Clinicopathological significance of gastric poorly differentiated medullary carcinoma

(低分化髄様胃癌の臨床病理学的特徴)

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Abstract

Poorly differentiated gastric adenocarcinoma of solid type is known to show a clinicopathological diversity, but its morphological characteristics have rarely been investigated. In this study, we defined poorly differentiated medullary carcinoma indicating the following three characteristics: (i) more than 90% of the entire tumor were composed of poorly differentiated adenocarcinoma in a medullary growth, (ii) the tumor exhibited an expansive growth at the tumor margin, and (iii) special types such as an α-fetoprotein-producing carcinoma, neuroendocrine carcinoma, and carcinoma with lymphoid stroma were excluded. Based on the definition, we subclassified the poorly differentiated gastric adenocarcinoma of solid type into the two groups: medullary carcinoma and non-medullary carcinoma, and clinicopathologically analyzed 23 cases of medullary carcinomas and 38 cases of non-medullary carcinomas. The medullary carcinomas less frequently displayed lymphatic invasion, venous invasion, and lymph node metastasis, compared with the non-medullary carcinoma \( (P < 0.001, P = 0.002, \text{ and } P < 0.001, \) respectively). The patients with medullary carcinomas significantly showed better disease-free survival \( (P = 0.017) \). This is the first study to demonstrate that poorly differentiated adenocarcinoma of solid type can be subclassified into tumors with low and
high malignant potentials. Gastric poorly differentiated medullary carcinoma is considered to be a novel histological type predicting good patients’ prognosis.
There are various histopathological classifications of gastric cancer. The Japanese classification of gastric carcinoma classifies it into the differentiated and undifferentiated types (11, 19), while the Lauren classification categorizes gastric carcinomas into the intestinal and diffuse types (15), which mostly correspond with differentiated and undifferentiated types, respectively. However, gastric carcinomas often consist of a mixture of various histological patterns. According to the Japanese and Lauren classifications, the most quantitatively superior histological pattern became the diagnostic category (histopathological diagnosis), but the other histological patterns are not reflected in the diagnostic category. Histological types according to the World Health Organization (WHO) classification indicates tubular/papillary/mucinous adenocarcinoma, and poorly cohesive carcinoma (5). The WHO classification recommends that the diagnostic category includes not only the most quantitatively superior histological pattern, but also the other histological patterns.

According to the Japanese classification of gastric carcinoma, poorly differentiated carcinomas are divided into two groups, i.e., solid type and non-solid type. The solid type exhibits a sheet-like solid growth pattern with scanty stroma, whereas the non-solid type shows a small alveolar, cord-like, or isolated pattern with abundant fibrous stroma. The
clinicopathological features of the poorly differentiated carcinoma of solid type less
frequently exhibit lymphatic invasion and lymph node metastasis, and show better patients’
prognosis compared to the non-solid type carcinoma (9, 14, 21, 25). However, poorly
differentiated carcinomas of solid type are thought to be a heterogenous histological group,
because the Japanese classification defines the histological type of gastric carcinomas as a
quantitatively superior histological pattern. In this study, therefore, we have defined
histological criteria of “poorly differentiated medullary carcinoma” as a carcinoma of pure
(homogenous) poorly differentiated carcinoma of solid type without the other histological
patterns. First of all, we devided the poorly differentiated carcinoma of solid type into the two
groups, *i.e.* medullary carcinoma and non-medullary carcinoma. The medullary carcinoma
was composed of homogenous poorly differentiated carcinoma in a medullary growth pattern.
Moreover, we evaluated the clinicopathological characteristics and prognosis for medullary
carcinoma and non-medullary carcinoma.
Materials and methods

Patients

This study evaluated 61 consecutive surgical cases of poorly differentiated gastric adenocarcinoma of solid type treated between January 2005 and December 2014 at the Hirosaki University Hospital after obtaining each patients’ informed consent to use their clinical records and pathological specimens. The case series comprised 39 males and 22 females with a median age of 74 years (range, 59–94 years). The carcinomas were located in the lower third (26 cases), middle third (26 cases), and upper third (9 cases) stomach according to the anatomic location (11). Curative resection and regional lymph node dissection were dependent on the location of primary tumors. Distal gastrectomy was performed for 35 patients, and total gastrectomy was performed for 26 patients. Borrmann classification type 1 or 2 was observed in 31 cases and type 3, 4, or 5 was seen in 30 cases (5). The mean tumor diameter was 66.2 mm. Survival data were obtained from hospital medical records. The median observation period for 35 cases was 36.5 months.

