Case Report
Genetic alterations and expression of epithelial-mesenchymal transition markers in gastric sarcomatoid carcinoma: report of a case and review of literature

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Abstract: Sarcomatoid carcinoma arising from stomach is rare and its molecular alterations are largely unknown. We reported one case of gastric sarcomatoid carcinoma in a 69-year-old man who was hospitalized for upper abdominal discomfort and melena. Endoscopic examination revealed an infiltrative and ulcerated mass in the body of stomach. A huge gastric mass was also evident on CT scan. Histologically, the tumor was composed of diffuse undifferentiated cells with high mitotic activity and pleomorphism. Immunohistochemistry revealed intense and diffuse staining for vimentin and p53, focal staining for cytokeratin and loss of cadherin and β-catenin in tumor cells indicative of epithelial-mesenchymal transition. Differential diagnosis was excluded by negative expression of CD56, CgA, CD3, CD20, CD10, bcl-6, S-100, CD34, desmin, SMA and HMB45. Mutations of nine genes could be found by next generation sequencing including TP53, ETV1, SOX21, GATA6, FAT1, NORCH2NL, MED12, SRC and NSD1. In addition, four genes were shown to have amplification, including SOX2, GATA2, RPTOR and CCND1, while RB1 exhibited loss of gene copy number. The patient was diagnosed as sarcomatoid carcinoma and given one cycle of chemotherapy with oxaliplatin and S-1. One month later, the tumor progressed rapidly with ascitis and liver metastasis and the patient died for suspected disseminated intravascular coagulation (DIC).

Keywords: Sarcomatoid carcinoma/genetics, stomach neoplasms, epithelial mesenchymal transition, next-generation sequencing

Introduction
Sarcomatoid carcinoma of the stomach is an extremely rare biphasic tumor which consists of both epithelial and mesenchymal elements. There have been more than 50 cases reported so far who were recently reviewed by Cirocchi to have a poor prognosis of median survival to be only 6.5 months in spite of surgery and chemotherapy [1]. In order to understand and develop new tailored therapeutics to improve the prognosis for this rare disease, it is essential to explore its oncogenic mechanisms. Herein, we presented a case of gastric sarcomatoid carcinoma and analyzed its molecular characteristics by next-generation sequencing (NGS) of 416 cancer-related genes together with epithelial-mesenchymal transition (EMT) markers by immunohistochemistry staining.

Case report

Clinical history
A 69-year-old man was admitted to our division reporting a history of epigastric discomfort with intermittent melena and weight loss (10 kilos over 3 months). The physical examination revealed emaciation, marked pallor and a 10 cm mass in epigastric region. Routine laboratory parameters were found to be normal except for leukocytosis, anemia and hypoalbuminemia, white blood cell (WBC) 15.91×10³/L, hemoglobin 82 g/L, and albumin 34 g/L. The levels of tumor markers, carcinoembryonic antigen (CEA) and carbohydrate anti-gen (CA 19-9) were 0.81 ng/ml and 7.68 ng/ml, respectively (normal CEA <5 ng/ml, CA 19-9 <37 ng/ml).
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Figure 1. A, B: Abdominal CT scan showed diffuse wall thickening and stiffness of the gastric body to form a huge mass at diagnosis (2014-12-6). C, D: The mass of gastric body enlarged with liver metastasis and ascites after one cycle of chemotherapy (2015-1-8).

Figure 2. Immunohistochemical staining of EMT-related proteins and TP53 in gastric sarcomatoid carcinoma. HE staining, the tumor was composed of diffuse undifferentiated cells with pleomorphism (A); Diffuse staining of vimentin (B); Focal staining of CKpan (C); Diffuse and intense staining of p53 (D); Negative staining of E-cadherin (E) and β-catenin (F).
Table 1. Genetic alterations in analyzed 416 genes by NGS

