Abstract. Background: Ectopic HLA-G expression in tumor cells may indicate immune escape from the host immune defense via the inhibitory receptor on natural killer (NK) cells. However, there is little information on HLA-G expression in gastric cancer. Patients and Methods: HLA-G expression was immunohistochemically analyzed in 115 gastric cancer patients and the clinical implications of its expression in gastric cancer were assessed. Moreover, NK cell infiltration into the primary tumor was evaluated using anti-CD57 antibodies. Results: The HLA-G-positive group had a more differentiated histology, less nodal invasion and earlier clinical stage than the HLA-G-negative group and these differences were significant. The 5-year survival rate in the HLA-G-positive group was 78%, which was significantly higher than that in the HLA-G-negative group (51%). NK cell infiltration into the tumor tended to be negatively correlated with HLA-G expression. Conclusion: Our results suggest a high frequency of HLA-G expression in early gastric cancer. However, this may not be directly related to aggressive tumor behavior via escape from the host antitumor immune defense. Further investigation is required.

HLA-G is classified as a non-classic HLA-class I, which is also termed a class Ib structure (1). HLA-G is expressed in trophoblasts and helps to maintain pregnancy as part of the fatal protection system (2).

HLA-G binds to natural killer (NK) inhibitory receptors and is able to protect target cells lacking HLA class Ia expression from NK cell-mediated cytolysis (1, 3). It has been suggested that HLA-G plays a direct role in the inhibition of NK cell cytotoxicity through this mechanism. In addition, HLA-G can interfere with the T-cell-mediated lysis of target cells by counteracting the cytotoxic T lymphocyte (CTL) lytic signal from T-cell receptor recognition of the HLA class I antigen-peptide complex.

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Patients and Methods

Patients. A total of 115 gastric cancer patients, who had undergone curative gastrectomy at Kagoshima University Hospital, Japan, between 1996 and 2001, were enrolled in the present study. The patient group comprised 79 males and 36 females, ranging in age from 31 to 82 years (mean 61 years). No patient had received pre-operative chemotherapy. Forty-eight patients had undergone total gastrectomy, 59 patients distal partial gastrectomy and the remaining 8 had undergone proximal gastrectomy (Table I). All the patients had received at least D1 lymph node dissection and R0 resection. The clinical factors were assessed by the general rules for gastric cancer (8).

Detection and evaluation of HLA-G expression. Avidin-biotin complex (ABC) immunohistochemistry techniques were performed. Namely, 4-μm paraffin-embedded sections of gastric cancer were deparaffinized and soaked in phosphate-buffered saline (PBS). The sections were treated with 1% H2O2 for 30 min in order to block endogenous tissue peroxidase, followed by treatment with rabbit serum for 30 min in order to reduce non-specific binding. The primary anti-HLA-G antibody (ab7759, Abcam, UK) was diluted at 1:1000 and incubated with the sections for 24 h. The sections were subsequenly rinsed in PBS and were visualized using standard techniques for labelled avidin-biotin immunoperoxidase staining.
Normal placenta sections were used as positive controls for HLA-G expression. HLA-G was observed, not only in the cytoplasm, but also in the nucleus. The 115 patients were divided into three groups according to the histological distribution of their HLA-G expressions. If the HLA-G positivity was found in either the nucleus or the cell, patients were regarded as HLA-G-positive.

**N K cell infiltration in cancerous tissue.** NK cell infiltration around the tumor cells was detected by immunohistochemical staining using CD57 (DAKO, Kyoto, Japan). CD57-positive cells were counted under a high-power objective (x200) and, as described in a previous report (9), 25 fields were selected and the cell numbers were averaged. Correlations between the intensity of HLA-G expression and the degree of NK cell infiltration were investigated.

**Statistical analysis.** Statistical analysis of the clinical features was performed by the $\chi^2$ test and comparisons of the degree of infiltration between the two groups were performed by the Student’s t-test. Survival curves were produced using the Kaplan-Meier method and statistical significance was calculated using the generalized Wilcoxon method. A $p$ value of less than 0.05 was considered to be statistically significant.