Pathological analysis

All surgically resected specimens were fixed using 10% formalin, embedded in paraffin, and
stained using hematoxylin and eosin (H&E) for pathological evaluation. Gastric carcinomas were evaluated according to the Japanese classification of gastric carcinoma (11) and staged using the TNM classification of the International Union Against Cancer (UICC) (23). The histological features assessed in the largest cross-sectional tumor section were as follows:

histological type, depth of invasion (T-grade), lymph node metastasis, lymphatic invasion, venous invasion, and infiltration pattern of tumor (INF). The degree of lymphatic and venous invasion was classified as follows: 0, no invasion; 1, mild invasion; 2, moderate invasion; and 3, severe invasion. INF was categorized into three groups: INFa, cancer nests showing expansive growth and presenting the clear borderline between tumor tissue and stroma; INFb, intermediate patterns of growth between INFa and INFc; and INFc, scirrhous growth with an unclear border at the invasive front. We defined a medullary carcinoma indicating the following three characteristics: (i) more than 90% of the entire tumor were composed of poorly differentiated adenocarcinoma of solid type; (ii) the tumor exhibited an expansive growth at the tumor margin, i.e. INFa; and (iii) the special types such as an α-fetoprotein (AFP)-producing carcinoma, neuroendocrine carcinoma, and carcinoma with lymphoid stroma were excluded. Based on the definition, we subclassified the poorly differentiated gastric adenocarcinoma of solid type into the two groups: medullary carcinoma and
non-medullary carcinoma.

Immunohistochemistry

Immunohistochemical examination was performed on deparaffinized sections using the standard avidin-biotin-peroxidase complex method with automated immunostainer (Benchmark XT; Ventana Medical System, Tucson, AZ, USA). Podoplanin (D2-40) was used for revealing lymphatic endothelium and clarifying lymphatic invasion. The antibody we used was D2-40 (1:100, clone D2-40; Dako, Glostrup, Denmark).

Statistical analysis

The Pearson’s chi-square test was used to assess potential associations between categorical variables with the use of adjusted residual analysis. The Kaplan–Meier method was used to construct survival curves and differences in survival were evaluated using the log-rank test. Differences were considered to be statistically significant if the $P$ value was <0.05. Statistical evaluations were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (12) and PASW statistics softwares (version 18.0; SPSS, Inc., Chicago, IL, USA).
Results

Gross and histological findings

Review of 61 cases of poorly differentiated adenocarcinoma of solid type identified 23 medullary carcinomas and 38 non-medullary carcinomas. Macroscopically, the most medullary carcinomas were expansively ulcerated tumors with sharply demarcated and raised margins, i.e., type 2 tumor according to the Bormann classification (Fig. 1A). Cross-sectional tumor segments showed expansive growth with clear margins (Fig. 1B). The most non-medullary carcinomas were infiltratively ulcerated tumors lacking definite margins, i.e., type 3 tumor according to the Bormann classification (Figs. 1C, D). Microscopically, medullary carcinomas showed expansive growth with few glandular structures and a distinct border demarcating the surrounding tissue, i.e., INFα (Fig. 2A). The medullary carcinomas showed a solid pattern (Fig. 2B). Medullary carcinomas usually had either no or mild lymphatic invasion and moderate to severe venous invasion (Figs. 3A, B). Non-medullary carcinomas showed not only a solid carcinoma component, but also the other histological components such as poorly differentiated adenocarcinoma of non-solid type (Figs. 2C, D). The non-medullary carcinomas usually exhibited moderate to severe lymphatic and venous invasion. Immunohistochemical staining for D2-40 revealed lymphatic endothelium, and
clarified lymphatic invasion (Figs. 3C, D). The histological components of medullary and non-medullary carcinomas are summarized in Table 1. More than half (52.2%, 12/23) of medullary carcinomas were composed of poorly differentiated adenocarcinoma of solid type, only. On the other hand, the majority (92.1%, 35/38) of the non-medullary carcinomas were composed of the plural histological components.

Clinicopathological findings

The clinicopathological findings pertaining to medullary carcinoma and non-medullary carcinoma are summarized in Table 2. There were significant differences in Borrmann classification, lymph node metastasis, lymphatic invasion, venous invasion, and INF between medullary carcinoma and non-medullary carcinoma ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.002$, and $P < 0.001$, respectively). In comparison with the non-medullary carcinoma, the medullary carcinomas less frequently showed lymph node metastasis, lymphatic invasion, and venous invasion. There was no significant difference in the depth of invasion ($P = 0.335$).

Detailed results of lymphatic and venous invasion are summarized in Table 3. In comparison with the non-medullary carcinoma, the medullary carcinoma less frequently showed lymphatic invasion (ly0, 1 & v0, 1 and ly0, 1 & v2, 3 in Table 3). In comparison with
medullary carcinoma, non-medullary carcinoma more frequently exhibited venous invasion (ly2, 3 & v2, 3 in Table 3).