<table>
<thead>
<tr>
<th>Genes</th>
<th>Nucleotide variation</th>
<th>Protein variation</th>
<th>Mutation frequency</th>
<th>Point mutation impact (COSMIC)</th>
<th>Gene function (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>c.G638A</td>
<td>p.R213Q</td>
<td>58%</td>
<td>Loss of function (never reported in gastric neoplasms)</td>
<td>Oncosuppressor, up-regulating both cell cycle arrest and apoptotic factors [2]</td>
</tr>
<tr>
<td>ETV1</td>
<td>c.A113G</td>
<td>p.D38G</td>
<td>52%</td>
<td>Unknown (never reported)</td>
<td>Oncogene, promote cell proliferation, motility and invasion [3]</td>
</tr>
<tr>
<td>SRC</td>
<td>c.A913T</td>
<td>p.M305L</td>
<td>6%</td>
<td>Unknown (never reported)</td>
<td>Oncogene, tumorigenesis and acquisition of the invasive phenotype [7]</td>
</tr>
<tr>
<td>NSD1</td>
<td>c.T548C</td>
<td>p.I183T</td>
<td>6%</td>
<td>Unknown (never reported)</td>
<td>Regulation of chromatin structure and function [8]</td>
</tr>
<tr>
<td>NOTCH2NL</td>
<td>c.G427A</td>
<td>p.D143N</td>
<td>5%</td>
<td>Unknown (reported in skin tumor)</td>
<td>Repressing transcriptional activities of Notch proteins [9]</td>
</tr>
<tr>
<td>MED12</td>
<td>c.O6226T</td>
<td>p.Q2076X</td>
<td>1%</td>
<td>Unknown (never reported)</td>
<td>Transcriptional regulation of the RNA polymerase II complex and maintain gene stability [10]</td>
</tr>
</tbody>
</table>

Figure 3. Point mutation G638A of TP53 gene discovered by NGS was validated by Sanger sequencing in a case of gastric sarcomatoid carcinoma.
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Endoscopic examination revealed a huge ulcerative lesion that infiltrated the posterior wall of the stomach and part of fundus. The abdominal computed tomography scan showed diffuse wall thickening of the gastric body to form a huge unevenly contrasted mass with a diameter of 18 cm, which infiltrated through serosa to the pancreas (Figure 1A, 1B). An endoscopically taken biopsy revealed the diagnosis of sarcomatoid carcinoma by pathological and immunostaining findings (Figure 2A, 2B).

The patient received one cycle of chemotherapy with oxaliplatin (150 mg) and S-1 (80 mg/day, 14 days). 25 days later, the patient was hospitalized again for the deterioration of abdominal distension and malaise. The physical examination revealed shifting dullness of abdomen. Blood tests showed severe anemia with hemoglobin concentration of 79 g/L, leukocytosis (21.46×10^9/L), and low albumin level (25.7 g/L). Platelet count was 165×10^9/L. Abdominal enhanced CT scan showed that the enlargement of gastric tumor, liver metastasis and peritoneal effusion (Figure 1C, 1D), indicative of rapid progression. Best supportive care was then given to the man due to his bad conditions. To make illness even worse, widespread petechia could be seen around the umbilicus 2 days after hospitalization and blood tests revealed a rapid decrease of platelet count (9×10^9/L) and coagulation abnormality (Pronthrombin time 18.30 s, Fibronogen 1.96 g/L and D-dimer 1.60 mg/L). Disseminated intravascular coagulation (DIC) was suspected to occur secondary to cancer and the man died soon in spite of fresh blood plasma and red blood cell transfusion. The total survival time from diagnosis was only one month.

Pathological findings and expression of EMT markers

Histologically, the tumor was composed of diffuse undifferentiated cells with high mitotic activity (Ki67 labeling as high as 90%) and pleomorphism (Figure 2A). No carcinomatous component was recognized. Immunohistochemistry staining revealed focal positivity for pan-cytokeratin Cam5.2, diffuse positivity for vimentin and p53 (Figure 2B-D), but negativity for CD3, CD20, HMB45, SMA (-), S-100, CD21, Bcl-6, CD10, CD3, CgA, CD56, CD117 and CD34. As for EMT markers, loss of E-cadherin and β-catenin was evident (Figure 2E, 2F) together with diffuse and intense staining of vimentin (Figure 2B). So these immunohistochemical findings led to a diagnosis of Sarcomatoid carcinoma of the stomach.

Genetic analysis by NGS

Genomic DNA was extracted from FFPE tissues with QIAamp DNA mini kit (Qiagen, Heidelberg, Germany). Fragment DNA was generated with Bioruptr (Diagenode, Bioruptor UCD-200) and Libraries were constructed with the KAPA Hyper DNA Library Prep Kit, (KAPA Biosystem, KK8504). Dual-indexed sequencing libraries were PCR amplified with KAPA HiFi Hot start-ready Mix (KAPA, KK2602) for 4-5 cycles, then cleaned up by 1x purification Beads (Corning, AxyPrep Fragment Select-I kit, 14223162). The 5’-biotinylated probe solution was provided as capture probes. The baits target 416 cancer-related genes (Geneseq Technology Inc.). Illumina HiSeq 4000 was used for 75×75 paired-end sequencing (80-100 per flowcell).

