normal tissues and carcinoma foci, including primary organs and regional lymph nodes. Each specimen was also used for normal controls as venous vessels and lymphatic ones. The extirpated specimens were fixed in formalin for two days and processed in the conventional manner. The latent period between extirpation and staining was less than one week and their antigenicity would not weaken.

Methods

Immunohistochemistry was done with reagents against vWF, CD31, and CD34 (DAKO, Denmark), and compared with EVG-stained results. Their surrounding normal tissues were also available for control. Routinely-processed paraffin-embedded

Fig. 1. Lymphovascular histochemistry by Elastika-van Gieson stain

characteristic	reagents Weigert resorcin-fuchsin	van Gieson picrinc acid	acid fuchsin
elastic fiber	black		
muscular fiber		yellow	
collagen fiber			red
colon	vascular wall		
	elastosis in ulcer		
breast	vascular wall		
	mesenchyma		
	peri-duct and adnexa		

sections were preincubated by heat pretreatment with a microwave to enhance their antigenicity. After the reaction with primary antibodies routine peroxidase-anti-peroxidase reaction was performed. (1)

Results

vWF immunoreactant was strongly and diffusely positive on endothelial cytoplasmic membrane with the partial negativity in tumor vessels. (Fig. 2) There were many negative vessels with CD31 immunostaining. (Fig. 2) Endothelial immunopositivity with CD34 was almost similar to that with vWF and, moreover, other mesenchymae were stained with CD34: e.g. dispersing mesenchymal fibers, periduct, periadnexa, and so on. (Fig. 2) The intensity of immunoreactants was slightly weaker in tumors than in normal controls. There was no staining difference among organs.

Discussion

The vessels were internally lined with endothelium. The endothelium was immunohistochemically confirmed with antibodies against endothelium-specific antigens: von Willebrand's factor (vWF, factor VIII), CD31, and CD34. Reaction was limited on the cell surface. CD31 immunopositivity was the weakest of all. CD34 reagent also crossreacted with non-vascular immature fibrous elements in addition to vascular endothelium. (Fig. 3) vWF reaction was the most intense among reagents and restricted in endothelium. vWF is the most reliable marker for endothelium in this study.

The endothelium was the common intima of both venous and lymphatic vessels, so each of endothelium-specific reagents could not differentiate venous vessels from lymphatic ones. vWF-positive lymphovascular vessels was provisionally differentiated into veins and lymph vessels according to the presence of erythrocytes in the lumen: the

Fig. 2. Immunohistochemistry with endothelium-specific reagents

characteristic	vWF, factor VIII	CD31	CD34
lymphovascular			
endothelium	+~±	±~−	+~−
mesenchymal			
immature fiber/mesenchymal	−	−	+
peri-ductal and adnexal			

Fig. 3. Immunohistochemistry from commercial reference (Review)

characteristic	vWF, factor VIII	CD31	CD34
lymphovascular			
endothelium	+	+	+
hemangioma	+	+	+
hematopoietic			
stem cell			+
dendritic cell			+
leukocyte, monocyte, platelet	+	+	
mesenchymal			
immature fiber/mesenchymal*			+
solitary fibrous tumor, stromal tumor of GI tract, leiomyoma, schwannoma, epithelioid sarcoma			+

* including periadnexal or periductal aggregate.

former contained erythrocytes and the latter lacked ones.

Acknowledgment

Grateful acknowledgement is made to technicians in Byori Center for their immunohistochemical staining.

Referrences

1) Immunohistochemistry. In Obstetrical and Gynecological Pathology ABC. ed. Ikarashi T, 10th ed, §I-E-e-3 (histochemistry in connective tissue), I-E-g-6-2-2 (immunoreactive antibody), Pimento Press, Nagaoka, 1997. (for Windows 95, CD-R)

原　著

血管内皮細胞特異抗体による癌の脈管侵襲の免疫組織化学的同定—von Wiellebrand因子（vWF、第8因子)、CD31、CD34—

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癌症例における脈管侵襲の判定において、従来より、われわれは、エラスチカ・ファン・ギーソン染色による血管弾性板の同定に基づいて診断してきた。乳癌並びに一部の臓器、特に、組織構成として付属器や導管を含む臓器の癌においては弾性線維が豊富な為、従来のエラスチカ・ファン・ギーソン染色のみによる弾性線維の同定だけでは脈管の同定が不可能である。血管内皮細胞特異抗体von Wiellebrand因子（vWF，第8因子）による血管内皮層の同定は脈管の同定に有効であり、脈管侵襲の判定に有意義であると判断された。現時点においては、内皮細胞の同定のみでは静脈とリンパ管の鑑別はできず、リンパ静脈管を合わせた脈管侵襲としてしか臨床病理組織学的報告ができない。

キーワード：脈管侵襲、免疫組織化学、von Wiellebrand's factor (vWF、factor VIII)，CD31，CD34

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