Ultrastructural Descriptions of Pericyte/endothelium Peg-socket Interdigitations in the Microvasculature of Human Gastric Carcinomas

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Abstract. *Background: Angiogenesis requires complex multistep signalling pathways and a high degree of spatial and temporal coordination among endothelial cells and pericytes. The two cell types exhibit numerous contacts in vivo and in vitro, including the occurrence of peg-socket junctions. Materials and Methods: Ultrastructural findings in 9 cases of advanced gastric carcinomas were reviewed with special emphasis on endothelium/pericyte peg-socket junctions. Results: The incidence of peg-socket junctions was approximately 8% in 5 out of 9 cases. The remaining 4 cases showed a very low rate, including two cases in whom interactions were totally absent. Peg-socket junctions consisted of cytoplasmic projection from the pericyte protruding into the endothelial indentation. The endothelial cells interacting with pericytes showed ultrastructural signs of partial stabilization such as continuous endothelial lining, regularly constructed interendothelial junctions, more or less integrated pericytes, and multilayered basement membrane. Conclusion: Our ultrastructural study confirms previous reports regarding pericyte/endothelial peg-socket interdigitations in murine and human granulation tissues and extends these findings to the microvasculature of human gastric carcinomas.*

Angiogenesis is essential for the primary and metastatic growth of tumours and thus has become a target for antineoplastic therapy (1, 2). However, angiogenesis in solid tumours does not lead to stable, quiescent and optimally functional vessels (2). Instead, the vessels remain in a continuous dynamic state of growth, regression and remodelling (3). Angiogenesis requires complex multistep signalling pathways and a high degree of spatial and temporal coordination among endothelial cells and pericytes (3).

Pericytes exhibit several types of surface contact with endothelial cells, including gap junctions, adhesion plaques and peg-socket junctions (4-6). Gap junctions provide direct connections between the cytoplasm of the two cells and allow the passage of ionic currents and small molecules (5). Adhesion plaques are sites of contact exhibiting membrane specialization (6). The peg-socket contacts involve reciprocal, interdigitating evaginations from each cell, which invaginate into each other (4). They are only noticeable by electron microscopy, occur by closeness or apposition without physical contact of plasma membrane, leaving a small space between cells, measuring 15 to 30 nm (4).

As a part of an ongoing study of the ultrastructural morphology of cell-to-cell interaction in human gastric carcinomas, we previously examined the microvasculature of both normal mucosa and carcinomas of the stomach (7-9). In view of the known importance of angiogenesis in tumour biology, we thought that it would be of interest to extend our investigation to the pericyte/endothelium peg-socket interdigitations in the microvasculature of human gastric carcinoma. In addition, some of the experimental data which are pertinent to the interpretation of ultrastructural observation will also be discussed.

Materials and Methods

In our Department, gastric tumours are routinely processed for both light and electron microscopic observations (7-9). Surgically resected specimens were obtained from 9 patients with advanced gastric carcinoma. All surgical specimens for light microscopy were fixed in 10% formalin and embedded in paraffin. Haematoxylin and eosin staining was used for general evaluation of tissue morphology. For electron microscopy, small pieces of the fresh tumour tissue were immediately fixed in 3% phosphate-buffered glutaraldeyde (pH 7.4) and post-fixed in 1% osmium tetroxide. Semi-thin araldite-embedded sections were stained with Giemsa's reagent for selection of fields. Two blocks per case were studied ultrastructurally. Thin sections were double-stained with uranyl acetate and lead citrate;

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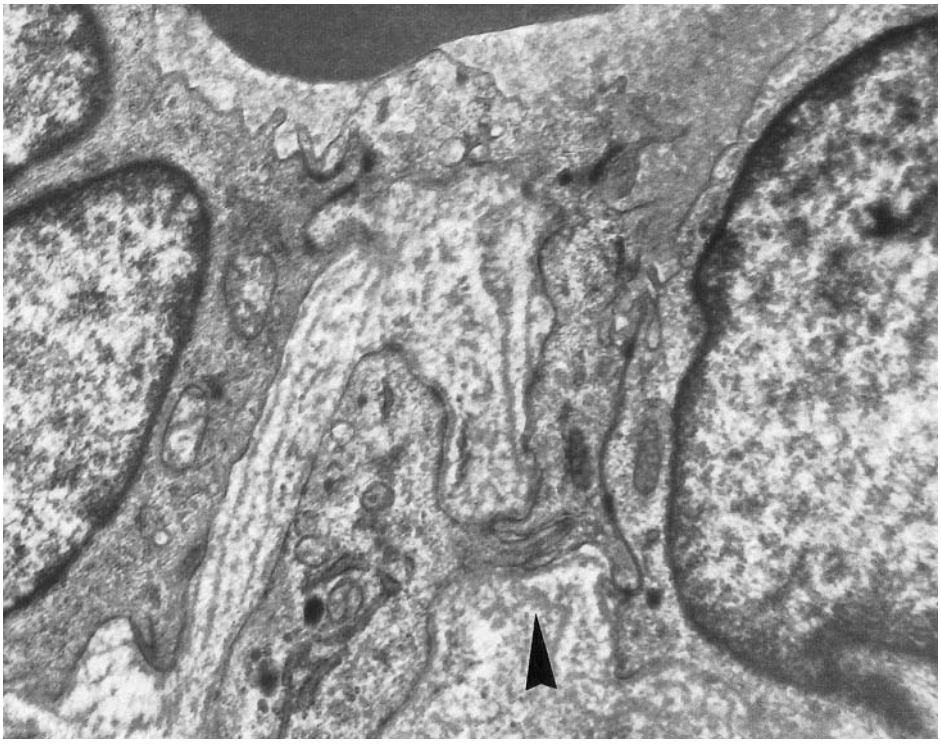

