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Two Types of Lymphatic Invasion in Lymph Node Metastasis with Special References to the Morphology of Gastric Carcinomas and Immunohistology of E-Cadherin and β-Catenin

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Two types of lymphatic invasion were distinguishable in lymph nodes (LN) with metastasis of a gastric carcinoma (GC). One was an embolic (E) type of lymphatic invasion that was characterized with embolus of carcinoma cells in collecting lymphatics. Infiltration of carcinomas as single cells or as tiny clusters through lymphatic capillaries were features of the other infiltrative (I) type of lymphatic invasion and were found in the interstitial spaces of peri-lymphnodal fat tissues. The latter was an early event during metastasis, even when not yet apparent in LN. Both types of lymphatic invasion coexisted, not infrequently, in advanced stages, but pure I type occurred exclusively in diffuse type GC ($\chi^2 < 0.005$), regardless of the existence of metastasis in LN. E type was more frequently encountered solely or predominantly in intestinal type GC ($\chi^2 < 0.05$). Of the destructive lesions of collecting lymphatics, fibrous occlusion or stenosis and large emboli were seen in intestinal type GC ($\chi^2 < 0.005$). Early I type lesions were more seen in diffuse type GC ($\chi^2 < 0.005$). The incidence of destruction of collecting lymphatics by carcinoma cells from outside and the existence of single cells in collecting lymphatics were seen more in diffuse type GC, but not significantly. Immunohistology revealed that E-cadherin (Ec) and β-catenin(βc) were expressed positively in cytoplasm of carcinoma cells of diffuse GC at 50.0% and 78.5%, respectively. However, βc of carcinoma cells exhibiting I type of lymphatic invasion were expressed negatively or much less. The mechanism of I type of lymphatic invasion, its low expression of βc, and the heterogeneity of GC were discussed. It is necessary to clarify the I type of lymphatic invasion to gain improvements in curability.

Lymphatic invasion connotes an embolus forming metastasis through the lymphatics and destructive lesions of the lymphatics themselves, and is a prognostically influenced factor[15,17,19,23)]. Carcinoma cell emboli occur in the lymphatic capillaries of primary sites, flow in the collecting lymphatics, enter the lymph node (LN) via afferent or efferent lymphatics (retrograde metastasis[5)]) , are deposited in the sinus, proliferate and spread over

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all parts of a LN. This mode of LN metastasis as developed by lymphatic invasion is noted here as E type, of which the severest type is called lymphangiosis carcinomatosa[5]. Another mode of lymphatic invasion was distinguishable histologically. Early metastasis did not always give rise in the LN but in peri-lymphnodal fat tissues. Later this mode of metastasis coexisted in LN or independently, even when LN metastasis was absent. This mode of lymphatic invasion is noted here as I (infiltrative) type. During the metastatic route through the collecting lymphatics, several modes of destructive changes of the collecting lymphatics were seen in both types of lymphatic invasion. The incidences of alteration were different according to whether it was E or I type lymphatic invasion, and also to the type of gastric carcinoma (GC).

Serially connected adhesion molecules, E-cadherin (Ec) and β-catenin (βc), determine the morphology of GC, and 13 % of diffuse GC were shown to have mutations with defects of exon 9 of Ec[1]. Also, phosphorylation of βc by tyrosine kinase type growth factor receptor has been said to cause carcinoma cell isolation[24]. There is evidence of the role of βc on carcinogenesis, which represents the phenomenon as an accumulation in the nucleus or cytoplasm in some carcinoma[7].

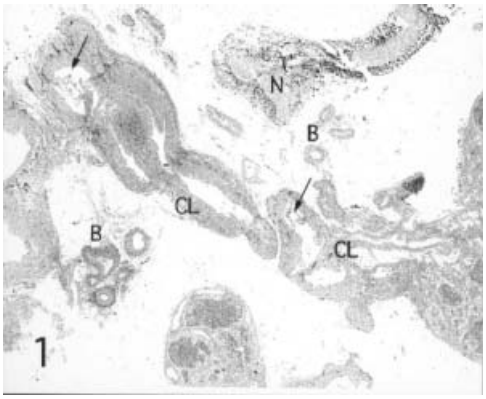
One purpose of this study was to elucidate whether there were relationships of each type of lymphatic invasion to any special type of GC, and the accompanying destruction of the collecting lymphatics or not. Another purpose was to examine the differences in the adhesion molecules that are involved in the two types of lymphatic invasion. To our knowledge, no reports of I type lymphatic invasion in diffuse type GC, from such a standpoint, have been published.

