Association of *MUC16* Mutation With Tumor Mutation Load and Outcomes in Patients With Gastric Cancer

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IMPORTANCE *MUC16*, which encodes cancer antigen 125 (CA-125), is frequently mutated in gastric cancer (GC); however, its association with tumor mutation load (TML) and outcome in patients with GC has not been established, to date.

OBJECTIVE To investigate whether *MUC16* mutations are associated with TML and prognosis in patients with GC.

DESIGN, SETTING, AND PARTICIPANTS Statistical analysis of genomic data from 437 GC samples obtained from The Cancer Genome Atlas (TCGA) and 256 samples from an Asian cohort. Both cohorts contained data of patients with GC involved in previous genomic studies. Data were obtained from TCGA on September 3, 2017, and from the Asian cohort on March 5, 2013, and analyzed from September 3 to December 1, 2017. The TCGA cohort was used as a discovery set and the Asian cohort as a validation set. Kaplan-Meier survival analysis and multivariate Cox and logistic regression models were applied. Regression models addressed confounding factors; Bayesian variant nonnegative matrix factorization was used to extract mutational signatures. The MutSigCV algorithm was used to identify significantly mutated genes.

MAIN OUTCOMES AND MEASURES Primary outcomes were mutation frequency, overall

Gastric adenocarcinoma (herein referred to as gastric cancer [GC]) is the leading cause of cancer-related death worldwide. Despite progress in *Helicobacter pylori* eradication and early cancer screening, the 5-year survival rate for GC remains 29.6% worldwide.[1]

Gastric cancer is genomically heterogeneous, with varying tumor mutation loads (TMLs). Recent studies have shown that GC samples with microsatellite instability-high (MSI-H) or *POLE* (OMIM 174762) mutations had DNA mismatch repair (MMR) signatures and higher TMLs.[2,3] Tumor mutation load is an important determinant in molecular subtyping of GC in The Cancer Genome Atlas (TCGA). With the use of GC samples in TCGA, 4 molecular subtypes have been identified, each defined by distinct genomic characteristics.[3] Previous studies of GC showed that clonal complexity and driver mutation patterns were associated with survival.[2,4] Recent advances in immunotherapy show that MMR-deficient tumors are more sensitive to immune checkpoint blockade, irrespective of tissue of origin.[5]

MUC16 is a type I transmembrane mucin protein with 3 components: a C-terminal domain, a tandem repeat region, and an extracellular N-terminal section.[6,7] Cancer antigen 125 (CA-125), used to monitor disease progression in ovarian cancer, is part of the tandem repeat domain.[7]

MUC16 (OMIM 606154) is one of the most frequently mutated genes in GC; however, its associations with TML and prognosis remain unclear. In this study, we investigated whether *MUC16* mutations are associated with TML and prognosis in patients with GC.

Key Points

Question Are *MUC16* mutations associated with tumor mutation load and prognosis in gastric cancer?

Findings In this analysis of 437 samples from The Cancer Genome Atlas and 256 samples from an Asian cohort of patients with gastric cancer, *MUC16* mutations were significantly associated with greater tumor mutation load and better outcomes among gastric cancer samples in The Cancer Genome Atlas cohort. These findings were independently validated in the Asian cohort.

Meaning *MUC16* mutations appear to be associated with tumor mutation load and can be used to stratify patients with gastric cancer into prognostically distinct groups.

Methods

Genomic Data of GC

Somatic mutation and gene expression data for 437 GC samples in the TCGA were downloaded from Genome Data Commons (https://portal.gdc.cancer.gov). For the Asian cohort, clinical and somatic mutation data were obtained from a previous study.[2] The Asian cohort contained data from 256 patients with GC, comprising 78 patients from northern China (1 sample from this group had no mutation in its exomic region and was excluded),[4] 100 from Hong Kong,[8] 49 from South Korea,[9] and 30 from Japan.[10] Gene expression data for the Asian cohort are not available, and survival data were only available for the 78 patients from northern China. We did not include esophageal adenocarcinoma in our study because it differs substantially from GC with respect to mutational signatures, driver mutations (eg, *TP53* mutation was present in 140 of 171 esophageal adenocarcinoma samples [81.9%] vs 165 of 347 GC samples [47.6%]; χ^2 test, $P < .001$), and genomic ploidy (genomic doubling event was present in 153 of 365 GC samples [41.9%] vs 97 of 163 esophageal adenocarcinoma samples [59.5%]; χ^2 test, $P < .001$). This study was approved by the Tianjin Medical University Cancer Institute and Hospital Institutional Review Board, which waived additional informed consent because all data used in this study were obtained from public databases. Participants in the original genomic studies provided informed consent.