Figure 1. *Endothelial cell process extending through the subendothelial basement membrane to form a junction with an overlying pericyte (magnification ×6,000).*

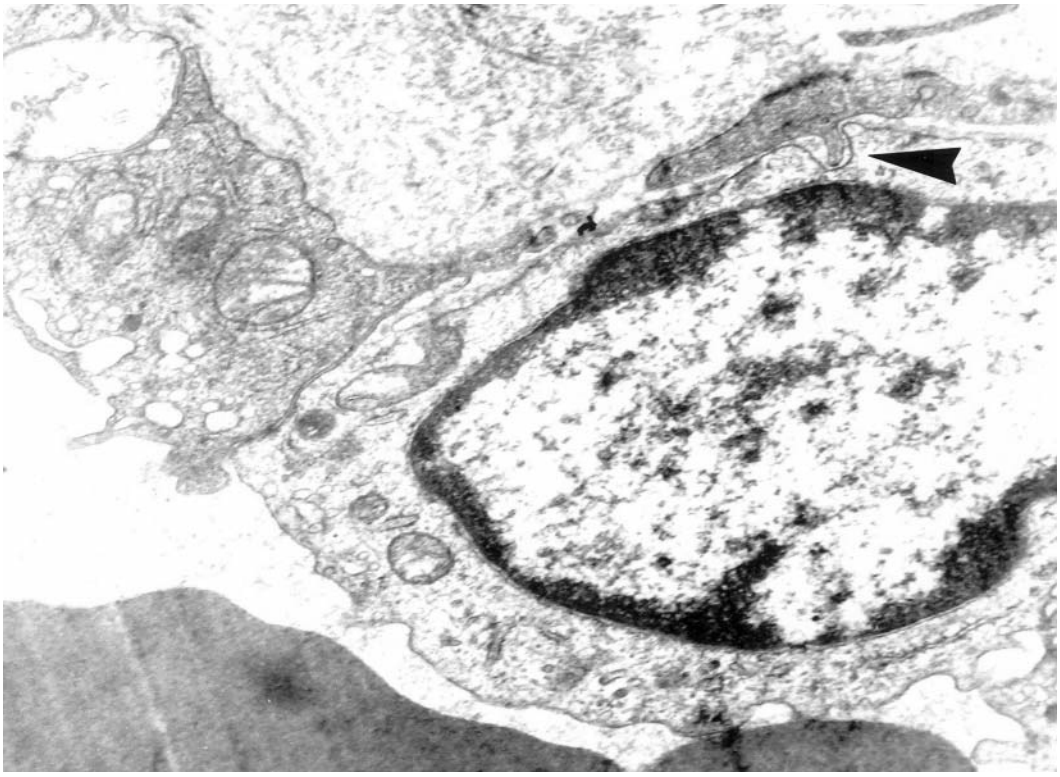

Figure 2. *Transmission electron micrograph showing a blood vessel in the stroma of a human adenocarcinoma. Finger-like pericyte interdigitation (arrowhead) extending through basement membrane deficiencies formed a peg-socket junction with the adjacent endothelial cell (magnification ×10,000).*

