Recent Incidence Trend of Surgically Resected Esophagogastric Junction Adenocarcinoma and Microsatellite Instability Status in Japanese Patients

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Keywords

Esophagogastric junction · Barrett's esophagus ·
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Molecular subtype

Abstract

Background: The incidence trend of esophagogastric junction (EGJ) adenocarcinoma in Japan has not been sufficiently investigated. Little is known about the microsatellite instability (MSI) status of this tumor. **Summary:** Previously published studies analyzing the trend of EGJ adenocarcinoma in Japan were reviewed. And a trend of surgically resected cases (Siewert type I–III) utilizing a retrospective multicenter cohort of 379 patients from 4 academic institutions in Japan investigated. Although an increasing trend in the last 2 reports was considered controversial, our cohort demonstrated a growing number of EGJ adenocarcinoma cases between 2006 and 2013. This trend was evident, especially in

Siewert type I cases. In the previous 16 studies that performed MSI testing, MSI-high tumors ranged 0–8.3%, though there were no fixed microsatellite markers on EGJ adenocarcinoma. In a recent comprehensive genetic analysis by The Cancer Genome Atlas, MSI testing using the following 7 markers, BAT25, BAT26, BAT40, D2S123, D5S346, D17S250 and TGF- β II showed a favorable correlation with hypermutated tumors. We performed MSI testing using 6 of those markers, except TGF- β II, on 206 cases from one institution, and detected 15 cases (7.3%) with MSI-high. The prevalence of MSI-high was 0% in Siewert type I, 7.6% in type II, and 16.7% in type III. **Key message:** The number of surgically resected EGJ adenocarcinoma cases gradually increased, and MSI-high was infrequent in Siewert type I–II tumors in our Japanese cohort. Considering MSI-high as a predictive biomarker for emerging immune checkpoint inhibitors, MSI status is becoming more beneficial in EGJ adenocarcinoma.

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Introduction

In Western countries, the incidence of esophagogastric junction (EGJ) adenocarcinoma has been increasing gradually over the past few decades, whereas that of non-cardiac gastric cancer decreased with declining prevalence of *Helicobacter pylori* infection [1]. EGJ adenocarcinoma, also called as esophageal adenocarcinoma, generally develops from a premalignant lesion of esophageal columnar metaplasia of Barrett's esophagus (BE) in the West. Despite the recent advancements in multimodal treatments, this tumor is still an aggressive malignancy with poor outcome.

In a number of clinical trials of conventional chemotherapeutic agents or molecular targeted drugs, patients with EGJ adenocarcinoma often share treatment strategy with gastric adenocarcinoma cases, since these 2 diseases are considered to be similar. Recently, immune checkpoint inhibitor has emerged as a novel effective chemotherapeutic option across a broad spectrum of malignant tumors and gastrointestinal adenocarcinoma. The phase 3 trial ONO-4538-12 (ATTRACTION-2) revealed the efficacy and safety of programmed cell death protein 1 (PD-1) blockade by monoclonal antibody nivolumab in Asian patients with esophagogastric ($n = 30$) and gastric adenocarcinoma ($n = 272$) [2]. Currently, in terms of a predictor of response to immune checkpoint inhibitor, much focus is being placed on the microsatellite instability (MSI) status [3]. However, little is known about the MSI status in EGJ adenocarcinoma.

In this study, we reviewed the previous studies investigating the recent incidence trend of EGJ adenocarcinoma cases in Japan, and also examined its trend utilizing 379 surgically resected Siewert type I, II, and III EGJ adenocarcinoma cases (type I, tumor epicenter located within 1–5 cm above the anatomical EGJ; type II, within 1 cm above and 2 cm below the EGJ; type III, between 2 and 5 cm below the EGJ with esophageal infiltration) from our multicenter retrospective cohort study in Japan. In addition, we reviewed previous reports related to the MSI status of this tumor and investigated the MSI status on 206 cases of EGJ adenocarcinoma from one institution.

Recent Trends of EGJ Adenocarcinoma in Japan

In Japan, Kusano et al. [4] first investigated the incidence trend of Japanese cases of EGJ adenocarcinoma, analyzing 520 cases with surgically resected Siewert type I–III tumors in a single institution of National Cancer

Center (Tokyo) in 2008, one of the highest volume cancer centers in Japan. They demonstrated that the proportion of Siewert type II increased between 1962 and 2005, but that of Siewert type I remained around 1% over 44 years. Recently, Yamashita et al. [5] showed that the number of surgically resected cases of EGJ adenocarcinoma were gradually increased between 2001 and 2010, using a questionnaire-based national retrospective study in Japan. In their article, EGJ adenocarcinoma was defined as tumors with epicenter located within 2 cm proximal or distal to the anatomical EGJ by the Japanese classification of gastric cancer (14th edition, Japanese only).