MATERIALS AND METHODS

Extirpated metastatic LNs, which were accompanied with many collecting lymphatics, were histologically investigated in 100 cases of GC (Kagawa Medical University Hospital and Saiseikai Tondabayasi Hospital 1991-1999). Immunohistology of Ec and βc were carried out on 18 specimen of 14 cases of diffuse GC with I type lymphatic invasion in formalin fixed paraffin embedded specimen. Morphology of GC was classified with modification into 8 categories according to the criteria defined by The Japanese Classification of Gastric Cancer Association 1999. These were poorly differentiated adenocarcinoma, solid (por 1), non-solid, scirrhous (por 2), moderately differentiated tubular adenocarcinoma, scirrhous (tub 2, sci), and nonscirrhous (tub 2), and well differentiated tubular adenocarcinoma (tub 1), papillary adenocarcinoma (pap), signet ring cell carcinoma (sig) and mucinous carcinoma (muc). Tub 1, which we investigated here, were all scirrhous (tub 1, sci). When two or three categories coexisted, the predominant type was adopted. If The Japanese Classification was applied to that of Lauren[4,6,16], the diffuse type was equivalent to por 2 and sig, intestinal type to por 1, tub 2, pap and muc and mixed type tub 2, sci and tub 1, sci.

Some difficulties existed in discriminating collecting lymphatics from veins to identify emboli and destructive changes of the collecting lymphatics. Lymphatic vessels were divided into 3 categories[14]. One was lymphatic capillaries with few smooth muscles and anchoring filaments. Another was medium sized muscular lymphatics called collecting lymphatics. They had attenuated endothelium and valves and their luminal shapes were circular, stellate or cruciate[2]. The third category was ductus thoracicus. Collecting lymphatics, including afferent and efferent lymphatic vessels, were along with small arteries and veins in the hilum (Fig. 1) of LN, and compared with blood vessels but were discriminated by the following histological features: in collecting lymphatics and lymphatic capillaries, an absence or scarcity of red blood cells, the existence of valves, thin and a sparseness of endothelial cells,

and an uneven thickness of muscular layers with portions of thin walled areas that were merged into lymphatic capillaries and parted from the interstitial spaces with thin walls (Fig. 2), or rarely opened. Silver impregnated fibers traversed irregularly between muscles, with a lack of thick adventitial bundles being found in veins. FactorⅧ related antigen (FⅧRA) and CD31 (PECAM: platelet endothelial cell adhesion molecule) of the lymphatic endothelium were stained much less than blood vessels. Lymphatic capillaries were also discernible immunohistologically from blood capillaries and were only visible by the extension of anchoring filaments, when dilated by carcinoma cells emboli or an edematous state. Immunohistology was undertaken to elucidate how cell adhesion molecules, Ec and βc were expressed and involved with what type of lymphatic invasion. Antibodies utilized for immunohistology by ABC methods were FⅧRA (polyclonal, DAKO, 50X), CD31 (monoclonal, DAKO, 3 X with trypsin digestion), laminin (polyclonal, Bioscience, 50 X with trypsin digestion), E-cadherin (Takara Co, Kyoto, Japan, 4 ℃ overnight), β-catenin (Zymed, Ca. USA, 4 ℃ overnight).