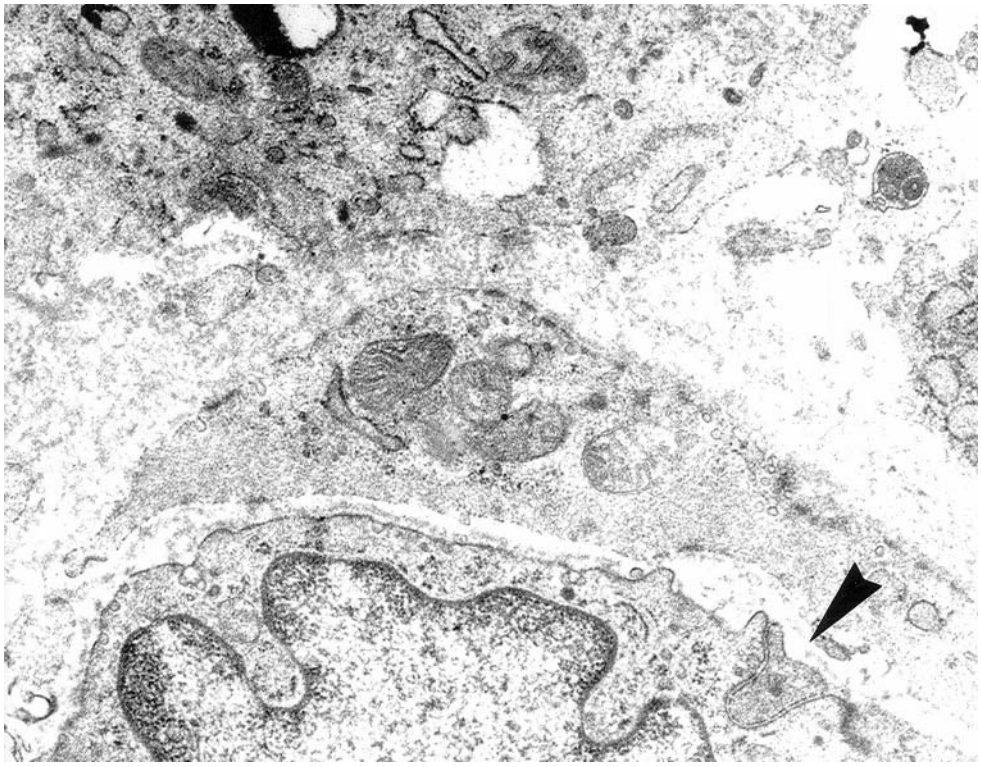

Figure 3. *Transmission electron micrograph demonstrating the intimate relation between the pericyte process and the endothelium. The pericyte process (arrowhead) fits into the indentation in the endothelium. These pericyte processes were derived from a flattened secondary process (magnification ×10,000).*

they were then examined and photographed in a Zeiss EM 902 electron microscope (Carl Zeiss, Oberkochen, Germany). Whole areas of each section were observed. A total of 200 microvessels were identified within the tumours.

Inclusion criteria for peg-socket junctions were the presence of a distinct interdigitation of a cell process into a socket of a neighboring cell; in order to exclude simple surface folds, only the structures that were separated by a constant, narrow gap, not containing a basal lamina or connective tissue components were selected.

Results

The 7 men and 2 women with advanced gastric carcinoma ranged in age from 60 to 75 years. Histologically, tumours were classified into intestinal (6 cases) and diffuse-type (3 cases) according to the Laurèn classification. All of the cases showed invasive growth beyond the muscularis mucosae.

Peg-socket contacts among the endothelium and pericytes were identified in 5 out of 9 cases, with an incidence of approximately 8% . The remaining 4 cases showed a very low rate, including two cases in which interactions were totally absent.

In these 7 cases, there were two types of cytoplasmic interdigitation between the endothelium and pericytes. The first was composed of a cytoplasmic projection from the endothelium in close apposition with a pericyte, with no intervening basement membrane (Figure 1). The cytoplasmic projection of this latter type was relatively short, 0.2 to 0.5 μm long on average, and could have a rather dull and shallow membrane indentation of the pericyte. The second consisted of a straight and/or curved cytoplasmic projection from the pericyte combined with a deep and narrow cytoplasmic indentation of the endothelium (Figure 2). The projection of this type was measured to be 0.5 to 1.3 μm in length. We also found interdigitations where pericyte pegs fitted tightly into endothelial sockets (Figures 3 and 4). Endothelial/pericyte interdigitations were found in microvessels showing ultrastructural signs of partial stabilization such as continuous endothelial lining, regularly constructed interendothelial junctions visible as electron-dense zones, more or less integrated pericytes, and multilayered basement membrane (Figures 3 and 4). The nuclei of endothelial cells were large in comparison to the cytoplasm, and showed a prominent nucleolus and nuclear bodies. Mitoses and cytoplasmic sproutings were not seen.

