Observational Study

**CD24 genetic variants contribute to overall survival in patients with gastric cancer**

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**METHODS:** We genotyped 3 tagging SNPs of **CD24** gene in the promoter region (P-534), in the coding region of exon 2 (P170) and in the 3′ untranslated region (P1527) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in specimens from 679 histologically-confirmed GC cases, 111 gastric atrophy (GA) cases and 976 tumor-free controls.

**Abstract**

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immunoglobulin G antibodies to *Helicobacter pylori* (*H. pylori*) of all subjects were detected by enzyme-linked immunosorbent assay. CD24 expression was evaluated by immunohistochemistry in 131 GC specimens. Correlations between SNPs and risk of GC or GA were shown by *P* values and odd ratios (ORs) with 95% confidence intervals (95%CI) compared with the most common genotype of each SNP using the unconditional logistic regression model after adjusting for age, sex and *H. pylori* infection. Survival within each SNP group was plotted by Kaplan-Meier method and compared by log-rank test (recessive model). Hazard ratios with 95%CIs were computed by Cox regression model after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operative chemotherapy.

RESULTS: All of the three loci were in Hardy-Weinberg equilibrium in the control group. Median follow-up time for the 600 GC patients included in the survival analysis was 36.2 mo (range, 2.1-66.7 mo; 95%CI: 34.3-36.5 mo). Patients with the P-534 A/A genotype had significantly shorter survival (HR = 1.38, 95%CI: 1.01-1.88, *P* = 0.042) than did the C/C or C/A genotype carriers after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operative chemotherapy. This trend was more evident in patients who lived longer than 2.5 years (HR = 7.55, 95%CI: 2.16-26.32, *P* = 0.001). The P170 T/T genotype was associated with a shorter lifespan than the non-T/T genotypes, but not significantly so. None of the three genetic variants was found to be associated with risk of GC (including tumor stage, grade and distant metastasis) or with risk of gastric atrophy. Furthermore, no difference of CD24 expression was found among the genotypes.

CONCLUSION: The P-534 site in CD24 gene affects the overall survival of gastric cancer and may serve as a prognostic marker for gastric cancer.

Key words: Gastric cancer; CD24; Single nucleotide polymorphisms; Gastric atrophy; Overall survival

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Core tip: We evaluated the role of three genetic variants of CD24 in gastric cancer (GC) risk and prognosis using 679 GC cases and 976 controls. We observed that GC cases with the A/A genotype of P-534 (which lies in the CD24 promoter) had a significantly shorter survival (HR = 1.38) especially among patients who lived longer than 2.5 years (HR = 7.55) after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operative chemotherapy. Our study provides the first evidence that P-534 site in CD24 may serve as a prognostic marker for gastric cancer.

**INTRODUCTION**

Gastric cancer (GC) is one of the most common malignancies and the third cause of cancer-related death worldwide[11]. *Helicobacter pylori* (*H. pylori*) infection has been established to cause GC and was classified as carcinogenic to humans (Group 1) by IARC in 1994[3]. Although over half of the world’s population are estimated to be infected with *H. pylori* [3], relatively few develop GC, and gastric damages induced by *H. pylori* infection vary widely, which together imply a role for host genetic factors in response to chronic *H. pylori* infection and subsequent GC development.

CD24 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface glycoprotein with functions in signal transduction and cell adhesion. It is expressed in a large variety of human malignancies. Over-expression of CD24 results in a more aggressive malignant phenotype with greater proliferative and cell migration capabilities, whereas its down-expression shows a less malignant phenotype[4-6]. CD24 is reportedly associated with tumor growth, invasion, metastasis, recurrence and treatment response in various cancers, including breast cancer[7,8], prostate cancer[9], colorectal cancer[10,11], hepatocellular carcinoma[12], esophageal squamous cell carcinoma[13] and GC[14,15]. CD24 is also a potential marker of cancer stem cells, which possess capabilities for tumorigenesis, self-renewal and producing differentiated progeny[16-18].