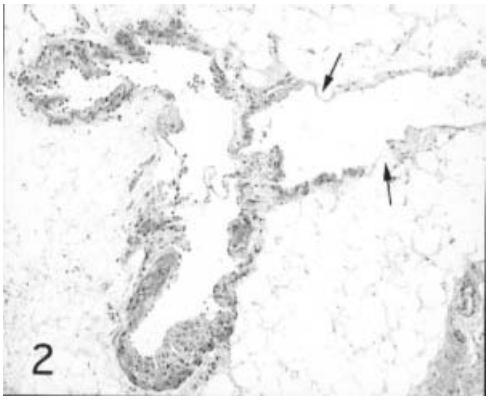


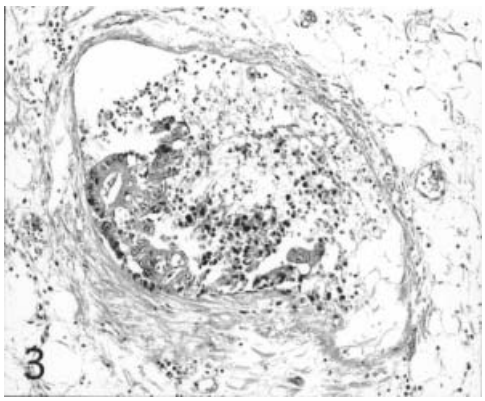
Fig. 1. Large collecting lymphatics (efferent lymphatics) which was connected with the sinus of the lymph node and had valves (arrows), blood vessels (B) and nerves (N) in the hilum of the lymph node.(original 4 X)

Fig. 2. Terminal end of collecting lymphatics parting from the interstitial spaces with concave thin walls (arrows).(orig. 33X)

RESULTS

1) *Relation of morphology of GC to types of lymphatic invasion*

The incidence of two types of lymphatic invasion as classified by both the Lauren and Japanese Classifications in 100 cases of GC is shown in Table Ⅰ. In E type lymphatic invasion; rather large cell nests consisting of several tens of carcinoma cells or preserving a glandular configuration were in collecting lymphatics (Fig. 3). While, in I type, carcinoma cells were found as single cells or in tiny clusters of 2 or 3 cells in lymphatic capillaries and in interstitial spaces of peri-lymphnodal fat tissue (Fig. 4), which were seen early even when no metastasis was found in LN (Fig. 5). This lymphatic invasion was likely to start at the hilar region and spread out in fat tissue (Fig. 6). Notwithstanding the absence of carcinoma emboli, single cells were not uncommon in collecting lymphatics (Fig. 7). The coexistence of both types of lymphatic invasion was found in 59% of cases, 22% being equally frequent, 26% more I type, and 11% more E type. The frequency of cases with only I type in diffuse and mixed type carcinomas were significantly higher ($\chi^2 < 0.005$ by Yates correction for



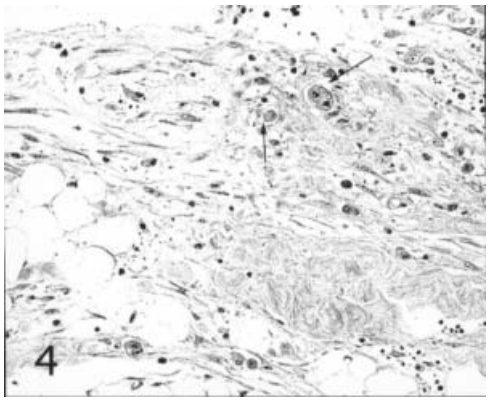
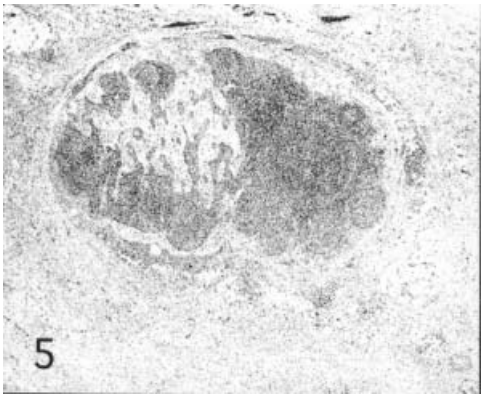


Fig. 3. E type of lymphatic invasion. Large embolus preserving glandular configuration in collecting lymphatics. (orig. 33 X)

Fig. 4. I type of lymphatic invasion. Single cells or tiny clusters of carcinoma in lymphatic capillaries (arrows) and in interstitial space of peri-lymphnodal fat tissue. (orig. 66 X)



