The increase of MICA gene A9 allele associated with gastric cancer and less schirrous change

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Since surgical resection is the principal treatment of gastric cancer, early detection is the only effective strategy against this disease at present. Recently, a new polymorphic gene family, the major histocompatibility complex class I chain-related (MIC) genes located about 40 kb centromeric to HLA-B gene has been proposed. This family consists of five genes (A, B, C, D and E). Among them, MICA has five various alleles (A4, A5, A5.1, A6 and A9), which can be used as a polymorphic marker for genetic mapping and for disease susceptibility. The MICA polymorphism was studied in our gastric cancer patients to see if there is any possible correlation with genetic predisposition and clinicopathological factors. Genomic DNA was extracted from fresh or frozen peripheral blood leukocytes in 107 patients with gastric adenocarcinoma who underwent gastrectomy in our hospital and 351 noncancer controls. MICA polymorphism was analysed by using PCR-based technique. The results showed both phenotypic and allele frequencies of allele A9 in patients with gastric cancer were significantly higher than controls (33 vs 17.6%, P = 0.005; 17 vs 9.9%, P = 0.02). Gastric adenocarcinoma with allele A9 was associated with less schirrous change than those without (P = 0.014). MICA gene A9 allele might confer the risk of gastric cancer and associate with less schirrous change. The mechanisms among them deserve further investigation.

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Patients and Methods

Subjects

In all, 107 consecutive gastric cancer patients who underwent gastrectomy in Taipei-VGH were enrolled into this study and their clinicopathological factors were recorded according to our prospective database. A total of 351 control subjects were selected from people who came for routine physical check up. Those with autoimmune disorders, blood disease and previous malignancy

Although the global incidence of gastric cancer is decreasing, gastric cancer is still one of the leading cancers in most Asian countries. Its current incidence in Taiwan is 15.19 per 100 000. Since surgical resection is the principal treatment, early detection is the only effective strategy against this disease at present. Human leukocyte antigen (HLA) has been reported to be associated with tumour susceptibility (Lee et al, 1996b), lymph node metastasis (Ogoshi et al, 1996), induction of cytotoxic T-lymphocytes (Nabeta et al, 2000) and HER-2/neu overexpression (Kono et al, 2002) in patients with gastric adenocarcinoma. However, its application in tumour screening or prognosis remains to be investigated.

Recently, a new polymorphic gene family, the major histocompatibility complex (MHC) class I chain-related genes located about 40 kb centromeric to HLA-B gene have been identified (Bahram et al, 1994). This family consists of five genes: MHC class I chain-related gene A (MICA), gene B (MICB), gene C (MICC), gene D (MICD) and gene E (MICE). MICC, MICD, MICE are pseudogenes, while MICA and MICB encode proteins that are involved in cellular responses to stress (Bahram and Spies, 1996; Groh et al, 1998).

Among them, MICA has a triplet repeat microsatellite polymorphism (GCT)n in the transmembrane region, which consists of five alleles, A4, A5, A5.1, A6 and A9 (Mizuki et al, 1997). According to the open reading frame of the MICA cDNA, the microsatellite encodes polyalanine and therefore the number of alanine residues differs by the number of triplet repeats. For example, an A4 is defined to contain four GCT repeats and A5.1 contains five triplet repeats plus one additional nucleotide insertion (GCCT) causing a frameshift mutation. The alleles vary among individuals, and hence polymorphism of MICA can be used for genetic mapping and analyzes of disease susceptibility. For example, increased frequency of MICA A6 allele was found in patients with oral squamous cell carcinoma (Liu et al, 2002), Behcet’s disease (Molinotti et al, 2001), and ulcerative colitis (Sugimura et al, 2001). In addition, increased frequency of A9 allele was reported in psoriatic arthritis (Gonzalez et al, 2001) and type 1 diabetes (Lee et al, 2000).

We investigated the MICA polymorphism associated with gastric cancer patients in Taiwan in order to see if there is possible correlation with genetic predisposition and clinicopathological factors.
were excluded. After an informed consent was obtained, blood was
drawn from the subjects to extract genomic DNA.

