

## STRUCTURAL STUDIES OF INITIAL LYMPHATICS ADJACENT TO GASTRIC AND COLONIC MALIGNANT NEOPLASMS

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### ABSTRACT

*Invasion and metastases are the main causes of death from cancer, and prognosis is best correlated with invasion of malignant cells into initial lymphatics and dissemination to regional lymph nodes. Using both light and transmission electron microscopy, we examined human gastric and colonic cancers and their relation to initial lymphatics. Invasion of malignant tumor cells into the initial lymphatics was characterized by interdigitating and overlapping endothelium giving way to open junctions as lymphatic endothelial cells were apparently dissolved and destroyed. Cytoplasmic vesicles, mitochondria, and rough endoplasmic reticulum were qualitatively increased as demonstrated by image analysis.*

Cell invasion and metastases are the primary mechanisms leading to patient death with malignant tumors. The lymphatic system is a major route of cell dissemination for adenocarcinomas as well as for melanomas, neuroblastomas, and malignant teratomas (1). Indeed, prognosis in cancer is often linked to invasion of initial lymphatics and regional lymph nodes (2,3). Accordingly, to examine the interaction between tumor cells and lymphatic endothelium, we examined the morphology of metastases in lymphatic vessels in specimens removed from patients undergoing resection of gastric and colonic malignancies.

### MATERIALS AND METHODS

Specimens were obtained in six patients with colon cancer and five patients with gastric cancer undergoing operation. Light microscopy samples were fixed in 10% formalin, embedded in paraffin wax, and serially cut (6 $\mu$ ) using a rocking microtome and stained with hematoxylin and eosin. Samples of electron microscopy were fixed in 3% glutaraldehyde for 6h, then post-fixed in osmium tetroxide for 1h, washed, dehydrated in a graded series of acetone and embedded in epoxy 812 (epoxy resin).

Initial lymphatics and adjacent collectors in semithin sections (0.5-1.0 $\mu$ m) were observed by light microscopy. Ultrathin sections 500-700 $\text{\AA}$  were obtained with glass knives on microtome and the sections stained with uranyl acetate and lead citrate and observed with a JEOL XI1200 electron microscope. Sixty films were photographed by electron microscopy and analyzed by an image analysis system.

### RESULT

#### *Light Microscopy*

Carcinoma cells were readily evident invading initial lymphatics. The lumina of lymphatic vessels were irregular, with diameters between 10.56-51.54 $\mu$ m. Lymphatic vessel walls were often dissolved and essentially destroyed, whereas cancer cells

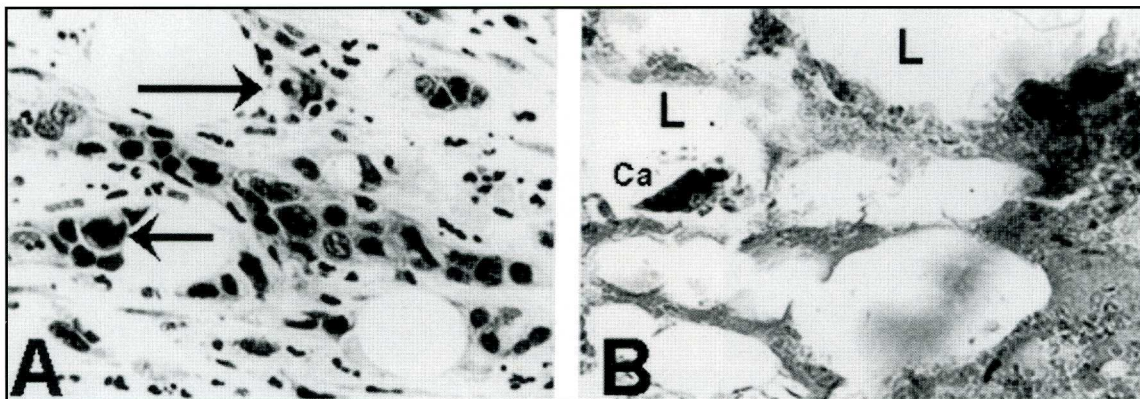


Fig. 1. Light microscopy showing A) a colonic cancer cell invading an initial lymphatic (arrows), and B) a gastric cancer cell (Ca) within a lymphatic lumen (L). The lymphatic is dilated and its wall has disintegrated. Ax100; Bx200 (H&E).

located near the junction of the lymphatic endothelium could be seen to enter the lymphatic lumen (Fig. 1).