Patient’s survival rates

There were significant differences in disease-free survival between the patients with medullary carcinoma and those with non-medullary carcinoma (Fig. 4A, $P = 0.017$), but there was no significant difference in overall survival between these groups (Fig. 4B, $P = 0.079$).
**Discussion**

We defined a medullary carcinoma indicating the following three characteristics: (i) more than 90% of the entire tumor were composed of poorly differentiated adenocarcinoma of solid type; (ii) the tumor exhibited an expansive growth at the tumor margin, *i.e.* INFα; and (iii) special types were excluded. Based on the definition, we subclassified the poorly differentiated gastric adenocarcinoma of solid type, into the two groups: medullary carcinoma and non-medullary carcinoma. Medullary carcinomas showed significantly reduced lymphatic invasion, venous invasion, and lymph node metastasis, resulting in a better prognosis compared to non-medullary carcinoma.

There are a few reports on the subclassification of poorly differentiated adenocarcinoma of solid type (1, 25). Adachi *et al.* divided it into 2 groups: pure poorly differentiated medullary carcinoma, in which a solid pattern occupied more than 80% of the tumor area, and mixed poorly differentiated medullary carcinoma, in which a solid pattern occupied 50%–80% of the tumor area (1). Compared to mixed poorly differentiated medullary carcinoma, pure poorly differentiated medullary carcinoma showed expansive growth and inflammatory infiltration. However, the pure poorly differentiated medullary carcinoma did not indicate low frequency of lymphatic invasion/venous invasion/lymph node metastasis.
metastasis, and there was no statistical significance in patients’ outcomes between the two groups. Their “pure poorly differentiated medullary carcinoma” is similar to our category, but they did not mention the infiltration pattern at the tumor margin indicating INF, and exclusion of the special types of gastric carcinoma. Song et al. has reported that INFα of the gastric carcinoma was associated with good patients’ prognosis (24). INF has been shown to be a useful prognostic factor not only for gastric carcinoma, but also for colorectal, gallbladder, and urothelial carcinomas (8, 18, 20). We excluded the special types of gastric carcinoma such as AFP-producing carcinoma, neuroendocrine carcinoma, and carcinoma with lymphoid stroma. AFP-producing carcinomas are known to be high-grade malignancy with frequent liver metastasis, and show a poor patients’ prognosis (10, 13). Neuroendocrine carcinomas also exhibit a poor patients’ prognosis (17, 22). In contrast, carcinomas with lymphoid stroma are distinct entities with better patients’ prognosis (26, 27). In the present study, we defined medullary carcinoma with three histological characteristics, and assumed that it showed clearly distinct clinicopathological features, compared to the non-medullary carcinomas.

Recent studies have demonstrated that microsatellite instability represented a hypermutable phenotype caused by a DNA mismatch repair deficiency, one of the initiating pathways for gastric cancer (4, 6, 16). The microsatellite instability in gastric tumors was
associated with the histological types such as poorly differentiated adenocarcinoma of solid type, and papillary adenocarcinoma, typically indicating expansive growth (2, 7). The poorly differentiated gastric adenocarcinoma with microsatellite instability is considered to be a counterpart of colorectal medullary carcinoma, which is representative of colorectal carcinomas with microsatellite instability (3). In the near future, we will analyze any relationships between the microsatellite instability and the gastric medullary carcinoma that we have proposed in this study.

In conclusion, we should classify the poorly differentiated gastric adenocarcinoma of solid type, into the two distinct groups: medullary carcinoma and non-medullary carcinoma. The gastric poorly differentiated medullary carcinoma is a novel histological type predicting good patients’ prognosis.

Acknowledgements

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References


**Tables**

**Table 1.** Histological components of medullary carcinoma and non-medullary carcinoma

<table>
<thead>
<tr>
<th>Histological subclassification†</th>
<th>Medullary carcinoma (n = 23)</th>
<th>Non-medullary carcinoma (n = 38)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>por (solid), only</td>
<td>12 (52.2%)†</td>
<td>3 (7.9%)</td>
<td></td>
</tr>
<tr>
<td>por (solid)&gt;por (non-solid)</td>
<td>1 (4.4%)</td>
<td>12 (31.6%)†</td>
<td></td>
</tr>
<tr>
<td>por (solid)&gt;well, mod</td>
<td>9 (39.0%)</td>
<td>18 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>por (solid)&gt;muc, sig</td>
<td>1 (4.4%)</td>
<td>5 (13.2%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

†Histological subclassification according to the Japanese classification system for gastric carcinomas: por (solid), poorly differentiated adenocarcinoma of solid type; por (non-solid), poorly differentiated adenocarcinoma of non-solid type; well, well differentiated tubular adenocarcinoma; mod, moderately differentiated tubular adenocarcinoma; muc, mucinous adenocarcinoma; sig, signet-ring cell carcinoma.