Polymorphysim analysis

A PCR-based polymorphism analysis was used in this study.
Genomic DNA was extracted from fresh or frozen peripheral blood
leukocytes by standard technique (Buffone and Darlington, 1985;
Lee et al, 1996a). Primers (MICA5F, 5'-CTTACCAGGGAAG
TGCTG-3' and MICA5R, 5'-CCCTACACTCCAGGAAACTGC-3')
flanking the transmembrane region were designed based on the
reported sequence (Bahram et al, 1994; Ota et al, 1997). The
MICA5F primer corresponds to the intron 4 and exon 5 boundary
regions, and MICA5R is located in intron 5 (Ota et al, 1997).
MICA5R was 5' end-labelled with fluorescent dye (Applied
 Biosystems, Foster City, CA, USA) was used to do the PCR reaction. The
amplification reaction mixture (15 µl) contained 50 ng genomic
DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM of each dNTP, 0.5 µM of each primer and 0.5 U Prozyme DNA polymerase (Protech Enterprise,
Taipei, Taiwan). A GeneAmp PCR system (Perkin-Elmer Corporation,
Foster City, CA, USA) was used to do the PCR reaction. The
reaction mixture was denatured at 95°C for 5 min followed by 10
cycles at 94°C for 15 s, 55°C for 15 s, 72°C for 30 s, then by an
additional 20 cycles at 89°C for 15 s, 55°C for 15 s, 72°C for 30 s,
and by a final extension at 72°C for 10 min.

Then the PCR products were denatured for 5 min at 100°C,
mixed with formamide-containing stop buffer, and subjected to
electrophoresis on 4% polyacrylamide gel containing 8-M urea in
an ABI Prism 377-18 DNA sequencer (Applied Biosystem). The
number of microsatellite repeats was estimated automatically with
an ABI Prism 377-18 DNA sequencer (Applied Biosystem). The
polymorphysim analysis

Analyses with clinicopathological factors

Any possible significant alteration of MICA allele will be analysed
with their clinicopathological factors, which are based on Japanese
criteria (Japanese Gastric Cancer Association, 1998) and include
age, sex, tumour location, tumour size, cellular differentiation,
gross appearance, histological patterns, stromal reaction (cancer–
stroma relationship), depth of invasion, lymph node status and
tumour stage to see if there is correlation among them. Based on
the amount of stromal tissue, stromal reaction (cancer–stroma
relationship) of gastric cancer was classified into scirrhous,
medullary and intermediate types by observation of H&E stained
pathological sections (Japanese Gastric Cancer Association, 1998).
In this study the scirrhous type was quantitatively defined as
tumour stroma occupied more than 50% of tumour area, less than
10% in medullary type and 10–50% in the intermediate type. The
three categories were determined under ×40 (low power field)
magnification field.

Statistical analysis

The difference of phenotype and gene frequencies between patients
and normal controls were analysed by using χ² test. Significant
alteration of MICA allele also was analysed with clinicopathological
factors by using χ² test. Statistically significant difference was
defined as P<0.05.

RESULTS

To establish the phenotypic frequencies of MICA alleles in
Taiwanese population, we have analysed 351 normal samples.
The controls’ ages ranged from 22 to 71 years (mean + s.d. = 42.1 ± 10.7). Age did not affect the MICA alleles distribution.
The gender distribution of the control was 185:166, male to
female. The analyses concluded that A4 is 31%, A5 is 50%, A5.1 is
36%, A6 is 8% and A9 is 18%. This is important for this study,
since the frequencies are different in various areas (Table 1). With
this information available to us, we then analysed and compared
samples from 107 patients with gastric adenocarcinoma who
underwent gastrectomy in our hospital. Although no significant
difference of frequency of A4, A5, A5.1 and A6 alleles was found
between normal controls and gastric cancer patients, both the
phenotypic and allelic frequencies of A9 were significantly higher
than those in normal controls (33 vs 17.6%, P = 0.005; 17 vs 9.9%,
P = 0.02) (Tables 2 and 3).

We further examined whether the A9 allele might contribute to
the clinicopathological factors of these patients. Several clinicopathological factors such as age, sex, tumour location, size, gross
appearance, histological patterns, depth of invasion, lymph node
status and TNM staging were included in the analyses. Among
these factors, we found that gastric cancer with allele A9 was
strongly associated with less schirrous reaction (stromal reaction)
compared with ‘non-A9’ gastric tumours (P = 0.014) (Table 4),
suggesting that gastric cancer patients with allele A9 associated
with less schirrous reaction.

Table 1 Phenotypic frequencies of MICA alleles in various countries

| Allele | Taiwana (n = 351) (%) | Japanb (103) (%) | Spainb (342) (%) | Swedenb (153) (%) |
|--------|----------------------|-----------------|-----------------|-----------------|
| A4     | 31                   | 20              | 33              | 26              |
| A5     | 50                   | 52              | 21              | 56              |
| A5.1   | 36                   | 17              | 43              | 66              |
| A6     | 8                    | 46              | 55              | 9               |
| A9     | 18                   | 31              | 29              | 18              |

*Current study. bPetersdorf et al (1999).