To be aware of current incidence trends of EGJ adenocarcinoma, we retrospectively collected a total of 379 patients who underwent surgical resection between 2006 and 2013 in 4 academic institutions (Department of Gastroenterological Surgery, the Cancer Institute Hospital [Tokyo]; Department of Gastroenterological Surgery, Kumamoto University Hospital [Kumamoto], Department of Surgery and Science, Kyushu University Hospital [Fukuoka], and Department of Surgery, National Hospital Organization Kyushu Cancer Center [Fukuoka]) in Japan. Ethical approvals for this retrospective observational study were obtained from all institutions (approval number 2015-1107 for The Cancer Institute Hospital, 858 for Kumamoto University Hospital, 747-00 for Kyushu University Hospital, and 2015-26 for National Hospital Organization Kyushu Cancer Center). The annual numbers of surgically resected EGJ adenocarcinoma cases were counted according to the tumor locations by Siewert classification. During the 8-year study period, the annual number of surgically resected cases increased gradually (Fig. 1a). Of note, the increase in Siewert type I tumors was evident. While that of Siewert type III tumors decreased (Fig. 1a). These tendencies were clearer in terms of proportions by Siewert classification (Fig. 1b). Although the report from Yamashita et al. [5] and our data showed growing trends of EGJ adenocarcinoma, these were attributed to an increase of the elderly population in Japan. The search in PubMed revealed that there were no reports examining the incidence trend of EGJ adenocarcinoma in other Asian countries, such as Korea or China. Further population-based study is warranted to clarify the trend of this tumor.

Anatomically, EGJ adenocarcinoma includes tumors arising from BE, and those arising from cardiac mucosa. Theoretically, tumors adjacent to BE can presumably originate from the BE (BE-associated EGJ adenocarcinoma). We also investigated the annual number of surgically resected cases with EGJ adenocarcinoma, according to the presence or absence of BE in our cohort ($n = 379$).

Fig. 1. Recent trends of surgically resected cases of esophagogastric junction adenocarcinoma in 4 institutions in Japan ($n = 379$). **a** and **b**, patient numbers (**a**) and proportions (**b**) according to tu-

mor location by Siewert classification. **c** and **d**, patient numbers (**c**) and proportions (**d**) according to the presence or absence of Barrett's esophagus (BE).

The existence of BE was defined by endoscopic findings, or by surgical pathology reports. The proportions of tumors coexisting with BE were 35/47 (74.5%). Siewert type I tumors, 69/273 (25.3%) in Siewert type II, and 2/59 (3.4%) in Siewert type III. Type I tumors were significantly associated with coexistence of BE ($p < 0.0001$). Over the 8 years, we observed a growing trend of tumors adjacent to BE, in terms of numbers and ratios (Fig. 1c, d). Our data may suggest that BE-associated EGJ adenocarcinoma cases are increasing in Japan, similar to what was observed in Western countries a few decades ago. However, as our cohort were collected in a retrospective manner, a future larger scale prospective study in collaboration with endoscopists and pathologists would be needed to address the trend of BE-associated tumors.

MSI and Gastrointestinal Cancers

Genetic instability is regarded as a hallmark of malignant tumor. MSI is a form of genetic instability in cancer, caused by DNA mismatch repair deficiency (MMR) [6]. Microsatellites are short tandem repeats of simple DNA sequence, generally 5–60 times of mono-, di-, tetra- penta- or hexanucleotide throughout the genome. Around 50,000–100,000 microsatellites occur in human genome, commonly in introns, promoter regions, as well as in exons. DNA synthesis is always at risk of replicative error of DNA repairing or replication. This nature of microsatellites results in a susceptibility to replication errors caused by slippage of DNA polymerase over short tandem repeats. In normal cells, these replicative errors can be ef-

ficiently corrected by MMR proteins, such as MLH1, MSH2, MSH6, and PMS2 [7]. The MMR deficiency, usually caused by genetic or epigenetic alterations, is one of the major genetic predispositions to cancer.

High-level MSI (MSI-high) was approved as a biomarker for pembrolizumab in many types of solid malignancies by Food and Drug Administration (FDA). In general, MSI-high tumors possess remarkably high mutational burden, potentially producing a numerous number of neoantigens [8]. Displaying these tumor-specific neoantigen by antigen-presenting cells recruit CD8⁺ T cells (effector T cells) into tumor microenvironment. Simultaneously, tumor cells promote immune evasion by suppressing T-cell mediated tumor cell lysis, through programmed death ligand 1 (PD-L1) [9]. Immune check point inhibitors, such as nivolumab and pembrolizumab, restore T-cell activation by blocking the interaction between PD-L1 on tumor cells and PD-1 on activated T cells, leading to antitumor response. This is why MSI status can currently be used as a predictor of PD-1 blockade in gastric and colorectal cancers in gastrointestinal malignancies in the US.