In GC, CD24 can mediate carcinogenesis and promote GC progression[14,15]. CD24 expression gradually increases in the progression of normal gastric mucosa, non-atrophic chronic gastritis, chronic atrophic gastritis (CAG), CAG with intestinal metaplasia, dysplasia and finally, GC[14]. Mice with normal CD24 expression show more gastric inflammation, parietal cell atrophy and gland hyperplasia following *Helicobacter felis* infection compared with CD24-null mice which have the genetic background of inbred CD24-normal mice[19].

Given the important role of CD24 in GC, we tested whether single nucleotide polymorphisms (SNPs) in the CD24 gene are associated with genetic susceptibility to GC tumor progression and prognosis in a Chinese population.

**MATERIALS AND METHODS**

**Subjects**

From July 2008 to December 2012, patients with
Table 1  Primer sequences used for polymerase chain reaction

| Primer | Sequence | Length of product | Endonucleases | Bands |
|--------|----------|-------------------|---------------|-------|
| P-534F | 5'-AGAGATAAACCCTGCCGAG-3' | 209 bp | BsrFI | C: 126 bp + 83 bp |
| P-534R | 5'-CCAGGTCTTCCGTGCCCC-3' | 1882 bp | - | - |
| outerF | 5'-CCACTTGGCAGTATTGGACATCT-3' | 404 bp | BstXI | T: 275 bp + 129 bp |
| outerR | 5'-TGCTGCTGAGCCAGTTGAAAG-3' | 847 bp | BsrI | TCG: 645 bp + 202 bp |
| P170F | 5'-CTAAAGAGAAGTACCTTGTTGGCTGAG-3' | 202 bp fragments | - | - |
| P170R | 5'-GGATTGGGTTTAGAAGATGGGGAAA-3' | - | - | - |
| P1527F | 5'-GCCAGCGCAAATGAGAATGAG-3' | - | - | - |
| P1527R | 5'-TTGTTGCGGCGAGTTGAAAGAT-3' | - | - | - |

The full length of the CD24 gene was first identified by our previous study and was mapped to Chromosome 6q21 by fluorescence in situ hybridization (submitted to NCBI database, accession number FJ226006)[20]. Although SNP data on CD24 was unavailable in HapMap project or dbVar database, we have identified three linkage disequilibrium (LD) blocks that cover the promoter region and exons 1-2 of the CD24 locus[20]. Haplotype tagging SNPs (tag SNPs) were identified from the three LD blocks with the pairwise $r^2 ≥ 0.9$ and minor allele frequency $> 0.05$.

Three tagging SNPs, P-534C/A, P170C/T and P1527TG/del were genotyped to evaluate the association of CD24 and genetic susceptibility to GC. P-534C/A is located in the promoter region of CD24 and is 534bp away from the translation-starting site. P170C/T is located in the coding region of exon 2 and its C-to-T transition leads to an alanine to valine substitution at codon 57 of the CD24 protein. P1527TG/del, 1527bp down from the translation-starting site, is located in the 3' untranslated region.

Genotypes of the selected sites were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described by Li et al[21] and our previous study[20]. Genotyping was performed after PCR reaction. PCR products were then digested with endonuclease BsrFI overnight at 37 ℃ and the restriction site indicated the presence of the C allele (126 bp and 83 bp) (All restriction endonucleases were bought from New England Biolabs, United States). For P170 and P1527, a nested PCR in which one of the primers for the first PCR mapped to an intron was performed to increase specificity as the exon 2 of CD24 shows high homology with the intronless pseudogenes in Chromosomes 1, 15 and Y[22]. The second PCRs were amplified independently for P170 and P1527 using the 1000-fold-diluted products of the first PCR as templates (all reagents for PCR were from Tiangen, Beijing, China). These PCR products were then digested overnight with the restriction enzyme BsrXI (for P170, 37 ℃) and BsrI (for P1527, 65 ℃). The T allele of P170 produced two fragments: 275 bp and 129 bp; the T allele of P1527 produced 645 and 202 bp fragments. All products were separated by electrophoresis on 1.5% agarose gels with ethidium bromide staining and scanned on gel imaging system (Gel Doc TM XR+ system, Bio-Rad, United States). Fifty samples were randomly selected to be genotyped by direct sequencing to confirm the validity of PCR-RFLP;
three of them with different genotypes were used as positive controls for each PCR-RFLP run. The overall concordance was 100%.