*Statistically significant association by adjusted residual analysis (P < 0.05)
Table 2. Clinicopathological characteristics of medullary carcinoma and non-medullary carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Poorly differentiated adenocarcinoma of solid type (n = 61)</th>
<th>Medullary carcinoma (n = 23)</th>
<th>non-medullary carcinoma (n = 38)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td>0.777</td>
</tr>
<tr>
<td>≥70</td>
<td>15</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>8</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>0.137</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastric location</td>
<td></td>
<td></td>
<td></td>
<td>0.523</td>
</tr>
<tr>
<td>Lower</td>
<td>11</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle or upper</td>
<td>12</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borrmann classification</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type 1 or 2</td>
<td>22</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 3, 4, or 5</td>
<td>1</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor diameter (mm)</td>
<td></td>
<td></td>
<td></td>
<td>0.902</td>
</tr>
<tr>
<td>&lt;50</td>
<td>7</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥50</td>
<td>16</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth of invasion†</td>
<td></td>
<td></td>
<td></td>
<td>0.335</td>
</tr>
<tr>
<td>T2 or T3</td>
<td>15</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>8</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pN (+)</td>
<td>6</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphatic invasion‡</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ly0 or ly1</td>
<td>16</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ly2 or ly3</td>
<td>7</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous invasion‡</td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>v0 or v1</td>
<td>9</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>v2 or v3</td>
<td>14</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INF‡</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>a</td>
<td>23</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b or c</td>
<td>0</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Depth of invasion according to TNM classification
‡ Lymphatic invasion, venous invasion, and INF according to the Japanese classification of gastric carcinoma
Table 3. Lymphatic and venous invasion of medullary carcinoma and non-medullary carcinoma

<table>
<thead>
<tr>
<th>ly† &amp; v‡</th>
<th>Medullary carcinoma (n = 23)</th>
<th>Non-medullary carcinoma (n = 38)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ly0, 1 &amp; v0, 1</td>
<td>5 (21.7%)*</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>ly0, 1 &amp; v2, 3</td>
<td>11 (47.8%)*</td>
<td>4 (10.5%)</td>
<td></td>
</tr>
<tr>
<td>ly2, 3 &amp; v0, 1</td>
<td>4 (17.4%)</td>
<td>2 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>ly2, 3 &amp; v2, 3</td>
<td>3 (13.0%)</td>
<td>32 (84.2%)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

†ly, lymphatic invasion according to Japanese classification of gastric carcinoma: 0, no invasion; 1, mild invasion; 2, moderate invasion; and 3, severe invasion.

‡v, venous invasion according to Japanese classification of gastric carcinoma: 0, no invasion; 1, mild invasion; 2, moderate invasion; and 3, severe invasion.

*Statistically significant association by adjusted residual analysis ($P < 0.05$)
Figure legends

Figure 1. Gross findings of medullary carcinoma (A, B) and non-medullary carcinoma (C, D). (A) Medullary carcinomas were ulcerated tumors with sharply demarcated and raised margins. (B) A tumor cross-section of medullary carcinoma showed expansive growth with a clear margin. (C) Non-medullary carcinoma comprised an ulcerated tumor without a definite border. (D) A tumor cross-section of non-medullary carcinoma showed an unclear margin.

Figure 2. Histological findings of medullary carcinoma (A, B) and non-medullary carcinoma (C, D). (A) Medullary carcinoma showed a fungating tumor with expansive growth, i.e., type 2 tumor according to the Borrmann classification and a distinct border with the surrounding tissue, i.e., INFa. (B) Medullary carcinoma indicated only a solid pattern with few glandular structures. (C) Non-medullary carcinoma showed an ulcerated tumor with infiltrating growth, i.e. type 3 tumor according to the Borrmann classification. (D) A non-solid pattern in non-medullary carcinoma indicated a small alveolar and isolated pattern with abundant stroma.

Figure 3. Vascular invasion of medullary carcinoma and non-medullary carcinoma. (A, B)
Medullary carcinomas usually display moderate to severe venous invasion (A, H&E staining; B, Elastica van Gieson staining). (C, D) Non-medullary carcinomas usually showed not only moderate to severe venous invasion but also lymphatic invasion.

Immunohistochemical staining for D2-40 revealed the lymphatic endothelium and clarified lymphatic invasion (C, H&E staining; D, immunohistochemistry for D2-40).

Figure 4. Patients’ prognosis using Kaplan-Meier survival curves. (A) Patients with medullary carcinoma and non-medullary carcinoma had significantly different disease-free survival ($P = 0.017$). (B) There was no significant difference in overall survival between patients with medullary carcinoma and non-medullary carcinoma ($P = 0.079$).
Figure 2
Figure 4

(A) ma

P = 0.017

(B) Probability

OS

P = 0.079

: medullary carcinoma

: non-medullary carcinoma