MMR deficiency can be detected by genetic testing or immunohistochemistry (IHC). Genetic testing has been employed for screening hereditary non-polyposis colorectal cancer, which are caused by germline mutations of MMR genes, by the Bethesda panel (BAT25, BAT26, D2123, D5S346, and D17S250) in clinical practice [10]. Although mononucleotide markers (BAT25 and BAT26) are stable and easy to realize when shifted in a fragment analysis, amplicons of dinucleotide markers (D2123, D5S346, and D17S250) are less sensitive due to the effect of loss of heterozygosity. In recent times, Promega MSI Analysis System (ver.1.2, Madison, WI, USA) using 5 mononucleotide markers alone (BAT25, BAT26, MONO27, NR21, and NR24) are becoming more common.

The reproducibility of MSI genetic testing has already been verified to be quite high across 71 clinical laboratories [11]. Genetic testing allows visualization of amplified fragments of MSI markers, resulting in a better agreement. On the contrary, the sensitivity of IHC methods always depends on the antibodies used, as well as on the quality of formalin-fixed paraffin-embedded (FFPE) tissue. It is sometimes difficult to judge whether uneven staining is caused by loss of expression, or simply due to degraded protein on FFPE tissue. In addition, when a mutation occurs outside *MLH1*-*PMS2* interacting domain, both MLH1 and PMS2 expressions can be detected theoretically. Because IHC method sometimes miss MMR de-

ficiency in some situations, genetic testing would be recommended rather than IHC methods, for detecting MMR deficiency.

Previous Studies Examining MSI Status in EGJ Adenocarcinoma

There is still no consensus on which microsatellite markers should be used for detecting MSI-high in EGJ adenocarcinoma. In colorectal adenocarcinoma, the Bethesda panel mentioned above, was developed for detecting hereditary non-polyposis colorectal cancer at National Cancer Institute-sponsored MSI workshop in 1997 [10]. To our best knowledge, there are 16 studies that investigated the MSI status, focusing on EGJ adenocarcinoma (Table 1) [12–27]. All those studies used genetic testing. Among them, 13 studies (81.3%) employed at least 1 microsatellite marker listed in the Bethesda panel. In a recent comprehensive genomic study of esophageal cancer by The Cancer Genome Atlas (TCGA) Research Network, microsatellite markers of BAT40 and TGFR-II were added to the Bethesda panel, and demonstrated that 7 cases (7/172, 4.1% of esophageal adenocarcinoma) with MSI-high tumors were hypermutated in their data set, harboring 32–103 mutations occurred per mega base [17]. Hence, the following 7 microsatellite markers, BAT25, BAT26, BAT40, D2S123, D5S346, D17S250 and TGFR-II, are suitable for detecting MSI-high tumors in EGJ adenocarcinoma.

Among the 16 studies, 8 studies investigated the prevalence of MSI-high tumors (Table 1). Although the definitions of MSI-high tumors were not common across the studies, all studies marked low prevalence of MSI-high in EGJ adenocarcinoma, ranging from 0 to 8.3% (Table 1). The number of patients analyzed was very small ($n \leq 80$), and the statistical power was limited, except 1 study from TCGA ($n = 172$, data were obtained from supplementary data) [17]. There were only a few studies investigating the association between MSI-high and other molecular status, or clinicopathological characteristics in EGJ adenocarcinoma.

Kulke et al. [19] did not detect any MSI-high tumor (defined as 3 or more unstable microsatellite markers of 5 markers) in their cohort ($n = 70$). They suggested a better prognosis of MSI-low tumors ($n = 13$, defined as 1 or 2 unstable microsatellite markers), compared to MSI-absent cases ($n = 57$), but it did not reach a statistical significance ($p = 0.12$) [19]. They did not find any outstanding clinicopathological features of MSI-low.