### Testing for H. pylori infection and diagnosis of gastric atrophy

Serum immunoglobulin G (IgG) antibodies to *H. pylori* were detected by enzyme-linked immunosorbent assay (ELISA) using *H. pylori*-IgG ELISA kits (Biohit, Finland) according to the manufacturer’s protocol. Titers higher than the cut off value of 30 EU were considered positive for *H. pylori* infection. The inter-day coefficient variations (CV) of the negative and the positive control samples were 4.5% and 1.4%, respectively.

Diagnosis of GA was described elsewhere[23]. Briefly, serum pepsinogen I (PGI) and II (PGⅡ) were quantified by ELISA kits (Biohit, Finland). Individuals with PGI < 82.3 ng/mL and PGI/PGⅡ < 6.05 were diagnosed as GA.

### Immunohistochemistry of CD24

CD24 expression was assessed in tumor tissue of 131 gastric cancer patients by immunohistochemistry (IHC) method. The detailed procedure is described elsewhere[24]. Briefly, the 4-μm-wide tissue sections were deparaffinized and stained using a streptavidin-biotin immunoperoxidase technique. All slides were then incubated with anti-human CD24 polyclonal antibody (1:100 diluted, sc-7034, Santa Cruz, United States) and developed by 3, 3-diaminobenzidine (DAB). As negative controls, the slides were treated with the IgG isotypes in place of primary antibodies and all negative controls demonstrated negligible background staining. The stained slides were independently evaluated by two pathologists (MSJ and YPW) who were blinded to clinical data and outcomes. The HSCORE system was used to assess the staining results and was calculated by a following equation: 

$$HSCORE = \sum Pi(i)$$

(i = 0, 1, 2, 3, Pi = 0-100). The i means the intensity of staining (no staining: 0; weak staining: 1; moderate staining: 2; and strong staining: 3). Pi represents percentages (0-100) of stained cells with intensities. The HSCORE ranges from 0 to 300.

### Statistical analysis

Continuous data were summarized as medians (25th to 75th percentiles) and compared by Mann-Whitney U test or Kruskal-Wallis test. Categorical variables were described as frequencies and percentages and compared using \( \chi^2 \)-test. Correlations between SNPs and risk of GC or GA were demonstrated by \( P \) values and odd ratios (ORs) with 95% confidence intervals (95%CI) compared with the most common genotype of each SNP. The \( P \) values and ORs with 95%CIs were calculated using the unconditional logistic regression model after adjusting for age, sex and *H. pylori* infection. Survival functions of the GC patients within each SNP were plotted by Kaplan-Meier method and compared by log-rank test using the recessive model. Hazard ratios (HRs) with 95%CIs were used to quantify the influence of genotypes of each SNP on overall survival and were calculated with Cox regression model after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operative chemotherapy. For haplotypes with frequencies > 1%, their associations with risk of GC or GA were assessed compared to the most common haplotype using the logistic regression model with the HAPSTAT software 3.0[25]. Unless otherwise stated, analyses were performed in SAS 9.1.3 software (SAS Institute Inc, United States). A two-tailed \( P \) value < 0.05 was considered to be statistically significant.

### RESULTS

#### Subject characteristics

A total of 679 GC cases, 111 GA cases and 976 tumor-free controls were included in the study. The baseline characteristics of the subjects are summarized in Table 2. The GC group was oldest in the three group and the control group was youngest (median age: 61.0 years vs 50.0 years vs 48.5 years, \( P < 0.001 \) for all pairwise comparisons). And there were more males in the cancer group (71.7% vs 59.5% vs 59.2%, \( P < 0.001 \) for comparisons to the atrophy cases or the controls). In the GC group, 67.8% were positive for *H. pylori* infection, significantly higher than the control...
Table 2 Characteristics of subjects included n (%)  