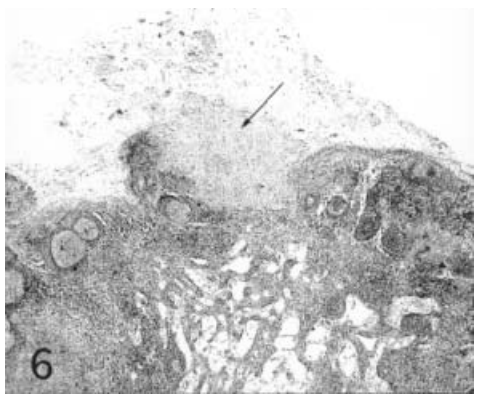
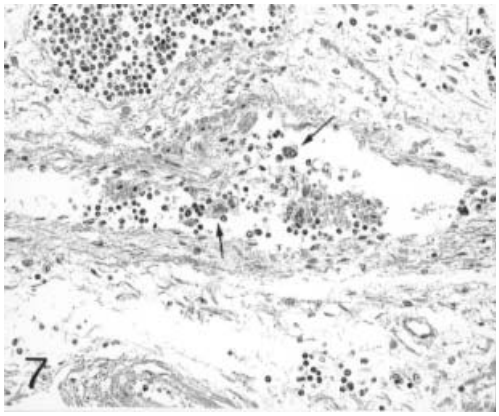


Fig. 5. I type of lymphatic invasion. Widespread of infiltration of carcinoma in peri-lymphnodal interstitial spaces even when no metastasis was found in the lymph node. (orig. 6.6 X)

Fig. 6. Early I lesion (arrow) starts from the interstitial spaces near the hilum of the lymph node without metastasis. (orig. 6.6 X)



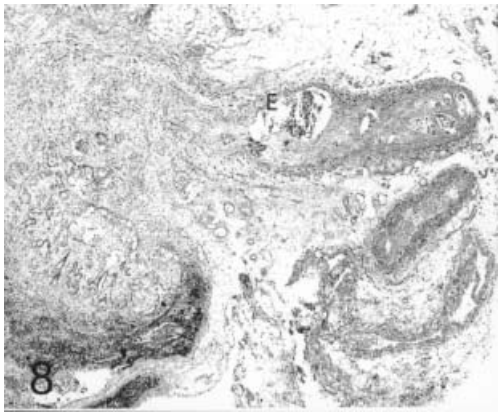
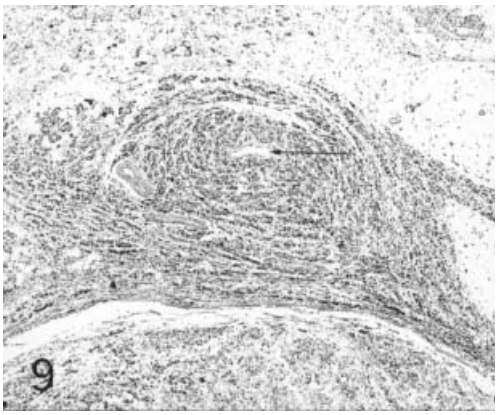


Fig. 7. Dispersed single carcinoma cells (arrows) in collecting lymphatics. (orig. 66 X)

Fig. 8. Fibrous obstruction of collecting lymphatics (efferent lymphatics) with large carcinoma embolus (E). (orig. 6.6 X)



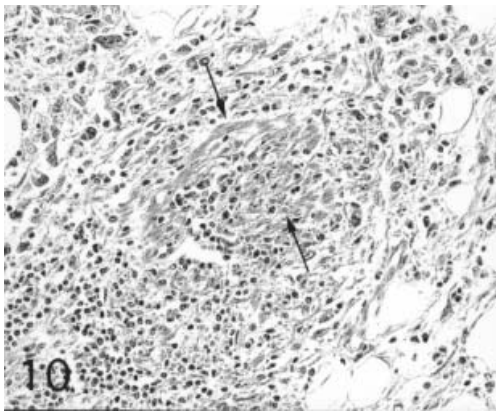
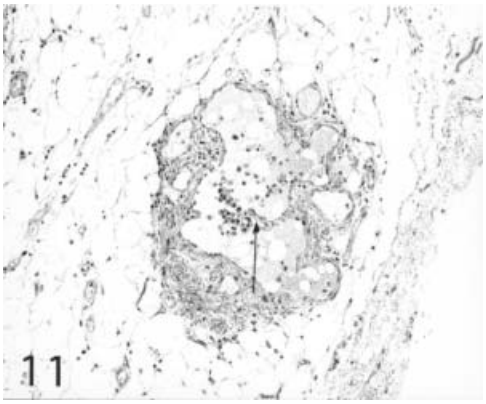