Table 2 Phenotype frequencies of MICA gene in gastric carcinoma patient and normal control

| Phenotype | Normal (N = 351) | Gastric ca (N = 107) | P-value | Corrected P |
|-----------|-----------------|---------------------|---------|-------------|
| A4        | 107             | 35                  | 0.663   | 3.315       |
| A5        | 174             | 59                  | 0.313   | 1.565       |
| A5.1      | 127             | 40                  | 0.821   | 4.105       |
| A6        | 27              | 13                  | 0.153   | 0.765       |
| A9        | 62              | 35                  | 0.001*  | 0.005*      |

*Statistical significance.
In this study, we have shown that the MICA allele A9 was significantly correlated with gastric adenocarcinoma and less schirrous change in gastric cancer tissue. These findings suggest that MICA allele A9 may be important in the etiology and immune reaction of gastric adenocarcinoma. Although status of stromal reaction is not routinely included in pathological report, it was reported to be a prognostic indicator of gastric cancer (Wu et al., 1997). Stromal reaction of tumour was shown to relate with cancer desmoplastic reaction (Ohtani et al., 1992), angiogenesis (Engels et al., 1997), tumour invasion and metastasis, tumour cell proliferation (Wernet, 1997), tumour cell adhesion molecules and production of matrix-degrading enzyme by stromal cells to facilitate tumour invasion and metastasis (Wernet, 1997). Currently, the cellular and molecular events of stromal reaction were proposed to be similar to those of wound healing (Dvorak, 1986) and inflammatory diseases, such as ulcerative disease and Crohn’s disease (Ohtani, 1998). In these inflammatory lesions, the aberration of the immune system is speculated to be the cause of the diseases. Stromal reaction of

Table 3 Allelic frequencies of MICA gene in gastric carcinoma patient and normal control

| Allele | Normal (N = 702) | Frequency | Gastric ca (N = 214) | Frequency | P-value | Corrected P |
|--------|-----------------|-----------|----------------------|-----------|---------|-------------|
| A4     | 161             | 22.9%     | 19                   | 1.22      |
| A5     | 262             | 37.3      | 34                   | 1.965     |
| A5.1   | 177             | 25.2      | 23                   | 2.455     |
| A6     | 32              | 4.5       | 7                    | 1.225     |
| A9     | 70              | 9.9       | 17                   | 0.02a     |

*aStatistic significance.

Table 4 Clinicopathological features of gastric cancer with A9 phenotype MICA

| Parameter | A9 (n = 35) | Non-A9 (n = 72) | P-value |
|-----------|-------------|-----------------|---------|
| Age       |             |                 |         |
| <65       | 15          | 26              | 0.617   |
| >65       | 20          | 46              |         |
| Sex       |             |                 |         |
| Male      | 26          | 55              | 0.978   |
| Female    | 9           | 17              |         |
| Tumour location |         |                 |         |
| Upper 1/3 | 7           | 9               | 0.512   |
| Middle 1/3| 10          | 28              |         |
| Lower 1/3 | 18          | 34              |         |
| Whole     | 0           | 1               |         |
| Tumour size |           |                 |         |
| <4 cm     | 19          | 30              | 0.287   |
| 4–8 cm    | 12          | 35              |         |
| >8 cm     | 4           | 7               |         |
| Cell differentiation |       |                 |         |
| Well      | 1           | 1               | 0.424   |
| Moderate  | 19          | 30              |         |
| Poor      | 15          | 41              |         |
| Borrmann type |       |                 |         |
| O         | 19          | 30              | 0.256   |
| II        | 5           | 11              |         |
| III+IV    | 11          | 31              |         |
| Infiltration type |     |                 |         |
| Alpha     | 12          | 24              | 0.466   |
| Beta      | 12          | 18              |         |
| Gamma     | 11          | 31              |         |
| Stromal reaction |     |                 |         |
| Medullary | 9           | 23              | 0.014a  |
| Intermediate | 25        | 33              |         |
| Schirrous | 1           | 16              |         |
| Ming classification |     |                 |         |
| Infiltrative | 15        | 26              | 0.560   |
| Expanding | 20          | 46              |         |
| Lauren’s classification |     |                 |         |
| Intestinal type | 22      | 36              | 0.191   |
| Diffuse type | 13         | 36              |         |
| Depth of tumour invasion |     |                 |         |
| T1        | 14          | 28              | 0.744   |
| T2        | 6           | 10              |         |
| T3+T4     | 15          | 34              |         |

**Table 4 (Continued)**

| Parameter | A9 (n = 35) | Non-A9 (n = 72) | P-value |
|-----------|-------------|-----------------|---------|
| Lymph node metastasis |       |                 |         |
| Negative  | 23          | 36              | 0.123   |
| Positive  | 12          | 36              |         |
| Liver metastasis |      |                 |         |
| Negative  | 33          | 71              | 0.221   |
| Positive  | 2           | 1               |         |
| Peritoneal dissemination |     |                 |         |
| Negative  | 34          | 70              | 1.000   |
| Positive  | 1           | 2               |         |
| TNM stage |             |                 |         |
| I         | 18          | 34              | 0.292   |
| II        | 7           | 6               |         |
| III       | 6           | 21              |         |
| IV        | 4           | 11              |         |

*aStatistic significance.