| n | Cancer | Atrophy | Controls | P value |
|---|--------|---------|----------|---------|
| Gender | | | | |
| Male | 487 (71.7) | 66 (59.5) | 578 (59.2) | < 0.0001 |
| Female | 192 (28.3) | 45 (40.5) | 398 (40.8) | |

| Age (yr) | | | | |
|< 55 | 54.0-70.0 | 47.0-57.0 | 44.0-55.0 | |
| 55-70 | 204 (30.0) | 76 (68.5) | 756 (77.5) | < 0.0001 |
| > 70 | 310 (45.7) | 28 (25.2) | 181 (18.5) | |

| H. pylori | | | | |
|Positive | 453 (67.8) | 84 (75.7) | 478 (49.7) | < 0.0001 |
|Negative | 215 (32.2) | 27 (24.3) | 483 (50.3) | |

Data are presented as frequency counts (percentage of total) or median (25th to 75th percentiles).

The GC cases were mainly of tubular adenocarcinoma type (82.2%), with low-grade differentiation (68.4%), at TNM stage II (35.1%) or III (34.6%). One third of the cases received chemotherapy after operation (32.8%); 14.7% received FOLFOX-4 and 6.9% received XELOX.

Association of SNPs with risk of gastric cancer or gastric atrophy

All of the three SNPs were in Hardy-Weinberg equilibrium in the control group (P-534: P = 0.612; P170: P = 0.413; P1527: P = 0.423). Distributions of genotypes and alleles are listed in Table 3. Compared with the most common genotype of each SNP, no difference was observed for the distributions of the three loci between the GC and control groups after adjusting for age, sex and H. pylori infection. No allele or haplotype was associated with risk of GC. Similar negative results were obtained for GA risk (Table 3). Moreover, no associations were observed between SNPs and risk of H. pylori infection (data not shown).

Association of SNPs with clinicopathologic parameters of GC

Genotypic distributions of SNPs were analyzed by clinicopathologic parameters such as histological type, tumor differentiation, TNM stage and distant metastasis in GC cases. However, no significant association was observed (Table 4).

Association of SNPs with survival of gastric cancer

Follow-up information was available for 610 of the 679 GC patients (89.8%). Ten patients died of postoperative complications within 30 d at the beginning of the study period (range, 0-29 d, median: 15.5 d) and these cases were excluded from analyses of effects of SNPs on survival. The median follow-up time for the remaining 600 GC patients was 36.2 mo (range, 2.1-66.7 mo; 95%CI: 34.3-36.5 mo). Two hundred and sixty patients (43.3%) died from GC during the follow-up, 272 patients (45.3%) lived and 68 (11.3%) died of other causes or were lost to follow up.

Survival curves were plotted and compared according to genotypes of each SNP using the recessive model. The patients who carried the A/A genotype of P-534 had shorter survival than those carrying C/C or C/A after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operational chemotheraphy (HR = 1.38, 95%CI: 1.01-1.88, P = 0.042; Table 5 and Figure 2A). This trend was more evident in patients who lived longer than 2.5 years (HR = 7.55, 95%CI: 2.16-26.32, P = 0.001). Similarly, the P170 T/T carriers tended to have shorter survival time than the C/C or C/T carriers, although not significantly so (Figure 2B). No meaningful correlation could be observed between the variation of “TG” deletion in P1527 and GC survival (P = 0.799).

Multivariate Cox regression analysis showed that three other factors-degree of differentiation, TNM stage and post-operational chemotherapy-were associated with the prognosis of GC. Patient whose GC had low-grade differentiation or advanced clinical stage or who did not received post-operational chemotherapy had shorter survival time (Table 5).