#### Transmission Electron Microscopy

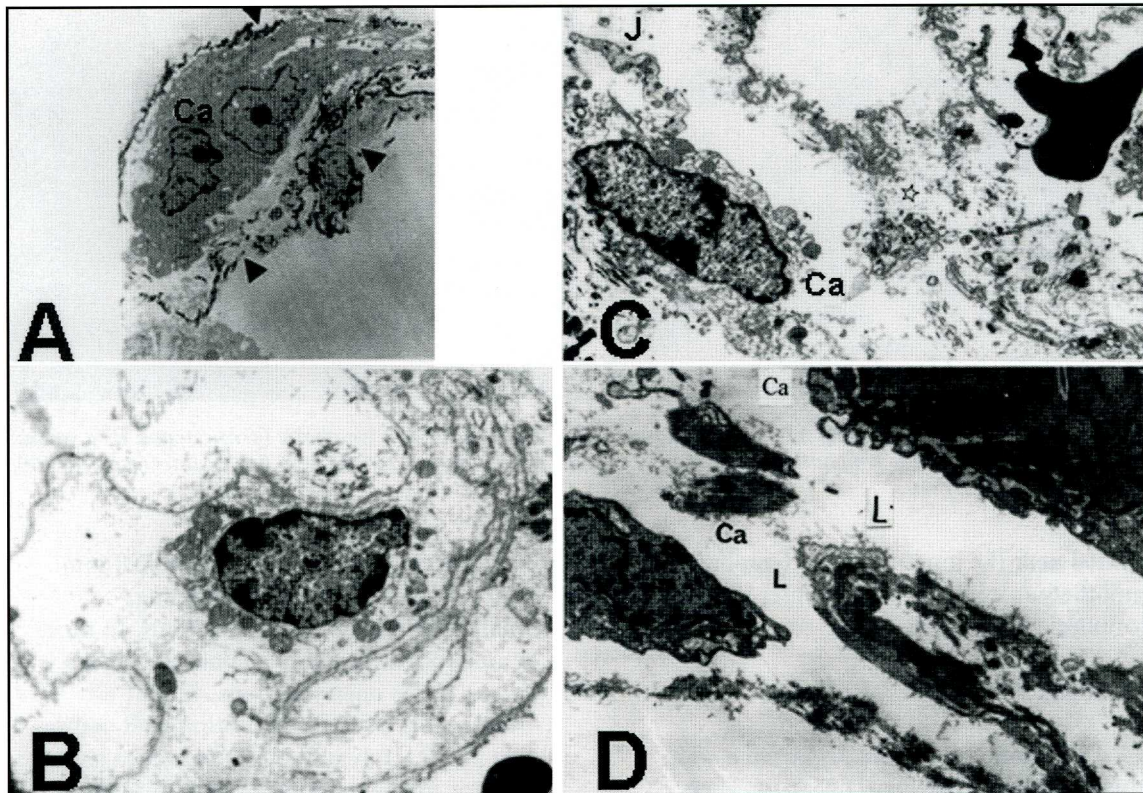
Lymphatic capillaries displayed a wide lumen and were irregularly shaped. There was an absent or discontinuous thin basement membrane along the outer endothelial surface. Anchoring filaments were frequently seen along the abluminal endothelial surface. Lymphatic endothelial cells were extremely flattened with the nuclei showing only a slight elevation above the surface of the surrounding cytoplasm. In the endothelium itself, there was little cytoplasm. Nonetheless, Golgi complex and cytoplasmic organelles such as mitochondria, rough endoplasmic reticulum (RER), and ribosomes were often seen in the perinuclear region (Fig. 2). The cytoplasm of endothelial cells was rich in vesicles. On the other hand, the membrane and cytoplasm of lymphatic endothelium were often destroyed. Some structures in the cytoplasm such as mitochondria and the RER had lost their shape and were disintegrating as seen by transmission electron microscopy (Fig. 3). Cancer cells in the lymphatic lumen or near the junction of the endothelium were readily depicted (Fig. 3).