Table 1. Previous 16 studies examining microsatellite instability (MSI) in esophagogastric adenocarcinoma

Ref. No.	First author	Year of publication	No. cases	No. MSI-high, <i>n</i> (%)	Definition of MSI-high	Tumor location	Microsatellite markers
12	Shiraishi	2006	12	1 (8.3)	30% unstable markers	NA	BAT25, BAT26, D2S123, D5S346, D17S250 , D17S579, D17S786, D17S796, D3S1300, D7S486, D9S161, D11S904, D13S268, D18S474
13	Pandilla	2013	30	2 (6.7)	≥2 unstable markers	NA	D2S123 , D3S1300, D5S346 , D9S1748, D17S250 , TP53CA
14	Farris	2011	76	5 (6.6)	≥2 unstable markers	BE-associated	BAT25, BAT26, D2S123, D5S346, D17S250
15	Chong	2013	54	3 (5.6)	≥2 unstable markers	Siewert type II/III	BAT25, BAT26 , NR21, NR24, MONO27, Penta-C, Penta-D
16	Dolan	2004	47	2 (4.3)	≥2 unstable markers	Distal esophagus	D3S587, D5S107, D5S346 , D9S171, D17S805, D18S70
17	TCGA	2017	172	7 (4.1)	≥40% unstable markers	NA	BAT25, BAT26 , BAT40, TGFR-II, D2S123, D5S346, D17S250
18	Evans	2004	27	0	≥5 unstable markers	NA	D2S123 , D10S197, D2S119, D11S904, D2S147, D3S1764, D7S1830, D7S1805, D2S434, D9S299, BAT25, BAT26, D5S346, D17S250 , TGF-β-RII
19	Kulke	2001	80	0	≥3 unstable markers	BE-associated	BAT25, BAT26 , D10S219, D10S541, D10S551
20	Shirasaki	2009	11	NA	NA	NA	BAT25, BAT26, D2S123, D5S346, D17S250 , D17S796, TP53, D17S579, D17S786, D3S1300, D7S486, D9S161, D11S904, D13S268, D18S474
21	Cai	2008	18	NA	NA	Distal esophagus	D2S123 , D3S1300, D3S1616, D5S346 , D17S787, D18S58, BATRII
22	Lin	2006	44	NA	NA	NA	D3S1754, D2S119, BAT26, D2S123 , BAT21, D3S3603, AFM072yb7, D3S2314, D3S3609, CI_4_1, D10S2314, D10S2318, D10S197, D10S541, D10S2309, D10S2311, D10S2317, D10S2312, D10S2310, D11S904, CTG-B37, D13S175, D14S72, D14S69, D17S1157, D17S250 , D17S1787, D17S856, D21S411, DYS19
23	Yanagi	2000	54	NA	NA	Siewert type I/III	D5S346 , D18S34, TP53, D14S65, D14S75, D14S80, D14S267
24	Rumpel	1999	28	NA	NA	NA	D4S392, D4S1543, D4S2915, D4S2932, D4S1538, D4S1534, D4S423, D4S1638, D4S1572, D4S407, IL2, D4S427, D4S1615, D4S1565, D4S1586, D4S1589, D4S620, D4S3028, D4S1607, D4S2951, D4S2920, D4S1554, D4S1535, D4S171, D4S426

Table 1 (continued)

Ref. No.	First author	Year of publication	No. cases	No. MSI-high, <i>n</i> (%)	Definition of MSI-high	Tumor location	Microsatellite markers
25	Muzeau	1997	26	NA	NA	BE-associated	D1S225, D1S229, D1S239, D1S306, D3S1282, D3S1297, D4S414, D5S393, D5S430, D6S309, D6S271, D6S277, D8S272, D8S277, D8S283, D9S152, D9S153, D9S156, D9S157, D9S158, D9S159, D9S161, D9S165, D9S168, D9S169, D9S171, D9S175, D9S197, D9S259, D10S199, D10S226, D13S175, D14S250, D15S128, D16S517, D17S784, D17S790, D18S53, D20S107
26	Gleeson	1996	19	NA	NA	BE-associated	139 loci were analyzed, but all loci are not listed in the manuscript.
27	Meltzer	1994	36	NA	NA	BE-associated	D2S123 , D10S197, D2S147, D2S119, D11S904

Microsatellite markers listed of the Bethesda panel (BAT25, BAT26, **D2S123**, D5S346, and D17S250) are bold. BE, Barrett's esophagus; NA, not applicable; TCGA, The Cancer Genome Atlas.