Fig. 9. Dense carcinoma infiltration in the wall of collecting lymphatics from outside and remaining narrow lumen (arrow). (orig. 13.2 X)

Fig. 10. Destruction and disapperance, with remaining smooth muscles (arrows), of collecting lymphatics by infiltration of carcinoma cells. (orig. 66 X)



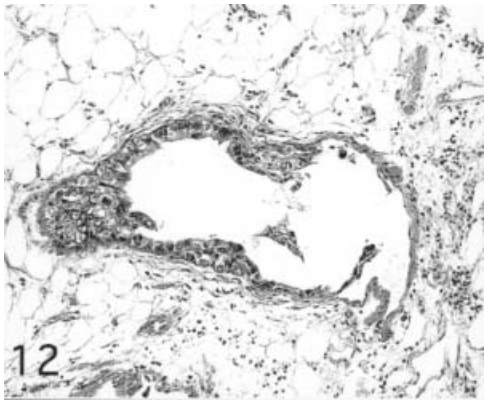


Fig. 11. Single carcinoma cells were caught on valves (arrow) in collecting lymphatics. (orig. 33 X)

Fig. 12. Carcinoma cells attach to and replace endothel cells of collecting lymphatics and remained up to the stage of complete obstruction. (orig. 33 X)

small numbers) as compared with those of intestinal type. Whereas, no cases with only E type were seen in diffuse and mixed GC, as compared with 20% in intestinal type. Cases with much more E type than I type were more frequent in intestinal type ($\chi^2 < 0.05$). (Table I).

2) *Histological alterations of collecting lymphatics in types of lymphatic invasion*

The early stage of I type lymphatic invasion, interstitial carcinoma infiltration in fat tissues near the hilum of LN without metastasis, was more frequently found in diffuse and mixed types of GC than in intestinal type, with a statistical significance ($\chi^2 < 0.005$).

Incidence of fibrous obstruction or stenosis of collecting lymphatics, probably due to E type lymphatic invasion (Fig. 8) ($\chi^2 < 0.025$), and obstruction by large carcinoma embolus ($\chi^2 < 0.005$) were significantly higher in intestinal type than in diffuse and mixed. The occurrence of carcinoma cell infiltration from outside followed by destruction (Fig. 9) and disappearance of collecting lymphatics (Fig. 10), and tiny nests of carcinoma cells in lymphatic capillaries or the existence of single cells in the collecting lymphatics were more

frequent in diffuse and mixed type GC than in intestinal type, but were not statistically significant (Table Ⅱ). Carcinoma cells or tissue in collecting lymphatics attached to and gradually replaced endothelial cells or valves (Fig. 11), and remained up to the stage of complete luminal obstruction (Fig. 12). The weaker expression of FⅧRA and CD31 in the

Table Ⅰ. Incidence of frequency of types of lymphatic invasion in types of GC.

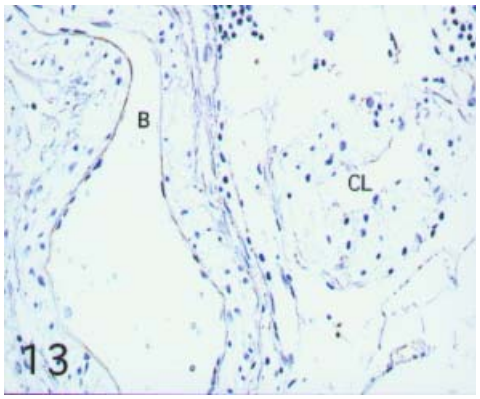
Lauren classification	Japanese classification	No cases	I *	I>E	I=E	E>I**	E
diffuse	por 2	38	19 50%	12 32%	7 18%	0 0%	0 0%
	sig	4	2 50%	1 25%	1 25%	0 0%	0 0%
	sum	42	21 50%	13 31%	8 19%	0 0%	0 0%
mixed	tub 2sci	15	6 40%	4 27%	2 13%	3 20%	0 0%
	tub 1sci	3	2 67%	1 33%	0 0%	0 0%	0 0%
	sum	18	8 44%	5 28%	2 11%	3 17%	0 0%
intestinal	por 1	14	2 14%	2 14%	5 36%	4 29%	1 7%
	pap	7	0 0%	1 14%	1 14%	2 29%	3 43%
	tub 2	12	1 8%	4 33%	3 25%	2 17%	2 17%
	muc	7	1 14%	1 14%	3 43%	0 0%	2 29%
	sum	40	4 10%	8 20%	12 30%	8 20%	8 20%
total		100	33 33%	26 26%	22 22%	11 11%	8 8%