CD24 expressions in tissues of GC

To assess whether SNPs were associated production of CD24 protein, CD24 expression was evaluated in cancerous tissue of 131 GC cases using IHC. Genotypic distributions of the three SNPs in selected cancer cases were similar to non-selected cases (data not shown). CD24 expression was seen mainly in membranes of tumor cells (Figure 3). However, CD24 expression did not observably differ among genotypes of each SNP (Figure 3 and Table 3).
Table 3  Distributions of genotypes in three groups \( \alpha (\%) \)

|    | Controls | Cancer  | OR\(^{1}\) (95%CI) | Atrophy  | \( P \) value | OR\(^{1}\) (95%CI) |
|----|----------|---------|---------------------|----------|---------------|-------------------|
| P-534 | C/C | 271 (27.8) | 181 (26.7) | - | Reference | 29 (26.1) |
|     | C/A | 488 (50.0) | 358 (52.7) | 0.099 | 1.1 (0.86-1.48) | 62 (55.9) |
|     | A/A | 217 (22.2) | 140 (20.6) | 0.145 | 0.9 (0.61-1.20) | 20 (18.0) |
|     | C | 1030 (52.8) | 720 (53.0) | 0.886 | - | 130 (54.1) |
|     | A | 922 (47.2) | 638 (47.0) | 1.0 (0.86-1.14) | 102 (45.9) |
| P170 | C/C | 419 (42.9) | 295 (43.4) | - | Reference | 60 (54.1) |
|     | C/T | 439 (45.0) | 308 (45.4) | 0.277 | 1.1 (0.82-1.38) | 37 (33.3) |
|     | T/T | 118 (12.1) | 76 (11.2) | 0.369 | 0.9 (0.60-1.29) | 14 (12.6) |
|     | C | 1277 (65.4) | 898 (66.1) | 0.673 | - | 157 (70.7) |
|     | T | 675 (34.6) | 460 (33.9) | 1.0 (0.83-1.13) | 65 (29.3) |
| P1527 | TG/TG | 826 (84.6) | 559 (82.3) | - | Reference | 95 (85.6) |
|      | TG/del | 142 (14.6) | 117 (17.2) | 0.069 | 1.3 (0.97-1.82) | 15 (13.5) |
|      | del/del | 5 (0.5) | 3 (0.4) | 0.162 | 0.4 (0.09-1.77) | 1 (0.9) |
|      | TG | 1794 (91.9) | 1235 (90.9) | 0.956 | - | 205 (92.3) |
|      | del | 158 (8.1) | 123 (9.1) | 1.1 (0.88-1.45) | 17 (7.7) |
| Haplotype\(^{2}\) | ACTG | 45.6 | 47.0 | - | Reference | 45.4 |
|      | CTTG | 33.6 | 33.7 | 0.946 | 1.0 (0.85-1.16) | 28.7 |
|      | CCTG | 11.4 | 10.2 | 0.382 | 0.9 (0.71-1.14) | 17.7 |
|      | CCdel | 8.0 | 9.1 | 0.350 | 1.1 (0.87-1.46) | 7.7 |

Data are presented as frequency counts (percentage of total). \(^{1}\)ORs of the genotypes were calculated adjusting for age, sex and \( H. \) pylori infection in logistic regression model; \(^{2}\)The haplotype was lined with P-534, P170 and P1527 and displayed as percentage.

Table 4  Distributions of genotypes according to clinical parameters in gastric cancer cases

|    | P-534 | \( P \) value | P170 | \( P \) value | P1527 | \( P \) value |
|----|-------|---------------|------|---------------|-------|---------------|
|    | C/C | 181 | 60 (53-70) | 61 (54-71) | 61 (55-70) |
|    | C/A | 358 | 0.715 | 61 (53-71) | 61 (54-70) | 63 (56-70) |
|    | A/A | 140 | 0.658 | 61 (54-70) | 61 (51-71) |
|    | Male | 27.3 | 23.8 | 28.7 | 30.2 | 28.6 |
|    | Female | 25.0 | 39.4 | 4.9 | 52.8 | 12.1 |
|    | H. pylori Positive | 26.3 | 42.6 | 11.3 | 42.6 | 17.7 |
|    | Negative | 27.0 | 45.6 | 10.7 | 45.6 | 17.7 |
|    | Poor Differentiation | 24.7 | 43.5 | 9.2 | 43.5 | 18.5 |
|    | Moderate to well | 30.2 | 45.0 | 14.8 | 45.0 | 28.6 |
|    | TNM stage 1-2 | 26.2 | 45.1 | 10.2 | 45.1 | 17.6 |
|    | III-IV | 26.3 | 42.7 | 12.1 | 42.7 | 17.6 |
|    | Pathologic type Tubular adenocarcinoma | 26.4 | 45.0 | 11.2 | 45.0 | 17.2 |
|    | Signet ring cell Other | 26.0 | 34.0 | 6.0 | 34.0 | 20.0 |
|    | Distant metastasis Positive | 32.3 | 40.4 | 15.1 | 40.4 | 20.2 |
|    | Negative | 25.1 | 44.3 | 10.5 | 44.3 | 17.1 |
|    | CD24 staining | 60 (0-120) | 60 (0-120) | 60 (20-140) | 60 (0-120) | 60 (40-100) |