Mutations of nine genes could be found including TP53, ETV1, SOX21, GATA6, FAT1, NORCH2NL, MED12, SRC and NSD1 (Table1). Of note, the first five genes had high mutation frequency and TP53 mutation (exon 6, G638A) was validated by Sanger sequencing (Figure 3). In addition, gain of copy numbers could be seen in the genes of SOX2, GATA2, RPTOR and CCND1, while RB1 exhibited loss of gene copy number (Figure 4).

A literature review of gastric sarcomatoid carcinoma excluding gastric carcinosarcoma

In order to characterize the clinical features of true gastric sarcomatoid carcinoma, we dogged...
Table 2. A review of gastric sarcomatoid carcinoma diagnosed on both histological and immunohistological characteristics

<table>
<thead>
<tr>
<th>No</th>
<th>Year</th>
<th>Age</th>
<th>Sex</th>
<th>Location</th>
<th>D (cm)</th>
<th>EGDS</th>
<th>Symptoms</th>
<th>T</th>
<th>N</th>
<th>M</th>
<th>Vimentin</th>
<th>CK</th>
<th>EMA</th>
<th>CAM5.2</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1990</td>
<td>11</td>
<td>M</td>
<td>fundus</td>
<td>5</td>
<td>polyoid</td>
<td>Epigastric pain</td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Resection and chemotherapy for recurrence</td>
<td>45 m, dead</td>
</tr>
<tr>
<td>2</td>
<td>1990</td>
<td>11</td>
<td>F</td>
<td>GEJ</td>
<td>5</td>
<td>polyoid</td>
<td>swallowing pain</td>
<td>T4</td>
<td>Nx</td>
<td>kidney, lung, ovary, pleura, peritoneum</td>
<td>focal+</td>
<td>-</td>
<td>NA</td>
<td>Esophagogastrectomy, jejunostomy and splenectomy</td>
<td>6 m, dead</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1990</td>
<td>11</td>
<td>F</td>
<td>GEJ</td>
<td>5</td>
<td>polyoid</td>
<td>infiltrative ulcerative Epigastric distension</td>
<td>T4</td>
<td>N+</td>
<td>ovary</td>
<td>focal+</td>
<td>-</td>
<td>NA</td>
<td>Gastrectomy, bilateral salpingoophorectomy and chemotherapy</td>
<td>8 m, alive wd</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1993</td>
<td>12</td>
<td>M</td>
<td>Body, antrum</td>
<td>11</td>
<td>NA</td>
<td>NA</td>
<td>T4a</td>
<td>Nx</td>
<td>liver</td>
<td>+</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>gastric cancer resection</td>
<td>2 m, dead</td>
</tr>
<tr>
<td>5</td>
<td>1993</td>
<td>12</td>
<td>M</td>
<td>body</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>T3</td>
<td>Nx</td>
<td>M0</td>
<td>+</td>
<td>NA</td>
<td>-</td>
<td>focal+</td>
<td>gastric cancer resection</td>
<td>49 m, alive fd</td>
</tr>
<tr>
<td>6</td>
<td>1993</td>
<td>12</td>
<td>M</td>
<td>antrum</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>T3b</td>
<td>Nx</td>
<td>M0</td>
<td>focal+</td>
<td>NA</td>
<td>focal+</td>
<td>focal+</td>
<td>gastric cancer resection</td>
<td>60 m, alive fd</td>
</tr>
<tr>
<td>7</td>
<td>1993</td>
<td>12</td>
<td>F</td>
<td>body, antrum</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
<td>T4</td>
<td>Nx</td>
<td>liver</td>
<td>focal+</td>
<td>NA</td>
<td>+</td>
<td>focal+</td>
<td>gastric cancer resection</td>
<td>1 m, dead</td>
</tr>
<tr>
<td>8</td>
<td>1993</td>
<td>12</td>
<td>M</td>
<td>antrum</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
<td>T4</td>
<td>Nx</td>
<td>M0</td>
<td>+</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>gastric cancer resection</td>
<td>3 m, dead</td>
</tr>
<tr>
<td>9</td>
<td>1993</td>
<td>12</td>
<td>M</td>
<td>body, antrum</td>
<td>13</td>
<td>NA</td>
<td>NA</td>
<td>T3</td>
<td>Nx</td>
<td>M0</td>
<td>focal+</td>
<td>NA</td>
<td>focal+</td>
<td>focal+</td>
<td>gastric cancer resection</td>
<td>6 m, dead</td>
</tr>
<tr>
<td>10</td>
<td>2002</td>
<td>13</td>
<td>M</td>
<td>antrum</td>
<td>3.5</td>
<td>polypoid</td>
<td>hematemesis</td>
<td>T3</td>
<td>Nx</td>
<td>liver</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>Subtotal gastric cancer and liver wedge resection</td>
<td>8 m, alive fd</td>
</tr>
<tr>
<td>11</td>
<td>2007</td>
<td>14</td>
<td>F</td>
<td>Cardia, body</td>
<td>21</td>
<td>Infiltrative ulcerative Epigastric discomfort</td>
<td>T4</td>
<td>Nx</td>
<td>M0</td>
<td>+</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
<td>palliative gastric resection with chemotherapy</td>
<td>16 days, dead</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2007</td>
<td>15</td>
<td>F</td>
<td>body</td>
<td>15</td>
<td>polypoid</td>
<td>Epigastric pain</td>
<td>T3</td>
<td>N+</td>
<td>liver</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>gastric cancer resection</td>
<td>12 m, dead</td>
</tr>
<tr>
<td>13</td>
<td>2012</td>
<td>1</td>
<td>F</td>
<td>fundus</td>
<td>20</td>
<td>polypoid</td>
<td>Epigastric pain</td>
<td>T4</td>
<td>N+</td>
<td>liver</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NA</td>
<td>Gastrectomy and RFA for liver metastasis</td>
<td>4 m, dead</td>
</tr>
<tr>
<td>14</td>
<td>2013</td>
<td>16</td>
<td>M</td>
<td>cardia</td>
<td>4</td>
<td>polypoid</td>
<td>melana</td>
<td>Tx</td>
<td>Nx</td>
<td>M0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>Gastrectomy of remnant stomach</td>
<td>7 m, alive fd</td>
</tr>
<tr>
<td>15</td>
<td>2013</td>
<td>17</td>
<td>F</td>
<td>fundus</td>
<td>7.5</td>
<td>polypoid</td>
<td>Epigastric pain</td>
<td>T2</td>
<td>N+</td>
<td>M0</td>
<td>+</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
<td>palliative gastric resection with omentectomy</td>
<td>3 m, alive fd</td>
</tr>
<tr>
<td>16</td>
<td>2013</td>
<td>18</td>
<td>F</td>
<td>antrum</td>
<td>12</td>
<td>Infiltrative ulcerative Epigastric pain</td>
<td>T4</td>
<td>N+</td>
<td>peritoneum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>palliative gastric resection with chemotherapy</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2015</td>
<td>19</td>
<td>M</td>
<td>antrum, body</td>
<td>14</td>
<td>polypoid</td>
<td>melana</td>
<td>T4</td>
<td>N+</td>
<td>M0</td>
<td>focal+</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>resection of distal stomach, gallbladder and right hemicolon</td>
<td>2 m, alive fd</td>
</tr>
</tbody>
</table>