*χ^2<0.005 I: I type of lymphatic invasion, E: E type of lymphatic invasion
**χ^2<0.05 I>E: I type is much more frequent, I=E: equally frequent
E>I: E type is much more frequent

endothelium of collecting lymphatics was not altered when had carcinoma emboli (Fig. 13,14). Laminin in the walls of collecting lymphatics were much less than venules without carcinoma cell emboli, but sometimes increased in collecting lymphatics having carcinoma emboli (Fig. 15).

3) *Immunohistology of Ec and βc in the type of lymphatic invasion*

Immunohistology for Ec and *β*c were carried out in 18 specimen of 14 cases of diffuse type GC. Ec positive carcinoma cells were found in 7/14 (50.0%) cases. Not only carcinoma cells within LN and emboli in collecting lymphatics (E type lymphatic invasion), but also those in interstitial tissues (I type lymphatic invasion) were positively stained to the same degree in cytoplasm in 6/7 (85.7%) cases (Fig. 16). On the other hand, *β*c were stained positively in the cytoplasm and sometimes in the nuclei in 11/14 (78.5%) cases, but carcinoma cells in the interstitial tissues (I type lymphatic invasion) were negative, or at least much less positive (Fig. 17), in 11/11 (100.0%) of the positively stained cases .



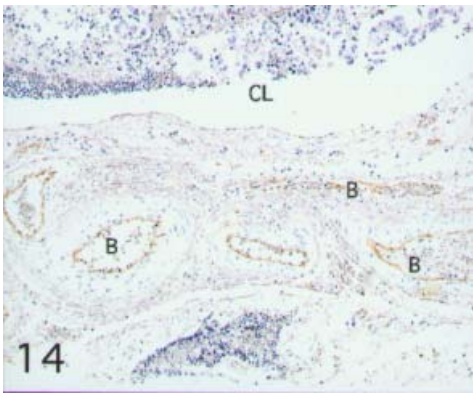
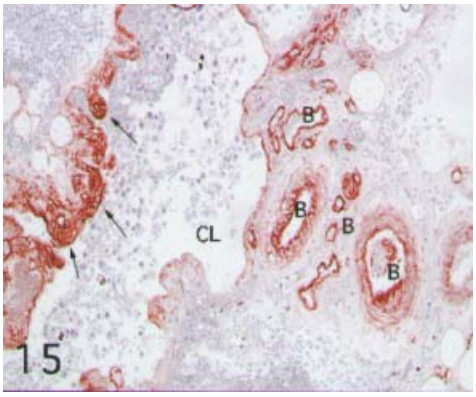


Fig. 13. Immunohistology of CD31 of vein (B) and collecting lymphatics (CL). Reaction of the endothelium of the latter is much weaker than the former. (orig. 66 X)

Fig. 14. Same as Fig. 13. Carcinoma embolus in collecting lymphatics (CL) (B:blood vessels). (orig.33 X)



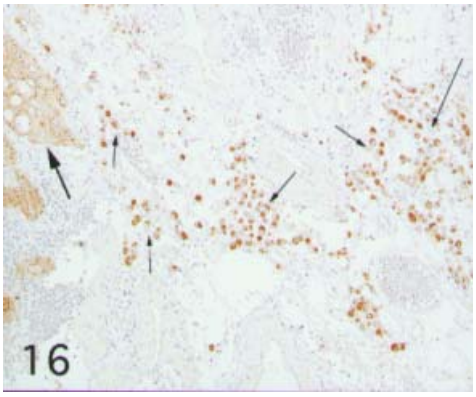


Fig. 15. Immunohistology of laminin. Laminin sometimes increases (arrows) in the wall of collecting lymphatics (CL)(B:blood vessels) having carcinoma emboli. (orig. 33 X)