Farris et al. [14] demonstrated that patients with MSI-high tumors were significantly associated with advanced age (76.2 ± 5.9 years old in MSI-high [$n = 5$], 66.4 ± 11.0 years old in MSI-absent [$n = 71$], $p = 0.006$), and higher number of tumor infiltrating lymphocytes (42.9 ± 5.9 per high power field in MSI-high, 5.2 ± 13.0 per high power field in MSI-absent, $p < 0.0001$). They also addressed the pathological characteristics according to MSI status. Generally, MSI-high tumors are associated with medullary morphology in gastric and colorectal adenocarcinoma, suggesting similar histological characteristics across gastrointestinal adenocarcinoma. Expectedly, in their observation, 2 of 5 (40%) cases with MSI-high tumor exhibited medullary component. The remaining 3 cases (60%) included one with conventional adenocarcinoma without any additional component, another with signet-ring cell carcinoma, and the other with mucinous histology. Signet-ring cell and mucinous component are reportedly related to CpG island methylator phenotype, as well as MSI-high in colorectal adenocarcinoma [28]. MSI-high can be also induced by epigenetic silencing of *MLH1* in colorectal adenocarcinoma. Further study would be needed to address the relationship between signet-ring cell or mucinous histology, epigenetic alterations, and MSI status in EGJ adenocarcinoma.

Prevalence of MSI-high Tumors in Cancer Institute Hospital Cases

Using FFPE-derived DNA from 206 cases with EGJ adenocarcinoma who underwent surgical resection at Cancer Institute Hospital, MSI testing was performed by fragment analysis. After microdissection of the tumor area marked by pathologists (Y.I. and M.T.) under a microscope from hematoxylin and eosin stained tissue section, DNA was extracted using QIAamp DNA FFPE Tissue Kit (QIAGEN, Duesseldorf, Germany) [29]. DNA fragment analyses were done by ABI PRISM 3030xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using 6 microsatellite markers (BAT25, BAT26, BAT40, **D2S123**, D5S346, and D17S250; primer sequences conducted are shown in Table 2), as previously described [30]. All these 6 markers overlap the 7 markers used in the TCGA esophageal cancer study. This study was approved by the research institutional review board at Cancer Institute Hospital (approval number 2015-1107). MSI-high was defined as 2 or more unstable markers, MSI-low as one unstable marker, and microsatellite stable as no unstable marker. MSI-high, MSI-low, and microsatellite stable were detected in 15 (7.3%), 19 (9.2%), and 172 cases (83.5%), respectively. Notably, MSI-high was not detected in Siewert type I tumors. As the tumor

Table 2. Primer sequences for MSI assay in this study

Markers	Repeating unit	Forward/reverse	5' Reporter dye	5'–3' sequence
BAT25	Mononucleotide	Forward	6-FAM	TCGCCTCCAAGAATGTAAGT
		Reverse	None	TATGGCTCTAAAATGCTCTGTTCTC
BAT26	Mononucleotide	Forward	6-FAM	GAAATTGGATATTGCAGCAGTCAGAG
		Reverse	None	CATTTTTTAACCATTCACATTTTTTAACCC
BAT40	Mononucleotide	Forward	6-FAM	AGCCAAGATTAACTTCCTACACCACAAC
		Reverse	None	GTAGAGCAAGACCACCTTGTCTC
D2S123	Dinucleotide	Forward	6-FAM	AAACAGGATGCCTGCCTTTA
		Reverse	None	GTGTCTTCCTTTCTGACTTGGATACCATCTA
D5S346	Dinucleotide	Forward	6-FAM	ACTCACTCTAGTGATAAATCGGG
		Reverse	None	GTGTCTTAGCAGATAAGACAGTATTACTAGTT
D17S250	Dinucleotide	Forward	6-FAM	TACACATACATAAACTTTCAAATGGTTTC
		Reverse	None	GTGTCTTGCCACTCAGCTGGCCATATATATATT

Fig. 2. Proportions of microsatellite instability (MSI) status according to tumor locations by Siewert classification. MSI testing was performed in the patients from Cancer Institute Hospital ($n = 206$). MSS, microsatellite stable.

epicenter location go down from the esophagus (Siewert type I) to the stomach (Siewert type III), the proportion of MSI-high tumors increased, and that of MSI-low remained stable (8–9%, Fig. 2). MSI-high was a relatively rare genetic instability in EGJ adenocarcinoma compared to that in gastric adenocarcinoma, reported to be around 10–25% [31, 32]. Further study on the features of MSI-low is warranted.

Conclusion

Here, we reviewed the incidence trend of surgically resected EGJ adenocarcinoma in Japan, and previous studies regarding the MSI status in this tumor. The number of patients with EGJ adenocarcinoma is on the rise in Japan, similar to the Western countries. Having high expectations of cancer immunotherapy, MSI status is becoming an essential molecular testing in gastrointestinal malignancies including EGJ adenocarcinoma. However, MSI status in EGJ adenocarcinoma has still not been well

examined or understood. Further studies examining the clinical, pathological, and molecular characteristics according to MSI status, would be beneficial to improve the treatment outcome of this tumor.

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Disclosure Statement

All authors have no conflict of interest to declare.

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