Ultrastructural Observations on Inflammatory Angiogenesis in Gastric Carcinomas with Massive Neutrophil Infiltration

ABSTRACT

Neutrophils are traditionally thought of as terminal effectors of inflammatory reaction, but experimental studies suggest that they play a direct role in the inflammatory angiogenesis of tumors. Thus, further evidence in humans is required regarding the mechanisms by which neutrophils induce tumor angiogenesis. In this study, 4 cases of human gastric carcinomas with massive neutrophil infiltration were studied by light and electron microscopy, focusing on the inflammatory angiogenesis in the tumor stroma. At light microscopy, the tumors were advanced gastric carcinomas in which various degrees of tubular differentiation were present. Under an electron microscope, pericytes exhibited two major differentiated states with distinct ultrastructural features: a contractile phenotype and a synthetic phenotype. The contractile phenotype was characterized by abundant microfilaments. Synthetic pericytes contained abundant rough endoplasmic reticulum, lipid bodies, and numerous membrane-bound vesicles. These ultrastructural findings extend concept of contractile/synthetic phenotype modulation, originally described in smooth muscle cells, to tumor microvascular pericytes. Tumor microvasculature was also characterized by abortive or slit-like lumina, endothelial cell mitoses, and replicating basement membranes. These qualitative and observational transmission electron microscopy findings provide additional morphological evidence of active inflammatory angiogenesis in gastric carcinomas with massive neutrophil infiltration.

Keywords: electron microscopy, gastric carcinoma, inflammatory angiogenesis, microvasculature, neutrophils

The importance of angiogenesis for the growth of tumors is widely recognized. The majority of vascularization data have been collected in fast-growing rodent tumors. There is, however, relatively little information in the literature by which one can judge the relevance of the experimental tumor systems to the corresponding situations in human primary tumors.

The role of neutrophils in progression of tumor angiogenesis has been somewhat neglected, perhaps due to their traditional characterization as terminally differentiated effectors of inflammation. Only recently has their important and regulatory role in angiogenesis and tumor progression entered the focus of attention, with several reviews published in the last three years.
Inflammation-dependent angiogenesis seems to be a central force in tumor growth and expansion, a concept supported by the observations that the use of “classic” anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs, leads to angiogenesis inhibition [5].

We retrospectively reviewed our institutional experience [14–17] on human gastric carcinomas showing massive neutrophil infiltration to describe the ultrastructural findings of inflammatory angiogenesis.

MATERIALS AND METHODS

Surgically resected specimens were obtained from the 4 patients with gastric carcinoma. The patients ranged in age from 55 to 77 years. They comprised 3 males and 1 female. The neoplasms ranged from 2 to 7 cm in diameter, and the histologic type was defined according to WHO classification [18]. All surgical specimens for light microscopy were fixed in 10% formalin and embedded in paraffin.

For electron microscopy, endoscopic specimens or small pieces of the fresh tumor tissue were immediately fixed in 3% phosphate-buffered glutaraldehye, pH 7.4, and postfixed in 1% osmium tetroxide. Semithin araldite embedded sections were stained with Giemsa’s reagent for selection of fields. Thin sections were double-stained with uranyl acetate and lead citrate; they were then examined and photographed in a Zeiss EM 902 electron microscope (Carl Zeiss, Oberkochen, Germany).

No quantitation regarding image analysis of microvascular endothelial cells and pericytes was undertaken and therefore no statistical data are presented regarding image analysis.