Fig. 16. Immunohistology of E-cadherin stained positively to the same degree as in cytoplasm of carcinoma cells within the lymph node (thick arrow) and in the interstitial tissues of I type lymphatic invasion (arrows). (orig. 33 X)

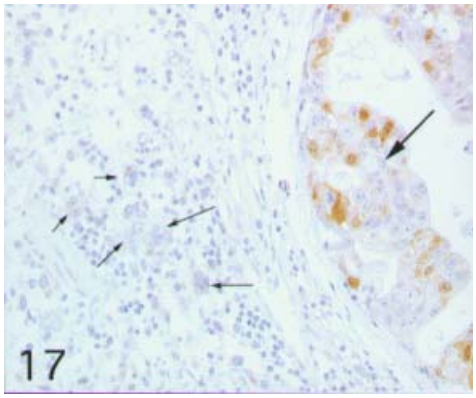


Fig. 17. Immunohistology of β-catenin stained positively in cytoplasm of carcinoma cells within the lymph node (thick arrow). However, negative or at least much weaker stained in the interstitial tissue of I type lymphatic invasion (arrows). (orig.66X)

Table Ⅱ. Incidence of frequency of histological alteration ratio of collecting lymphatics in two types of lymphatic invasion and types of GC.

Lauren classification	Japanese classification	NO	Infiltration from outside	Early I type lesion*	Destruction or disappearence of CL	Carcinoma cells in LC
diffuse	por 2	38	9 24%	20 53%	5 13%	19 50%
	sig	4	0 0%	3 75%	2 50%	2 50%
	sum	42	9 21%	23 55%	7 17%	21 50%
mixed	tub 2sci	15	5 33%	3 20%	2 13%	6 40%
	tub 1sci	3	0 0%	1 33%	0 0%	0 0%
	sum	18	5 28%	4 22%	2 11%	6 33%
intestinal	por 1	14	3 21%	3 21%	2 14%	3 21%
	pap	7	1 14%	0 0%	0 0%	2 29%
	tub 2	12	1 8%	2 17%	0 0%	5 42%
	muc	7	1 14%	1 14%	0 0%	2 29%
	sum	40	6 15%	6 15%	2 5%	12 30%
total		100	20 20%	33 33%	11 11%	39 39%

Lauren classification	Japanese classification	NO	Single carcinoma cells in CL	Fibrous obstruction & stenosis**	Obstruction of CL by large embolus*
diffuse	por 2	38	19 50%	7 18%	1 3%
	sig	4	1 25%	0 0%	1 25%
	sum	42	20 48%	7 17%	2 5%
mixed	tub 2sci	15	6 40%	5 33%	3 20%
	tub 1sci	3	0 0%	1 33%	0 0%
	sum	18	6 33%	6 33%	3 17%
intestinal	por 1	14	8 57%	6 43%	5 36%
	pap	7	0 0%	2 29%	3 43%
	tub 2	12	3 25%	8 67%	2 17%
	muc	7	3 43%	1 14%	1 14%
	sum	40	14 35%	17 43%	11 28%
total		100	40 40%	30 30%	16 16%

*χ^2<0.005 **χ^2<0.025 CL=collecting lymphatics, LC=lymphatic capillaries.