**Results**

HLA-G expression was observed in trophoblasts of the placenta, which served as a positive control (Figure 1), as well as in the cytoplasm and nuclei of tumor cells. According to the distribution of HLA-G positivity, the patients were classified into three groups: those with strong HLA-G expression (n=17) (Figure 1A); those with moderate HLA-G expression (n=19) (Figure 1B); and those with weak HLA-G expression (n=16) (Figure 1C). A total of 52 patients were regarded as being HLA-G-positive and 55% of stage I patients were HLA-G-positive. HLA-G positivity decreased as the clinical stage advanced (Figure 2) ($p<0.05$), and HLA-G-positive patients had less depth of invasion ($p<0.01$), less nodal involvement ($p<0.01$) and earlier clinical stage than HLA-G-negative patients (Table II). Among the 49 early gastric cancer patients, differentiated histology was significantly higher in the HLA-G-positive group ($p<0.05$) (Table III). The 5-year survival in the HLA-G-positive group was 67%, which was significantly better than the 45% observed in the HLA-G-negative group (Figure 3).
Figure 1. HLA-G expression in gastric cancer. Strong HLA-G expression was identified in the cytoplasm of cancer cells (n=17) (Figure 1A). Representative findings of moderate HLA-G expression (n=19) (Figure 1B) and weak HLA-G expression (n=16) (Figure 1C).
NK cell infiltration in the primary lesion was visualized using anti-CD57 antibodies (Figure 4). NK cell infiltration tended to correlate with intensity of HLA-G expression (none; 0.7/HPF, strong; 1.2/HPF, moderate; 1.1/HPF and weak; 0.7/HPF). However, this correlation was not statistically significant (Figure 5).

Discussion

Published data on HLA-G expression in cancer tissue (10,11) confirms 26% positivity in lung cancer and 25% in invasive breast cancer, while in the present study, HLA-G positivity was 57% in gastric cancer. HLA-G positivity was observed, not only in cancerous tissue, but also in gastric mucosa. However, it is reported that HLA-G shows ubiquitous expression, although skin tissue does not express HLA-G (12). This is the first report of HLA-G expression in gastric mucosa, although Torres et al. suggested that HLA-G plays an immunoregulatory role in inflammatory bowel disease (13). Gastric mucosa is constantly exposed to stimulation by food and is subjected to acidic conditions from gastric juices. Various types of immunological tolerance seem to occur in the gastrointestinal mucosa, in contrast to skin tissue.

In vitro data has suggested that HLA-G expression in tumor cells could protect them from CTL and NK cells (14). However, there has been little information about the clinical impact of HLA-G expression in cancer patients. We first investigated the correlation between clinicopathological features and HLA-G expression in gastric cancer. Surprisingly, early gastric cancer showed high HLA-G positivity. In addition, HLA-G-positive patients had fewer lymph node metastases and less depth of invasion, which did not support the notion that HLA-G expression in tumor cells protects tumor cells from antitumor effectors, in contrast with the results of other studies. Palmisano et al. suggested that HLA-G expression is correlated with high-grade histology in breast cancer (11). In the current study, limited to early gastric cancer patients, there was no significant difference in clinical aggressiveness between patients with or without HLA-G expression, except for tumor histology. HLA-G expression was not directly associated with the malignancy stage in melanoma and skin cancer, and thus Urosevic and Dummer (12) speculated that the immuno-escaping mechanism was a very plastic process and that HLA-G may be involved earlier in the course of malignant transformation, decreasing in later invasion stages. Our results support this hypothesis.

The accumulation of NK cells around the tumor tended to correlate with the intensity of HLA-G expression. It has been reported that NK cells had been found adjacent to HLA-G-positive tumor cells in lung cancer (12), as was the case in gastric cancer (9). Singer (15) et al. showed that soluble HLA-G plasma levels and ascites in cancer patients were significantly elevated and that local HLA-G secretion in the tumor nest inhibited the function of immuno-competitive effectors. Soluble HLA-G protected against NK-mediated cell lysis, thus maintaining antitumoral NK cell infiltration into the tumor. In the early stages, HLA-G expression may protect cancer cells from NK cells, even though NK accumulation is relatively high. We were unable to clarify the differences in the clinical features of HLA-G-positive early stage gastric cancer due to insufficient patient samples. Further study is warranted.
In conclusion, HLA-G expression in gastric cancer was confirmed in the early stages. HLA-G expression may not be correlated with aggressive behavior via the mechanism of escaping from host immune defenses; however, HLA-G expression may play a role in the early clinical stages by protecting cancer cells from tumor infiltrating effectors. Further investigation is necessary to resolve the clinical implications of HLA-G expression in gastric cancer.

References


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