Initial lymphatics were classified into four types: end-to-end, overlapping, interdigitating, and open junctions. In those lymphatics adjacent to colonic tumors we found 32.2% open junctions, 43.6% overlapping and interdigitating, and end-to-end 22.4%. In lymphatics adjacent to gastric tumors, open junctions numbered 57.1%, overlapping/interdigitating 22.0%, and end-to-end 20.9%.

Most cytoplasmic vesicles in the endothelial cells were uncoated but some were enclosed by a unit membrane and included low-density electron substances. The volume density of cytoplasmic vesicles was 0.42, and its number density was 399.51 ( $\uparrow/\mu\text{m}^3$ ). The medium diameter of cytoplasmic vesicles was 92.72 (nm). The volume density of mitochondria was 0.03 and its number density was 7.22 ( $\uparrow/\mu\text{m}^3$ ). The volume density of RER was 0.24, and its number was 61.19 ( $\uparrow/\mu\text{m}^3$ ). The ratio between membranous area of mitochondria and volume of mitochondria was 1.81. The ratio between membranous area and volume of RER was 2.28.

#### DISCUSSION

Lymphatic capillaries adjacent to colonic and gastric cancers have similar ultrastructural features to other lymphatics of the body.



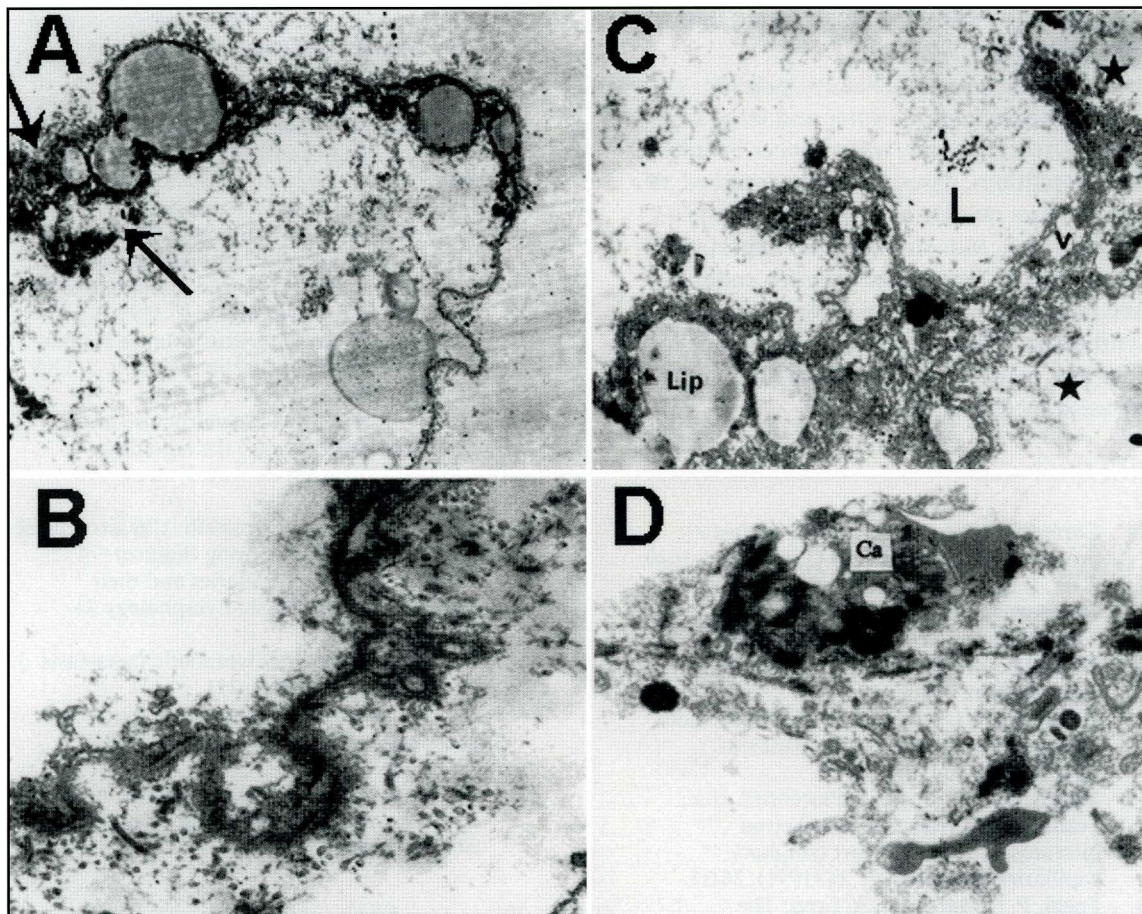
*Fig. 2. Transmission electron micrographs: A&B showing initial lymphatic adjacent to gastric cancer. A: Endothelial cytoplasm has been destroyed with many phytovesicles. The ribosome content is increased (arrows). Ax4,000; Bx20,000. C: Similar changes in lymphatic (L) adjacent to colonic cancer. The endothelial membrane has partly disintegrated and phagovesicles (V) are increased. Nearby connective tissue is destroyed (\*). A lipovesicle (Lip) is also seen. X10,000. D: Colonic cancer cell (Ca) invading a lymphatic endothelial cell. The Ca is rich in rough endoplasmic reticulum and mitochondria. Much of the endothelium has been destroyed. x5,000.*