EGDS: esophagogastroduodenoscopy; GEJ, gastroesophageal junction; NA, non-available. N+, lymphnode positive; RFA, radiofrequency ablation; wd, with disease; fd, free of disease; m, month.
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into the PubMed database from 1980 to 2015 and excluded the cases of carcinosarcoma on the basis of the following definitions. Keywords used were Gastric Sarcomatoid Carcinoma OR Gastric Carcinosarcoma OR Spindle cell carcinoma, Stomach OR Stomach neoplasms, Vimentin positive. Only those sarcomatous component stains positive, at least focally, for at least one epithelial marker could be diagnosed as true sarcomatoid carcinoma. By contrast, when the sarcomatous components do not express epithelial markers or reveal typical specialized differentiation, such as the obvious striation of rhabdomyosarcoma or osteoid production by malignant neoplastic cells, the diagnosis should be carcinosarcoma. In total, we found 18 cases of gastric sarcomatoid carcinoma (including our report) (Table 2) [11-19]. Females (8 cases) were similarly affected as males (10 cases). The median age was 62 years (range, 47-80) and median tumor diameter was 9.5 cm. It could arise from all areas of the stomach and it did not occur more frequently in any one area. 8 cases were polyloid and 4 cases were ulcerated in appearance. Most of cases were in advanced stage at diagnosis. 10 cases had T4, 7 cases had lymphnodes metastasis, 9 cases had distant metastasis and liver was the most often metastatic site. Although 17 patients had a surgical procedure and in most cases curative surgery was performed, the median survival was only 6.5 m. Of note, 3 patients survived as long as 45 m, 49 m and 60 m respectively, which might be due to their early tumor stage at diagnosis (T1b to T3, N0 or Nx, M0).