Data are presented as percentage of total or median (25th to 75th percentiles).
DISCUSSION

In this study, we explored the association between variants of CD24 gene and GC. We found that patients who harbored the P-534 A/A genotype tended to have shorter survival than those who carry P-534 non-A/A genotypes.

This is the first study on the association between SNPs of CD24 gene and GC, as no CD24 SNPs were included in any genome-wide association studies of GC [26,27]. Distribution of the P-534 genotypes of CD24 differs slightly from that of Caucasian populations. The minor allele C of P-534 in Caucasian population (37.2% [20]) was the major allele (52.8%) in Han Chinese in our study. Distributions of P170 and P1527 were similar to those of other ethnicities [28,29].

Numerous studies have reported that SNPs of CD24 gene are correlated with risk of various autoimmune diseases, such as systemic lupus erythematosus (SLE) [28-31], multiple sclerosis [28,32-35] and inflammatory bowel disease [28,36]. Li et al. [21] reported that P170 and P1527 of CD24 affected risk and progression of chronic hepatitis B infection and Sheng et al. [37] showed that the T/T genotype of P170 correlated with a 2.96-fold increased of risk of hepatocellular carcinoma. In our study, however, we did not observe any influence of CD24 polymorphisms on risk of H. pylori infection (data not shown, but available on request), gastric atrophy, precancerous lesions of GC that are induced partly by H. pylori infection [38,39], or GC (Table 3).

Polymorphisms of CD24 have been related to prognosis in several cancers. In breast cancer, CD24 expression was associated with adverse prognosis [7,8] and CD24 P170 polymorphism could predict response to chemotherapy [40-42]. In esophageal cancer, P170 of CD24 was involved in regional lymph node metastasis [43]. In our study, we found that P-534 of CD24 affected long-term survival of GC, as P-534 A/A genotype carriers have a 7.5-fold increased mortality risk compared with non-A/A carriers among patients who lived longer than 2.5 years (Figure 2A). P170 T/T carriers also tended to have shorter survival than did non-T/T carriers, although not significantly so (Figure 2B).

The P-534, which is located in the promoter region of CD24, may influence transcriptional activity. Our previous work showed that a hypomorphic haplotype that contained the C allele of P-534 was associated with risk of multiple sclerosis and this haplotype was involved in higher transcriptional activity and increased expression of CD24 in peripheral blood lymphocytes [20]. The non-synonymous variant P170 may alter the quantity and quality of CD24. Zhou et al. [32] found that the P170T/T genotype expressed more cell-surface CD24 than did the C/T or C/C genotypes using flow