DISCUSSION

E type lymphatic invasion was easily observable, and at primary sites has been used as a diagnostically important factor. Pure I type was likely to occur exclusively in diffuse or scirrhous GC, was found at an early stage in the interstitium of fat tissues near the hilar region of LN, even when no metastasis was found in the LN. This was probably because wide spread edema had been given rise to in the interstitium by diffuse GC. Hence, lymph fluid containing single carcinoma cells flowed reversely, leaked and spread in the interstitial spaces via the lymphatic capillaries, which was open to destruction and permeation before entering into LN. Recently, attention has been paid to micrometastasis, which is often overlooked or is difficult to find by microscopy, by attempting to inquire into it by genetic procedures[31]. Also, microscopic diagnosis is possibly misses the focus of I type lymphatic invasion without metastasis in LN. This study revealed a high incidence, and the characteristic histology of I type lymphatic invasion, in diffuse or scirrhous GC. However, the coincidence of both I and E types in 59% of cases suggested that GC might be composed of heterologously mixed clonality of tumor cells. Besides, there was a possibility that carcinoma cells detached from emboli were released into single cells and infiltrated like I type lymphatic invasion. Heterologous clonality of GC was also shown by Sepulveda et al[28] , in that 61% of GC consisted of mixed lineages of cells, by immunohistology using anti-surface cell mucin, anti-gastric gland cell mucin, and anti-intestinal cell mucin antibodies. It has been noted that it is necessary to discern true embolic occlusions of collecting lymphatics from pseudoembolus, in which carcinomas grew simply in interstitial tissues[17], because the former contributed to a poor prognosis[15,17,19,23]. However, this might be very difficult to discern, because carcinoma cells attached to the endothelium of collecting lymphatics, replaced and occluded them from our findings.

Thus, carcinoma with I type lymphatic invasion might be more aggressive and not be completely removable, and should be investigated in detail to find molecular or genetic abnormalities which could raise curability.

Immunohistology here revealed that the positive expression of Ec in carcinoma cells of diffuse GC was at a low rate, in agreement with other reports[3,20]. On the other hand, although expression of βc was high in metastases within LN and in emboli of collecting lymphatics (E type lymphatic invasion), it was diminished and declined in I type lymphatic invasion in the same cases. It is reasonable to consider that it was not due to the sensitivity of the stain but to molecular abnormality or mutation, because abnormal Ec or βc is not able to configurate adenomatous structures as is in I type lymphatic invasion. Several reviews have discussed abnormalities of Ec in diffuse and poorly differentiated GC[3,8,13,18,26,27]. Mayer et al[21] noted that 92% of sixty GCs had reduced Ec expression as an early event. But they found no significant correlation between Ec expression and lymphatic invasion or LN metastasis. Vlemincks et al[30] reported that the transfection of cDNA of Ec inhibited infiltration of a highly metastatic strain. Becker et al[1] reported recently that there were no differences of immunohistological expression between mutant and normal Ec, and that the monoclonal antibody reacted with only mutant Ec lacking exon 9 and that 13% of GC expressed this mutational Ec.

A notable achievement has been reported that protein of APC gene, an anti-oncogene of colon cancer, competes with Ec to bind βc, and formed complexes with βc thus inhibiting oncogenesis[8,12]. Ochiai et al[25] and Hoschultzky et al[11] noted that if cultured GC cells were stimulated by TGF-α, EGF, a receptor type kinase, and C-erbB_2 protein were phosphorylated, followed by phosphorylation of βc and thus cell attachment was lowered. Also, Ochiai found that such cell detachment by tyrosine phosphorylation of βc[20] took place transiently in an

advancing site of colonic adenocarcinoma[24]. These cell detachments were caused by the transfection of the γ-src tyrosine phosphorylation of βc. βc stabilization by β dephosphorylation was induced by the activation of the Wnt signal pathway, and by inhibition of GSK-3β. Hence, βc accumulated in the cytoplasm, translocated to nuclei and activated the transcription of the target gene of cMYC[9] or cyclin D_1[29]. The disruption of Wnt or a mutation in βc exon 3 has been suggested as being involved with carcinogenesis[7,22]. However, the accumulation of βc differed according to the kind of carcinoma. For example, there was much in highly aggressive anaplastic thyroid carcinoma cells but not much in colorectal or hepatocellular carcinomas[7,22]. Immunohistologically, the expression of βc in nuclei and cytoplasm was reported in anaplastic thyroid carcinoma in which frequent βc mutations occurred[7]. However, Candidus previously found no mutation of βc in GC[3]. If this is correct, nuclear or cytoplasmic expression of βc in metastatic cancer cells within LN or in emboli in E type lymphatic invasion is not brought about by mutation of βc, but by other genes such as APC or Axin[10,22]. The lack of, or lesser stainability of βc in carcinoma cells of I type lymphatic invasion in this study was probably due to intramolecular alterations such as the phosphorylation of βc that separate carcinoma cells into tiny clusters or single cells, and has easy access to lymphatic capillaries or interstitial spaces in fat tissues.

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