They are distinguished by a wider, more irregular lumen than blood microvessels and absence of a definite basement membrane. There are mitochondria, ribosomes, rough endoplasmic reticulum and cytoplasmic vesicles in the perinuclear region of the lymphatic endothelial cell. The nuclei of lymphatic endothelial cells appear as oval structures. Intercellular contacts include end-to-end, overlapping, interdigitating, and open junction (4-7).

We found that both the blood microvasculature and the number of lymphatic capillaries were increased in carcinomatous peripheral tissues. We suspect that carcinoma

cells secrete substances which promote hemangiogenesis and lymphangiogenesis and also lymphatic-venous neoanastomoses. The compression of local tissues and increased angiogenesis promotes opening of dormant lymphatics which help alleviate local interstitial edema, whereas secretory agents facilitate the opening of lymphatic-venous anastomoses (8). Lymphatic vessels are eroded by invading cancer cells and contain many lymphocytes as part of immunoresponsiveness to the adjacent cancer.

Dobbins and Rollins observed that the intercellular junction of initial lymphatics were normally open in roughly 1-6% (9) but



*Fig. 3. Transmission electron micrographs. A: Most of the initial lymphatic endothelial cell has been invaded by colonic cancer cells (Ca) and its structure has been destroyed (arrowheads). x10,000. B: Colonic cancer cells located in the lumen of an initial lymphatic. The endothelial cell has been destroyed. x8,000. C: Gastric cancer cell (Ca) invading and destroying an adjacent initial lymphatic (\*). Open junction (J) is seen in the upper left corner. x8,000. D: Gastric cancer cells (Ca) are seen in an adjacent initial lymphatic lumen (L). x11,500.*

different organs displayed different characteristics. Collin and Kalima suggested that the exact form of intercellular junction depends upon the structure of different lymphatic capillaries and ongoing function (10). We found that there was a continuum of initial lymphatic structure from a densely closed to a widely opened morphology. Most open junctions derived from end-to-end and overlapping, interdigitating junctions and seemed to relate to whether cancer cells were entering the initial lymphatics. It is likely that with ongoing angiogenesis and cancer

growth, lymphatic anchoring filaments are activated and lymphatic capillaries become dilated thereby facilitating the migration of cancer cells from the interstitium into initial lymphatics (11-13). With the invasion of the lymphatic endothelium by cancer cells, the endothelium becomes attenuated and destroyed. Thus, cancer cells were seen in the lumen of lymphatics or near the junction of dissolved parts of the lymphatic wall, findings that conform to earlier experimental results (14,15). Our data support that cancer cells pass through initial lymphatics by

ameboid movement and disrupt lymphatic structure (14,15).

Lymphatic endothelial cytoplasmic vesicles help transport water and large molecules (7,16). However, when cancer invades the interstitium, tissue fluid is reabsorbed as lymph more quickly as cytoplasmic vesicles are activated. Mitochondria are important for energy metabolism whereas rough endoplasmic reticulum is where secretory proteins are synthesized. Quantitatively, these organelle activities seem to be increased with lymphatic invasion by cancer cells.

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