### Table 5 Results of multivariate Cox regression analysis

|                | P value | HR  | 95% CI    |
|----------------|---------|-----|-----------|
| P-534          |         |     |           |
| C/C + C/A      |         | 1.0 |           |
| A/A            | 0.0416  | 1.38| 1.01-1.88 |
| Age            | 0.1875  | 1.01| 1.00-1.02 |
| Sex            |         |     |           |
| Male           |         | 1.00|           |
| Female         | 0.2315  | 0.83| 0.61-1.13 |
| Differentiation|         |     |           |
| Moderate to well| 0.0009 | 1.50| 1.11-2.04 |
| Poor           |         |     |           |
| Pathologic type|         |     |           |
| Tubular adenocarcinoma | 0.0003 | 5.50| 2.18-13.86 |
| Signet ring cell | 0.0001 | 22.29| 9.05-54.89 |
| Other          | 0.9469  | 1.01| 0.67-1.53 |
| TNM stage      |         |     |           |
| I              |         | 1.00|           |
| II             | 0.0003  | 5.50| 2.18-13.86 |
| III            | < 0.0001| 22.29| 9.05-54.89 |
| IV             | < 0.0001| 32.12| 12.63-81.70 |
| Chemotherapy   |         |     |           |
| No             |         | 1.00|           |
| FOLFOX-4       | 0.0195  | 0.64| 0.45-0.93 |
| XELOX          | 0.0021  | 0.43| 0.25-0.74 |
| Other          | 0.0004  | 0.45| 0.29-0.70 |

Figure 2 Survival plots of gastric cancer patients. A: Plot for P-534 using the recessive model (A/A vs C/C + C/A); B: Plot for P170 using the recessive model (T/T vs C/C + C/T).
cytometry, which showed an increased risk and more rapid progression of multiple sclerosis. In our study, we evaluated CD24 expression using IHC and found that CD24 was expressed mainly in the membranes of GC cells. However, we did not observe any differences of CD24 expression among genotypes of P-534, P170 or P1527.

Three limitations should be noted in our study. The first one is that some baseline characteristics such as age are different among the three study groups, and we cannot rule out the possibility that individuals in the control group could develop GC when they are older. However, we control the potential influences to the greatest extent by adjusting for these factors in the multivariate analysis. The second is that the follow-up time of the GC cases seems insufficient because most of cases on the right side of the survival plots are censored. This may explain that although individuals carrying P-534 A/A genotype tended to have shorter observable survival time, $P$ value from log-rank tests is not significant. However, when we adjust the potential influencing factors and divide the patients to subgroups who live shorter than 2.5 years and those who live longer than 2.5 years, the association of P-534 with long-term survival is statistically significant (HR = 7.5, 95%CI: 2.2-26.3). Nonetheless, longer follow-up time is needed to re-evaluate the role of CD24 SNPs in prognosis of GC. The last one is that we semi-quantified CD24 expression in tissue using IHC and the influence of SNPs on CD24 might be offset, as protein production can be regulated by factors known and unknown e.g., regulations of transcription, post-transcription and translation. Therefore, more rigorous design should be applied in our future study.

In summary, we find that polymorphisms of the CD24 gene affect the overall survival of GC, as patients who bear the P-534 A/A genotype tend to have shorter survival than do patients with P-534 non-A/A genotypes. However, we do not observe any associations between CD24 SNPs and risk of $H.\ pylori$ infection, GA or GC. More studies with larger samples and longer follow-up time are needed to clarify the role of CD24 in GC.

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**COMMENTS**

**Background**

CD24 expression as a potential biomarker is associated with poor prognosis in patients with gastric cancer (GC) but its genetic basis still remains to be elucidated.

**Research frontiers**

GC is one of the most common malignancies and the third cause of cancer-related death worldwide. CD24 mediates gastric carcinogenesis and promotes GC progression. Elucidation of the association between CD24 genetic variants and risk and prognosis of GC may provide novel biomarkers to discriminate individuals with higher risk of GC or to predict prognosis for GC cases.

**Innovations and breakthroughs**

This study for the first time evaluates the effect of CD24 genetic variations in GC carcinogenesis and prognosis. Its results indicate that CD24 variants may serve as a marker for GC prognosis, but not for carcinogenesis.

**Applications**

P-534 site of CD24 might be used as a prognostic predictive marker for GC.

**Terminology**

CD24 is a glycosylphosphatidylinositol-anchored cell-surface glycoprotein with functions in signal transduction and cell adhesion. CD24 over-expression is associated with a more aggressive malignant phenotype of greater proliferative and migration capability; down-expression shows a less malignant phenotype.

**Peer-review**

This study investigated the role of CD24 genetic variants in susceptibility and overall survival of GC and shows that P-534 of CD24 may serve as an independent prognostic marker for GC.

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