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Original contribution

Role and mechanism of vasculogenic mimicry in gastrointestinal stromal tumors☆

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Summary Vasculogenic mimicry (VM) is the formation of fluid-conducting channels by highly invasive and genetically dysregulated tumor cells. In this study, we collected specimens of 84 human gastrointestinal stromal tumors (GISTs) along with clinicopathologic data and another 42 GISTs with fresh tissue that was used for gelatin zymography. VM was found in 21 of the 84 GISTs using CD31/periodic acid–Schiff double staining and CD117 and CD31 immunohistochemical staining. There was a significant difference in the VM-positive rate between the lesions with a mitotic rate ≥5/50 high-power fields and those with a lower mitotic rate (P = .000) and between the cases with and without liver metastasis (P = .008). There was a significant difference in the VM-positive rate between the high-risk group (5.9%) and the very low/low-risk group (12.5%) (P = .010) or the intermediate-risk group (39.5%) (P = .020). Kaplan-Meier survival analysis showed VM indicated a poor prognosis (P = .0000). Cox proportional hazards model indicated that the presence of VM, tumor size 10 cm or greater, and hemorrhage were independent predictors of a poor prognosis (P = .000, .005, .032, respectively). The staining indexes of matrix metalloproteinase (MMP)–2 and MMP-9 were higher in the VM-positive than in the VM-negative group (P = .024 and .037, respectively). Gelatin zymography showed that the activity of MMP-2 and MMP-9 was significantly higher in the VM-positive lesions (P = .013 and .033, respectively). We conclude that VM in GISTs is an unfavorable prognostic sign and that patients with VM-positive tumors are prone to suffer liver metastasis. Both MMP-2 and MMP-9 play an important role in VM formation in GISTs.

1. Introduction

Gastrointestinal stromal tumors (GISTs), although rare neoplasms, represent the most common mesenchymal tumors of the gastrointestinal tract [1]. The annual incidence of GISTs is estimated at 20 cases per million according to a large retrospective study in Sweden, and this figure is thought to mean about 5000 new cases in the United States each year [2]. Although no detailed study has been carried out, it is believed that the incidence of GISTs is greater in China than in the United States. The sites of GIST are as follows: stomach (60%), small intestine (25%), rectum (5%), esophagus (2%), and other locations (5%), including the appendix, gallbladder, pancreas, mesentery, omentum, and retroperitoneum. A large

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number of GISTs are composed of fairly uniform spindle cells (70%), but some are dominated by epithelioid cells (20%), and a few consist of a mixture of these 2 morphologies. Genetic analysis reveals acquired mutations in the c-kit or platelet-derived growth factor receptor α genes [3].

Vasculogenic mimicry (VM) is the formation of fluid-conducting channels by highly invasive and genetically dysregulated tumor cells having a pluripotent embryonic-like genotype [4]. VM is seen in melanoma, ovarian and prostatic carcinoma, inflammatory breast cancer, hepatocellular carcinoma, and soft tissue sarcomas, including synovial sarcoma, rhabdomyosarcoma, and Ewing sarcoma [4-10]. VM, a kind of new blood-supplying model, has been confirmed to be an indicator of a poor prognosis in some malignant tumors in that patients with tumors demonstrating VM have a lower 5-year survival rate than patients having tumors without VM [11].

Matrix metalloproteinases (MMPs) are a family of zinc-dependent neutral endopeptidases that can degrade many components of the extracellular matrix and basement membrane. They can contribute to cancer growth and invasion and to angiogenesis [12]. Matrix metalloproteinase-2 and MMP-9 were found to co-localize with VM by immunohistochemical staining; the development of VM could be inhibited by anti-MMP-2 antibody but not by anti-MMP-9 antibody, so MMP-2 is thought to play an important role in VM formation [5,13-16]. Gelatin zymography, which reveals actual gelatinolytic enzyme activity at a cellular level, can be used to assess the expression and activity of MMP-2 and MMP-9 in entire tissues [17].