Discussion

Sarcomatoid carcinoma arising from stomach is a rare tumor of unclear etiology and pathogenesis. Sarcomatoid carcinoma and carcinosarcoma were often mixed up and most cases reported in literature did not make such a distinction. Only those sarcomatous component stains positive, at least focally, for at least one epithelial marker could be diagnosed as true sarcomatoid carcinoma, for sarcomatoid carcinoma was considered to develop through a “conversion theory” (known as epithelial-to-mesenchymal metaplastic transformation [20]. In the present case, loss of membrane E-cadherin and β-catenin indicated the occurrence of EMT and validated the conversion theory. Based on the above histological and immunohistochemical characteristics, we found more than half of 13 cases in the review by Cirocchi [1] should be diagnosed as carcinosarcoma, while the others could be classified as sarcomatoid carcinoma or undefined. Whether such distinction is of clinical value remains unknown. However, both of them seem to have dismal prognosis. Besides, few cases of gastric sarcomatoid carcinoma have been reported on their response to chemotherapy [14], the case we reported here was extremely aggressive and refractory to chemotherapy. Thus we need a comprehensive dissection of the molecular mechanisms underlying the initiation and progression of this disease and then develop novel therapeutic strategies to specifically target it.

Next-generation sequencing (NGS) is a powerful technology for elucidating the pathogenesis of human cancer and identifying potential therapeutic targets. As far as we know, this is the first case report analyzing its genetic alterations by NGS in gastric sarcomatoid carcinoma. For the sarcomatoid carcinomas from lung [21] and urinary tract [22], they shared the similar molecular characteristics as carcinomas, thus basically, sarcomatoid carcinoma was considered to be a special type of carcinoma. However, there have not been similar comparative study of genetic variations on gastric sarcomatoid carcinoma with carcinomas. The case presented here was found to have a high mutation load and nine genes were detected to have a point mutation. Of note, five genes had high mutation frequency which included TP53, ETV1, SOX21, GATA6, FAT1 and might be the candidate driver mutated genes in this case. Except for TP53, the other four gene mutations were uncommon in gastric cancer [23] which might result from the fact that gastric cancer is a highly heterogeneous disease and each patient has distinct genetic and molecular profile. Most of these point mutations in this case have not been observed in COSMIC database and their functional data were still lacking except TP53. However, considering the important roles in tumor growth and invasion of these four genes (ETV1, SOX21, GATA6, FAT1) [3-6], we could postulate that they contributed to the aggressiveness of this case. In addition, four genes (TP53, ETV1, GATA6, FAT1) were all involved in EMT [5, 6, 24, 25] and might contribute to EMT phenotype and chemotherapy resistance in the
present case. Unfortunately, the functional roles of these mutated genes are sparse and the road to targeted therapy against them are still long.

In addition to high load of genes mutation in this case, five genes exhibited copy number variation. Except GATA2, copy number variations of the other four genes (CCND1, SOX2, RPTOR and Rb1) have been reported in gastric neoplasms of COSMIC database with a low frequency. Of note, copy number gain of CCND1 and SOX2 genes which encode cyclin D1 and stem cell factor Sox2 respectively, as well as copy number loss of Rb1 could synergistically promote cancer cell proliferation [26-28]. Besides, GATA2 was considered to be a potential metastasis-driving gene [29], which might contribute to the rapid liver metastasis in this case.

By far, there are no specific treatment guidelines due to the limited number of cases of gastric sarcomatoid carcinoma. However, early diagnosis and radical surgery with adjuvant chemotherapy may be an important approach to improve the dismal prognosis. Since most cases will relapse and metastasize, new targeted therapies should be developed, which warrants whole-genome sequencing and comprehensive molecular profiling in more cases in the future.

Disclosure of conflict of interest

None.

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References


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