DISCUSSION

In this study, we have shown that the MICA allele A9 was significantly correlated with gastric adenocarcinoma and less schirrous change in gastric cancer tissue. These findings suggest that MICA allele A9 may be important in the etiology and immune reaction of gastric adenocarcinoma. Although status of stromal reaction is not routinely included in pathological report, it was reported to be a prognostic indicator of gastric cancer (Wu et al., 1997). Stromal reaction of tumour was shown to relate with cancer desmoplastic reaction (Ohtani et al., 1992), angiogenesis (Engels et al., 1997), tumour invasion and metastasis, tumour cell proliferation (Wernet, 1997), and immune reactions (Saiki et al., 1996). Tumour stroma is composed of new blood vessels, inflammatory cells and connective tissue (Dvorak, 1986). Tumour stromal reaction include many complicated interrelated processes, including production of cytokine (interleukine) to induce immune T cells to elicit tumour regression (Nabeta et al., 2000), expressing adhesion molecules and production of matrix-degrading enzyme by stromal cells to facilitate tumour invasion and metastasis (Wernet, 1997). Currently, the cellular and molecular events of stromal reaction were proposed to be similar to those of wound healing (Dvorak, 1986) and inflammatory diseases, such as ulcerative disease and Crohn’s disease (Ohtani, 1998). In these inflammatory lesions, the aberration of the immune system is speculated to be the cause of the diseases. Stromal reaction of
tumour can also be regarded as an immune response to a neogrowth. Therefore, the host immune reactions can be regarded as a factor in modulating the aggressiveness of a tumour. For desmoplastic reaction, it is still uncertain whether it is defensive for the host or it is facilitating the tumour growth, although a poorer survival was reported in patients with gastric cancer and breast cancer (Cardone et al., 1997; Caporale et al., 2001). As shown in the current study, less scirrhous (less desmoplastic reaction) type tumour appeared in A9 allele group (P = 0.014) and was probably resulted from some host immune mechanism.

MICA encodes a molecule similar to MHC class I antigens and may share the same capacity of binding to short peptides or small ligands. MICA is expressed in fibroblasts, epithelial cells (Bahram et al., 1994), keratinocytes, endothelial cells and monocytes (Zwirner et al., 1998), and may play a role in the immune response (Bahram et al., 1994). Its expression is regulated by a promoter heat shock element similar to those of heat shock protein (HSP) genes (Groh et al., 1996). High levels of MICA expression in epithelial cell lines together with upregulation of MICA after heat shock may represent a new molecular mechanism of exposing stressed epithelial cells to the immune system (Bahram et al., 1996). It is shown that HSPs are involved in the formation of malignancy (Bonay et al., 1994; Kawanishi et al., 1999), including gastric adenocarcinoma (Liu et al., 1999; Maehara et al., 2000). In addition, they are expressed by transformed/cancer cells, which are important targets for T lymphocytes. High levels of MICA expression in epithelial cells after heat shock (stress) may not be coincident. It may provide a mechanism of exposing transformed cells to the mucosal immune system allowing γδ T cells (Bahram et al., 1996; Groh et al., 1998), a subset of T cells expressing the γδ T cell receptors (TCRs) γδ heterodimer (Haas et al., 1993), to recognise and destroy transformed/damaged cells. Although γδ T cells constitute only about 5% of circulating T cells, they are distributed throughout the human intestinal epithelium and may function as sentinels that respond to self-antigens. Interestingly, MICA is almost exclusively expressed intestinal epithelium. Recently, it was shown that NK cells and antigen-specific effector T cells could be triggered by MIC engagement of NKG2D (Bauer et al., 1999), a receptor expressed on most NK cells, γδ T cells and CD8+ γδ T cells involved in the innate and adaptive immune responses (Bauer et al., 1999). However, circulatory MICA secreted by neoplasms can downregulate the expression of NKG2D and impair the responsiveness of effector T cells (Groh et al., 2001). Whether MICA A9 antigen product can result in altered immunity and susceptibility to gastric cancer via reactions with HSPs or γδ T cell or NKG2D need further investigation (Groh et al., 1998, 2001, 2002; Bauer et al., 1999).

In summary, all of the above findings suggesting MICA may relate with host immunity. Since its alleles vary among individuals and may confer variable disease susceptibility, analyses of MICA alleles maybe useful in cancer investigation. Our results demonstrated that Taiwanese carrying an A9 allele have higher risk to gastric cancer. Furthermore, gastric cancers with A9 allele are associated with less scirrhous change. Further investigation can study the mechanism of activity of MICA A9 allele. Identification of the mechanism of association of MICA A9 allele with gastric cancer could help the individuals most likely benefit from cancer screening and prevention program and may suggest novel treatment modality.

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