Ultrastructurally, tubular structures consisting of a lumen bound by GIST cells were found by Qian et al [18] in 2000. In some of these structures, red blood cells (RBCs) were present. These structures were similar to VM and considered to be primitive blood vessels. The objective of this investigation was to elucidate the existence of VM in GIST, the correlation between VM and the patient's prognosis, and the mechanism of VM formation.

2. Patients and methods

2.1. Patients

Between January 1982 and December 2003, 120 patients with a primary diagnosis of gastrointestinal or abdominal leiomyoma, leiomyosarcoma, schwannoma, or stromal tumor were chosen from the Tianjin Cancer Hospital database. Five cases were ruled out because the lesions were combined with other tumors. For the remaining 115 cases, hematoxylin and eosin (H&E), and CD117 and CD34 immunohistochemical staining were performed to exclude other diagnoses (GIST: positive for CD117, CD34 or both, as judged by distinct staining of the cytoplasm in at least 10% of the tumor cells). Only 84 tumors were confirmed to be GIST. These 84 patients had enough follow-up data for evaluation and were divided into 4 groups according to the risk of aggressive tumor behavior (very low risk: <2 cm and <5 mitoses/50 high-power fields [HPF]; low risk: 2-5 cm and <5/50 HPF; intermediate risk: <5 cm and 6–10/50 HPF or 5–10 cm and <5/50 HPF; and high risk: >5 cm and >5/50 HPF or >10 cm and any mitotic rate, or any size and >10/50 HPF) [19].

Between January 2003 and September 2006, another 42 GIST patients with fresh tissue available for gelatin zymography were chosen from the Tissue Banking Facility supported jointly by the Tianjin Medical University Cancer Institute and Hospital and the US National Foundation for Cancer Research. These patients were reassessed and confirmed to have GISTs according to the above methods.

2.2. Definition of VM and the grouping of patients

VM was defined as tumor-cell–surrounded channels in which RBCs were present. In H&E-stained slides, VM could be seen to be formed by tumor cells but not endothelial cells without hemorrhage, necrosis, or inflammatory cells infiltrating near these structures. In CD31/periodic acid–Schiff (PAS) double-stained slides, VM consisted of tumor cells (not endothelial cells) forming channels with PAS-positive materials and RBCs. In CD31-stained slides, there were no positive cells in VM. In CD117-stained slides, the cells forming VM were confirmed to be GIST cells.

The above 84 and 42 cases GIST were divided into 2 groups: VM-positive and VM-negative.

2.3. Immunohistochemical investigation

2.3.1. Main agents

The primary antibodies were rabbit polyclonal antiserum raised to human CD117 (dilution 1/500; catalogue no. A4502, Dako Cytomation, Glostrup, Denmark), mouse monoclonal antibody raised to human CD34 (1/100; clone no. QBEnd/10), CD31 (1/100; clone no. IA10), vascular endothelial growth factor (VEGF) (1/100; clone no. VG3), matrix metalloproteinase-2 (MMP-2) (1/100; clone no. 17B11), and MMP-9 (1/100; clone no. 15W2). These primary antibodies were purchased from Zhongshan Goldenbridge Biotechnology Co Ltd, Beijing, PR China). Heat-induced epitope retrieval in citrate buffer (0.01 mol/L; pH 6.0) was applied to all slides before immunohistochemical staining. The 0.5% periodic acid and Schiff solutions were made in the pathology department of Tianjin Cancer Hospital and confirmed to be effective in previous experiments.

2.3.2. Immunohistochemical staining and CD31/PAS double staining

2.3.2.1. Immunohistochemical assays. Staining with primary antibodies against CD117, CD34, CD31, VEGF, MMP-2, or MMP-9 was performed on formalin-fixed, paraffin-embedded tissues with the SP-9000 kit (Zhongshan Golden Bridge Biotechnology Co. Ltd., Beijing, PR China).