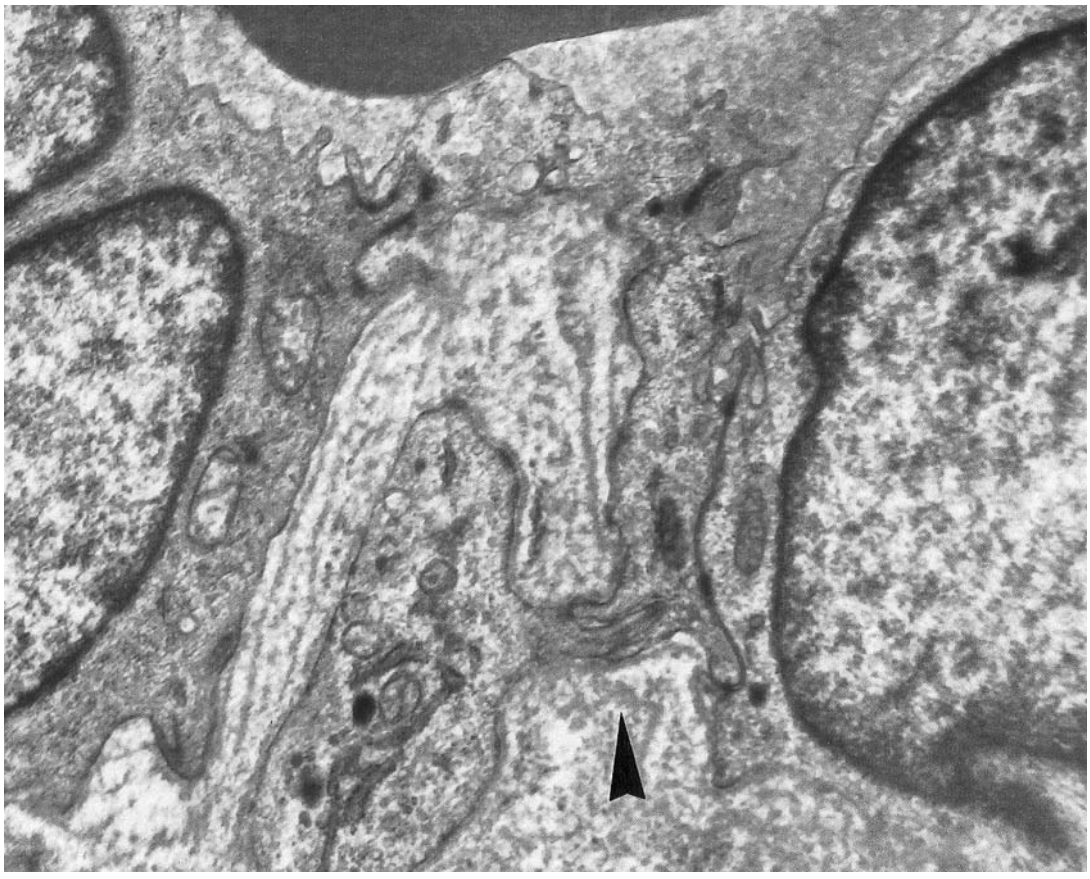

Figure 4. *Intimate interdigitations (arrowhead) between endothelial cell and pericyte. Note the pericyte peg fitting tightly into an endothelial socket (magnification ×16,000).*

Discussion

Pericyte/endothelium peg-socket junctions have been described in human and murine granulation tissue as well as in human retinal capillaries (10-14). The current ultrastructural analysis shows for the first time pericyte/endothelium peg-socket interdigitations in the microvasculature of human gastric carcinomas. We ruled out the possibility that this association was casual because interdigitation of cytoplasmic processes occurred at points of basement membrane interruption. An important feature of peg-socket junctions between pericytes and the endothelium was that the pericyte invariably provided the peg, while the endothelium formed the acceptor socket. Pericyte/endothelium cytoplasmic interdigitations may be anchoring sites. However, there were junctions where the pegs fitted tightly into their sockets. This geometry suggests the presence of synapse-like peg-socket contacts between pericytes and endothelial cells. Physical contact between pericytes and the endothelium has been reported to be necessary to prevent angiogenesis with inhibition of endothelial cell proliferation (15). Several growth factors, including epidermal growth factor, transforming growth factor-beta, and angiopoietin 1, are present in the intercellular pericyte/endothelium interdigitation space (11-14). Recently, Wakui *et al.* (10) showed that angiopoietin 1 may play a role in endothelial/pericyte interaction by recruiting pericytes to enhance microvessel maturation. Accordingly, our findings of endothelium/pericyte peg-socket junctions were associated with ultrastructural signs of partial stabilization such as continuous endothelial lining, regularly constructed interendothelial junctions visible as electron-dense zones, more or less integrated pericytes, and a multilayered basement membrane.

In conclusion, our ultrastructural observations with human gastric carcinoma confirm and extend previous reports regarding pericyte/endothelial peg-socket interdigitations in murine and human granulation tissues. The presence of endothelial/pericyte interdigitations may be interpreted as morphological expression of microvessel maturation and stabilization during angiogenic reaction in advanced gastric adenocarcinoma.

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