RESULTS

We identified 4 cases of advanced gastric adenocarcinomas in which various degrees of tubular differentiation were present (Figures 1, 2A). An extensive number of tumor-infiltrating neutrophils consisted of a single massive infiltrate of neutrophils (Figures 1, 2A). Neutrophil density was similar in deep and superficial portions of the same tumor. Some neutrophils were seen passing through the neoplastic epithelium, and others were found lying within neoplastic tubules similar to “crypt abscesses” (Figure 2B). A few macrophages and mast cells were seen in the tumor stroma (Figure 2B). Blood vessels were lined with plump endothelial cells with large nuclei (Figure 2B). Vascular dilatation and sometimes tight intraluminal packing of erythrocytes were present. Neutrophils were distributed in loose aggregates in these vessels. In all

DISCUSSION

Studies of tumor angiogenesis are based on several idealized experimental models, including well-defined growth conditions, uniform age, and genetic background of the host, and multiple stages of carcinogenesis [19–21]. Parallely, our ultrastructural study is focalized on gastric tubular adenocarcinomas, characterized by a nearly uniform neutrophil infiltration. The tumors reported here are similar to those previously reported as gastric carcinoma with prominent neutrophil infiltration: a subset of gastric carcinoma with an
incidence of about 7% [22]. Therefore, we think that human gastric carcinomas with prominent neutrophil infiltration provides a good model system for studying tumor inflammatory angiogenesis.

Recent in vivo studies using various models of angiogenesis have demonstrated that neutrophils promote neovascularization in experimental tumors. Neutrophils can contribute to tumor angiogenesis in vivo by releasing IL-8 and VEGF [23, 24]. Such tumor-promoting functions of neutrophils were also inferred in clinico-pathological studies in which high numbers of these cells in human tumors correlated with increased levels of tumor microvessel density [25]. However, microvessel density counting techniques assess the presence of blood vessels but do not give an indication of the degree of angiogenesis and the functional status of the tumor vascular bed [26]. Proliferation of endothelial cells may still be considered the single most reliable parameter to assess the presence of active angiogenesis [26]. Our ultrastrucural study now extends these findings and provide additional morphological evidence of active angiogenesis characterized by endothelial cell mitoses associated with margination and exudation of neutrophils. These data were also confirmed by the findings of abortive or slit-like lumina and replicating basement membranes, that have been also described in inflammatory angiogenesis [27].
The ultrastructural findings reported here show contractile and synthetic pericytes, which represent the two ends of a spectrum of pericytes with intermediate phenotypes. Contractile phenotype was characterized by abundant microfilaments. Pericytes with synthetic phenotype showed prominent cisternae of rough endoplasmic reticulum, numerous caveolae, and lipid bodies. Lipid bodies are dynamic fat-storing organelles present in virtually all eukaryotic cells and involved in many aspects of cell biology related to lipid metabolism and inflammation [28, 29]. The density of the lipid droplets as seen in electron micrographs varies from electron lucent to markedly electron dense [29]. Roughly, this may be correlated with the saturated and unsaturated fatty acid content of the triglyceride and the degree of unsaturation of fatty acid present [29]. This is an unusual finding in microvascular pericytes of human tumors [30]. The phenotypical modulation of tumor microvascular pericytes is similar to that described in the arterial smooth muscle cells in vivo and in vitro [31]. Smooth muscle cells are capable of major changes in its phenotype in response to changes in local environmental cues including growth factors/inhibitors, mechanical influences, cell–cell and cell–matrix interactions, and various inflammatory mediators [32]. According to Owens et al. [32], the process of phenotypic modulation is applicable not only to all smooth muscle cells but also to pericytes in tumor microvasculature. Therefore, our ultrastructural findings may be interpreted as a pericyte phenotypic modulation, at least in part, due to extensive areas of inflammation in the tumor stroma. However, additional

Figure 5. Synthetic pericyte characterized by abundant rough endoplasmic reticulum. Dilated rough endoplasmic reticulum is also present. × 8000.

Figure 6. Synthetic pericyte containing moderately electron-dense lipid bodies in the cytoplasm. × 12,000.

Figure 7. Synthetic pericyte shows numerous vesicles along plasmalemmal membrane. × 8000.
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studies with a larger series, including other forms of gastric carcinomas, are clearly needed.

In conclusion, our qualitative ultrastructural findings add new data and show an intimate association between neutrophil exudation and active angiogenesis. Our ultrastructural findings are quite suggestive for extending the concept of contractile/synthetic phenotype modulation, originally described in smooth muscle cells, also to tumor microvascular pericytes.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES