Clinicopathological features and differential diagnosis of gastrofibromatosis-like undifferentiated carcinoma

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Abstract
Objective: To study the clinicopathological features and differential diagnosis of gastrofibromatosis-like undifferentiated carcinoma (GFLUC).
Methods: Three patients with GFLUC underwent histological and immunophenotypic analyses and fluorescence in situ hybridization to detect human epidermal growth factor receptor (HER2) gene amplification.
Results: Among the three patients (2 male [36 and 44 years old], 1 female [58 years old]), two had lesions in the gastric body and one had lesions in the gastric antrum. Histological analysis revealed mixtures of aggressive fibromatosis and undifferentiated carcinoma in all three cases. Highly invasive fibromatous tissue, consisting of fibroblasts, proliferating myofibroblasts, and collagenous fibrous tissues, accounted for >90% of the tumor, with undifferentiated cancerous tissue accounting for <10% scattered in the gaps within the invasive fibromatous tissue, with no glandular ducts or nests. Immunophenotypic analysis showed that the undifferentiated cancerous cells were positive for pan-cytokeratin, CDX2, villin, and p53, while the cytoplasm of invasive fibromatous cells was positive for vimentin, β-catenin, and smooth muscle actin. No HER2 gene amplification was detected.
Conclusions: Unlike other gastric carcinomas, GFLUC shows specific histological, biological, and immunophenotypic characteristics.

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Introduction

Gastric carcinoma has diverse biological and genetic features and various etiological factors, including environmental and genetic factors. Gastric carcinoma is characterized by extensive morphological heterogeneity, including different structures, growth patterns, cell differentiation, and histogenesis. The World Health Organization (WHO) named undifferentiated types of digestive tumors as indeterminate carcinomas. Undifferentiated gastric carcinomas demonstrate many types of tissue morphologies, including diffuse small-cell type, spindle-cell type, and giant-cell type. Some scholars have divided undifferentiated gastric carcinoma into undifferentiated types with tubular components and simple undifferentiated types, based on differences in expression profiles of tumor suppressor proteins and tumor-related proteins, and significant differences in tumor metastasis and prognosis.

Compared with benign and malignant tumors, primary gastrofibrosis exhibits invasion, recurrence, and local destruction, with no signs of lymphatic or blood vessel metastasis. In 1994, the WHO defined such tumors as differentiated fibroblastic tumors, based on their specific biological characteristics. In the current study, numerous spindle fibroblasts, myofibroblasts, and collagen fibers, which are the main diagnostic feature of fibromatosis, were found in cancer tissues, in addition to undifferentiated gastric cancer cells. We therefore referred to this type of tumor as gastrofibromatosis-like undifferentiated carcinoma (GFLUC). We further examined the histopathological features and differential diagnosis of GFLUC.

Methods

Clinical data

Records of patients with lymph node metastasis of gastric cancer in the Pathology Departments of Shenzhen Hospital of Southern Medical University and The 990 Hospital of the Joint Logistic Support Force of the PLA between February 2018 and November 2019 were screened for GFLUC. The histopathological diagnostic criteria referred to the WHO classification of gastric cancer in digestive system tumors and gastric tumor pathology. This study protocol was approved by the Medical Ethics Committee of the 989 Hospital of the Joint Logistic Support Force of PLA. Written informed consent was obtained from all patients before the study.

Pretreatment of specimens

All specimens were fixed for 8 to 48 hours with 10% neutral buffered formalin solution within 30 minutes after surgery. In addition, all lymph nodes and cancer nodules were collected, and according to the depth of infiltration, color and texture, four to six pieces were collected from the central and peripheral areas of the tumor, including one piece from each of the deepest infiltration points and the closest serosal layer. The collected samples were prepared for histological and immunophenotypic
analyses and fluorescence in situ hybridization (FISH) analysis.

**Immunohistochemical (IHC) analysis**

IHC analysis was carried out using the EnVision two-step method using the following primary antibodies: pan-cytokeratin (CKpan), CDX2, villin, vimentin, β-catenin, smooth muscle actin (SMA), desmin, anaplastic lymphoma kinase (ALK), S-100 protein, CD99, Bcl-2, CD68, CD163, CD34, CD117, DOG1, p53, Ki-67, programmed cell death protein 1 (PD-1), and PD-1 ligand (PD-L1).Mismatch repair (MMR) proteins included MLH1, MSH2, PMS2, and MSH6. All antibodies were obtained from Shenzhen Dameng Biomedical Technology Co. Ltd. (China), and the procedures were performed in accordance with the kit instructions. HER2 protein was also detected using the same methods, with antibodies purchased from Fuzhou Maixin Biotech. Co., Ltd. (China).

**FISH analysis**

Paraffin-embedded sections were pretreated and FISH was carried out as described previously, and according to the manufacturers’ instructions (Paraffin Pretreatment Kit II and Vysion human epidermal growth factor receptor (HER2) probe kit; Vysis, IL, USA).

Positive IHC samples were analyzed by FISH. Briefly, hematoxylin–eosin-stained specimens were examined under a 10× objective lens, a suitable field was determined by a hybridization signal in >75% of cancer cell nuclei under a 40× objective lens, and >30 intact cancer cells with clear boundaries were then counted under a 100× objective lens. The FISH results were interpreted by calculating the ratio of the number of HER2 gene copies to the number of copies of chromosome 17 in 30 random carcinoma cells: a ratio >2.2 indicated that the HER2 gene was present, <1.8 indicated that the HER2 gene was absent, and the test was repeated for results ≤2.2 and ≥1.8.

**Results**

**Histopathological features**

GFLUC is a rare disease and only three cases were detected, two men (36 and 44 years old) and one woman (58 years old). Two had lesions in the gastric body and one had lesions in the gastric antrum. All three cases were diffuse infiltrative type. Regarding pathological stage, two cases were classified as pT4aN1Mx and the other was pT4aN2Mx. Tumor diameter ranged from 2.1 to 19.4 cm (mean 5.4 cm). The tumors were characterized by no capsule, invasive growth, and slightly hard, off-white sections. Ulcers were present on the surface of the gastric cavity, with a small amount of inflammatory necrotic tissue (Figure 1). All three cases showed a mixture of two histological structures: highly invasive fibromatous tissue, consisting of fibroblasts, proliferating myofibroblasts, and collagenous fibrous tissues, accounted for >90% of the tumor, while the undifferentiated cancerous tissue, which accounted for <10%, was scattered in the gaps of the invasive fibromatous tissue, with no glandular ducts or nests. The pattern of tumor growth was mainly characterized by invasive fibromatosis. Fusiform fibroblasts and collagen fibers were present in bundles or interlaced braided forms, and the collagen fibers sometimes formed conspicuous broad and long bands. After invasion and destruction of the mucosal muscle, the tumor grew into the submucosa and developed the morphological characteristics of fibromatoselike tissue, with accumulations of posterior blood vessels and lymphocytes in some areas. The tumor tissues invaded the
stomach either by mixing with smooth muscle fibers by dividing the fibers into nest-like or disjointed pieces of different sizes, or by growing invasively along the smooth muscle fibers outside the serosa, with no large flaky tumor components (Figure 2). Cancer cells were small and hard to detect; however, the IHC markers CKpan, CDX2, villin, and p53 were prominent. The tumor continued to grow in a crab-like fashion into the adipose tissues outside the serous membrane of the gastric wall (Figure 3). Cytologically, the undifferentiated carcinomas were medium-sized and irregularly round or ovoid in shape. The nuclei were deeply stained, but the chromatin was sometimes granular with a prominent nucleolus, and the nuclei were sometimes off-site, pulpy, signet-ring cell-like cancer cells, with mitotic indices of 3% to 7%. The tumors had a small amount of lymphocyte infiltration. GFLUC is extremely aggressive, but the tumor tissue is not necrotic and it does

Figure 1. Gastrofibromatosis-like undifferentiated carcinomas showing ulcers on the surface of the gastric cavity, with a small amount of inflammatory necrotic tissue (original magnification ×200 for fluorescence in situ hybridization analysis). Hematoxylin and eosin staining.

Figure 2. Gastrofibromatosis-like undifferentiated carcinomas showing some tumor cells growing invasively along the smooth muscle fibers outside the serosa without large flaky tumor components (left, original magnification ×200 for fluorescence in situ hybridization, hematoxylin and eosin). Immunohistochemical markers were positive for CKpan (right, original magnification ×200, EnVision staining).

Figure 3. In gastrofibromatosis-like undifferentiated carcinomas, the tumor continued to grow in a crab-like fashion into adipose tissues outside the serous membrane of the gastric wall (original magnification ×200 for fluorescence in situ hybridization). Hematoxylin and eosin staining.
not have a high lymph node metastasis rate. Although all three patients in this study had lymph node metastasis, the metastases had the same histological structure as the primary lesion. Other features mainly included fibrous connective tissue hyperplasia, singly scattered cancer cells, and no glandular duct or nest-like structures (Figure 4).

**Immunophenotypic analysis**

Undifferentiated cancerous cells were positive for CKpan, CDX2, villin, and p53, while the cytoplasm of invasive fibromatous cells was positive for vimentin, β-catenin, and SMA. In addition, the MMR proteins MLH1, MSH2, PMS2, and MSH6 were all detected in these cells. The Ki-67 index was 70% to 80% (Figure 5). Furthermore, 1% to 49% PD-L1 expression was only detected in one patient, while PD-1 was observed in tumor mesenchymal lymphocytes in two patients, but not in the other patient. HER2 protein was detected on the cell membranes of GFLUC cells by IHC analysis.

**FISH analysis**

The HER2 gene was not detected in these cells by FISH analysis.

**Follow-up data**

The three patients were followed-up every 3 months for 3 to 12 months, with no signs of recurrence.

**Discussion**

Some researchers have suggested dividing tumors originating in the epithelial and mesenchymal lobes of the stomach into two subtypes: differentiated and undifferentiated cancers, to improve the application of targeted therapies. Surgery is currently the main clinical treatment, and histomorphology and IHC analysis are the routine methods of pathological diagnosis. However, the morphology of undifferentiated gastric carcinoma varies, with a correspondingly different prognosis. Given that GFLUC had the characteristics of fibromatosis, we identified five key points relevant to its histopathological diagnosis: 1) GFLUC was extremely aggressive and destroyed the surrounding tissue, without...
a capsule, but did not cause tissue necrosis; 2) all three cases included mixtures of highly invasive fibromatous tissue, and undifferentiated cancerous tissue (<10%), scattered in gaps in the invasive fibromatous tissue, without glandular ducts or nests; 3) cytologically, medium-sized, irregularly round or ovoid undifferentiated carcinoma cells with deeply stained nuclei, but the chromatin was sometimes granular with prominent nucleoli, and the nuclei were sometimes off-site, pulpy, signet-ring cell-like cancer cells with mitotic indices of 3% to 7%; 4) the histological structure of the lymph node metastases was the same as that of the primary lesion; and 5) CKpan, CDX2, villin, and p53 were positive in undifferentiated cancerous cells, while vimentin, β-catenin, and SMA were positive in invasive fibromatous cells.

The key points for the differential diagnosis of GFLUC are described in Table 1 and the possible differential diagnoses are summarized below. 1) Aggressive gastric fibromatosis including proliferative spindle-shaped fibroblasts of uniform morphology and collagen fibers is characterized by strong invasion and destruction of smooth muscle, vasculature, and nerve tissue; immunohistochemically positive for vimentin, SMA, and β-catenin and negative for CKpan, CDX2, villin, CK20, and p53.17 2) Undifferentiated carcinoma of the stomach is a malignant tumor with no glandular structure or other clearly differentiated features. The tumor has been described in some studies as an indeterminate carcinoma,2 while others reported it as diffuse small-cell type, spindle-cell type, and giant-cell type.3–5 The proportion of mesenchyme in undifferentiated carcinomas was <50%, comprising proliferating spindle fibroblasts, fibroblasts, and collagenous fibroblasts. Structural pieces, nests, and cords composed of cancer tissues accounted for >50% of the tumor. Immunophenotypic analysis demonstrated that mesenchymal cells in differentiated carcinomas were negative for β-catenin and SMA.18,19 3) Fibromatosis-like metaplastic carcinoma (FLMC) generally occurs in the mammary gland and is rare in the stomach. Histologically, FLMC is composed of spindle cells with glandular, squamous epithelium, or heterologous components. The spindle cells are diverse in morphology, showing mild or regional polymorphism, and may be arranged in bundles. FLMCs include areas with vascular lumen structures and squamous cell differentiation. FLMC has a similar histological phenotype to desmoid fibromatosis and is a kind of low-grade metaplastic carcinoma. Immunophenotypic analysis demonstrated that this type of tumor was positive for CKpan, 34βE12, and vimentin.20,21 4) Malignant solitary fibrous neoplasms of the stomach are composed of unfixed tissue. The histological features include areas with an irregular distribution of sparse tumor cells and areas with abundant tumor cells, with many dense fibroids indwelling between the areas. Histologically, tumor cells are abundant, with moderate to severe atypical necrosis of the tumor tissue and lymph node metastasis. Immunophenotypic analysis demonstrated that these tumors were positive for CD34, signal transducer and activator of transcription (STAT)6, CD99, Bcl-2, and vimentin, focally weakly positive for CKpan, epithelial membrane antigen, SMA, S-100, and desmin, and negative for DX2 and villin.22,23 5) Schwannomas are rare in the stomach and occur with a frequency similar to that of gastric leiomyoma. They often occur in the elderly, forming a 2- to 5-cm tumor in the stomach wall. The clinical manifestations and gross features are similar to gastrointestinal stromal tumors, while the biological behavior of the tumors is generally benign, with rare recurrence. The tumor cells are composed of spindle cells, arranged in cross bundles, and the nuclei are arranged in a palisade pattern, with
| Tumor                                      | Definition                                                                 | Cytologic characteristics                  | Histopathological features                                      | Immunophenotype                                      |
|--------------------------------------------|----------------------------------------------------------------------------|---------------------------------------------|----------------------------------------------------------------|------------------------------------------------------|
| Gastric fibromatosis-like undifferentiated carcinoma | Undifferentiated gastric cancer with fibromatosis                           | Tumor cells irregular round or oval         | Histological structure of lymph node metastasis same as primary tumor | CK Pan (+), CDX2 (+), villin (+), p53 (+), vimentin (+), b-catenin (+), SMA (+) |
| Primary invasive fibromatosis of stomach    | Fibrous tumor caused by excessive proliferation of fibroblasts and myofibroblasts | Cell composition simple, and inflammatory cells rare | Tumor cells strong invasion, and easily damage surrounding tissue | b-catenin (+), vimentin (+), SMA (+)                  |
| Undifferentiated carcinoma of stomach       | Malignant tumor without glandular structure or other well-defined differentiation characteristics | Diffuse small cells, spindle cells, and giant cells common | Tumor tissues form blocks, nests, and cord structures, with proportion >50% | CK Pan (+), CDX2 (+), villin (+), CK 20 (+), p53 (+) |
| Fibromatoid metaplastic carcinoma           | Low-grade spindle cell metaplastic carcinoma                               | Squamous cell differentiation groups common | Tumor tissues consist of spindle cells, glandular or squamous epithelium, and other components | CK Pan (+), 34/ E12 (+), vimentin (+)                |
| Malignant solitary fibrous tumor of stomach | Fibroblastic tumor with differentiation of CD34 + dendritic stromal cells | Moderate or severe atypia of tumor cells common | More dense scar-like collagen fibers between different tumor groups | CD 34 (+), STAT 6 (+), CD 99 (+), Bcl-2 (-), vimentin (+) |
| Schwannoma                                  | Benign peripheral nerve sheath tumor                                       | Cytoplasm abundant and nucleus spindle shaped | Tumor tissues consist of bundles of spindle cells and collagen fibers | S-100 (+), SOX 10 (+)                                |
| Gastric plexiform fibromyxoma               | Mesenchymal tumor with myoid differentiation                              | Nucleus spindle or oval, and cytoplasm slightly eosinophilic | Tumor cells grow in nodules, and tumor tissues contain abundant mucinous or fibromyxoid matrix | ν-SMA (+), MSA (+)                                   |
| Inflammatory myofibroblastic tumor          | Myofibroblastic tumor in children and adolescents                         | Most cytological morphology mild with few slightly heteromorphic cells | Tumor tissue consist of spindle fibroblasts, myofibroblasts, and abundant inflammatory cells | Vimentin (+), SMA (+), ALK (+), desmin (+), calponin (+), CD 34 (+) |
| Synovial sarcoma                            | Spindle cell tumor with epithelial differentiation and unique chromosome translocation (x; 18) (p11; q11) | Epithelioid cells in clusters or nests common, and occasionally in the glandular structure | Tumor tissue consist of epithelioid cells and fibrosarcoma-like spindle cells | CK (+), EMA (+)                                     |
| Epithelioid gastrointestinal stromal tumor  | Tumor derived from gastrointestinal stromal stem cells                    | Two main forms of tumor cell cytoplasm eosinophilic and transparent | Tumor tissue consist of round, oval, or short spindle epithelioid cells with diffuse distribution | CD 117 (+), GOG 1 (+), CD 34 (+), SMA (+), S-100 (+) |
some collagen fibers between cells. Immunophenotypic analysis has demonstrated that schwannomas are positive for diffuse S-100 and CD34 and negative for CKpan, CDX2, villin, and β-catenin. Gastric plexus fibromyxomas are rare gastric mesenchymal-derived tumors with unique histological features, mainly occurring in the gastric antrum. The tumors grow in clusters or multiple nodular shapes of different sizes in the gastric wall, and are staggered with the smooth muscle of the gastric wall. The tumors may be abundant in small thin-walled vessels, and the cells are rich in myxoid or fibrous myxoid matrix. The nuclei of the tumor cells are spindle-shaped or oval, and the nucleoli are not obvious. The cytoplasm appears slightly eosinophilic, without obvious atypia. Immunophenotypic analysis showed that these tumors were positive for α-SMA and muscle-specific actin and negative for β-catenin. Fibroblast differentiation in the tumors was revealed by electron microscopy.

Inflammatory myofibroblast tumors mainly occur in children under 10 years old. They are composed of proliferating fusiform fibroblasts and myofibroblasts, along with many inflammatory cells infiltrating the mesenchyme. Most of the inflammatory cells are mature plasma cells, lymphocytes, and eosinophils, with a few neutrophils. Regional lymphocyte aggregation can be observed. In addition to spindle cells, round histiocyte cells are present in the lesions, with some irregular, polygonal, or bizarre cells in some cases. Eosinophilic or basophilic inclusion bodies can also be seen in the nuclei, similar to ganglion cells or R-S cells. Immunophenotypic analysis demonstrated that these tumors were positive for vimentin, SMA, desmin, MSA, and ALK and negative for CD117, DOG1, β-catenin, and S-100 protein, with an ALK-positive ratio of 50%. Synovial sarcomas are malignant mesenchymal tumors with varying degrees of epithelioid differentiation. Reverse transcriptase polymerase chain reaction revealed a characteristic SS18/SSX2 fusion transcript, t(X; 18) (p11.2; q11.2), consistent with synovial sarcoma. Synovial sarcomas can occur in many different locations throughout the body and are rarely found within the gastrointestinal tract. They typically form a bipolar structure with a mixture of carcinoid epithelioid cells and fibrosarcomatous spindle cells in different proportions. These tumors often occur in the gastric body and the fundus of the stomach, and occasionally in the gastric antrum and gastroesophageal junction. They range from 0.8 to 16.0 cm in diameter, with multiple nodules or single nodules. The tumor surface is uneven, and off-white or off-red in section. Ulceration may occur, with or without necrosis. Twenty-two cases of monophasic synovial sarcoma of the stomach and two cases of bipolar sarcoma have been reported in the literature.

Immunophenotypic analysis showed that the tumors were positive for CK and EMA, and molecular tests showed that the SYT-SST gene was fused. Gastrointestinal epithelioid stromal neoplasms are the most common mesenchymal neoplasms of the gastrointestinal tract. They can occur anywhere in the stomach, from small mural nodules to large complex masses with intra- and extraluminal growth. The tumors comprise many types, mostly spindle-cell type, but also a few epithelioid-cell type and a mixture of spindle-cell and epithelial-cell type. They have a specific morphology with sarcomatous characteristics, accompanied by a large number of nuclear atypia and mitotic findings, indicating a polymorphic cell type. Immunophenotypically, gastrointestinal epithelioid stromal neoplasms are mainly positive for CD117 and DOG1, partially positive for CD34 and S100 protein, and negative for MyoD1, myogenin, myoglobin, desmin, and actin. Molecular biology tests showed the presence of mutations in the KIT and PDGFRA genes.
Overall, gastric undifferentiated carcinomas show diverse histological morphologies, including tubular undifferentiated, simple undifferentiated, diffuse small-cell, spindle-cell, and giant-cell types. We named these tumors GFLUC, and discussed their histopathological characteristics, immunophenotype, and differential diagnosis. GFLUCs differed from other types of gastric cancer, and their differential diagnosis is critical. More records of GFLUC and longer follow-up times are needed to improve our understanding of the biological, genetic, and etiological characteristics of GFLUC and to further explore the differences in abnormal protein expression and clinicopathological parameters.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

Funding
This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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