Mutational Signature Extraction

We used SignatureAnalyzer[11] (https://software.broadinstitute.org/cancer/cga/Home) to extract mutational signatures by combining somatic mutation data from the TCGA and Asian cohorts rather than by extracting signatures in each cohort separately. SignatureAnalyzer uses Bayesian-based nonnegative matrix factorization that automatically determines the optimal number of mutational signatures. The Bayesian nonnegative matrix factorization method exploits a shrinkage or automatic relevance determination technique by iteratively pruning components that do not contribute to explanation of final mutation portraits. SignatureAnalyzer factorized the mutational portrait matrix A into 2 nonnegative matrices, W and H (ie, A equals approximately $W \times H$), with W representing mutational signatures and H representing mutational activities. The number of columns of matrix W is the number of mutational signatures. The rows of matrix A are the 96 mutational contexts, and its columns are the 693 GC samples of both cohorts. The 96 mutational contexts are derived from combinations of 6 mutational types (ie, C > A, C > G, C > T, T > A, T > C, and T > G) and their 5′ and 3′ adjacent bases. The pruning process is performed by introducing weight parameter λ_k, which is associated with the kth column of W and the kth row of H. During inference, the columns and rows of irrelevant components rapidly shrink to zero as λ_k approaches the optimal number of signatures, which is the number of nonzero columns of matrix W.[12] Mutational signatures were annotated by calculating cosine similarity against 21 independently validated mutational signatures in the Catalogue of Somatic Mutations in Cancer[13] and by manual review.

MUC16 Mutations vs TML

Because mutations in *BRCA1/2* (OMIM 113705 and OMIM 600185, respectively) and *POLE* and MMR deficiency increase mutation rates in the cancer genome,[3] we used a multivariate regression model to analyze associations between *MUC16* mutation and TML by including them as confounding factors. *Tumor mutation load* is defined as $\log_2$ transformation of mutation rate per megabase. The extracted MMR mutational signatures were treated as binary variables (ie, 0 and 1) in the multivariate model according to the principle used in a previous study: a signature was considered significant if it contributed to more than 100 substitutions or more than 25%

of total mutations.[13] We used stan_lm from the R package rstanarm, version 2.13.1 (https://cran.r-project.org/web/packages/rstanarm/index.html) to perform multivariate regression analyses.

Significantly Mutated Genes

We used the MutSigCV algorithm[14] to define significantly mutated genes (SMGs) in GC samples with and without *MUC16* mutations. Before performing MutSigCV analysis, we removed GC samples with substantial MMR signatures (>100 substitutions or >25% of total mutations) to avoid skewing the results. An additional procedure was performed to identify expressed SMGs in TCGA data[15] and an encyclopedia of cell lines[16]; a gene was considered to be expressed if it had 3 or more reads in 75% or more of the samples, as described in a 2013 study by Kandoth et al.[15]

Gene Set Enrichment Analysis

As in the analysis of SMGs, we first removed samples with significant MMR signatures and mutations in *BRCA1/2* and *POLE*. The R packages limma[17] and edgeR[18] were used to evaluate differential expression of each gene in GC samples with and without *MUC16* mutations. Specifically, read counts of gene expression data were downloaded from Genomic Data Commons (https://gdc.cancer.gov) and normalized by calcNormFactors in R package edgeR, and then fed to lmFit and eBayes functions in the R limma package. The differential expression statistics obtained from the eBayes function were used as input to perform gene set enrichment analysis for a list of cell-signaling pathways downloaded from MSigDB.[19] The fast gene set enrichment analysis algorithm[20] implemented in the Bioconductor R package fgsea was used. The *P* value was calculated based on 1 million permutations.

Prognosis

Kaplan-Meier survival and multivariate Cox regression analyses implemented in the R package survival were used to analyze associations between *MUC16* mutations and survival. The log-rank test was used to determine significant differences of survival curves stratified by *MUC16* mutations. A 2-sided $P < .05$ was considered statistically significant. Median overall survival time and 95% CIs are reported where relevant.

Results

TCGA Cohort

Of the 437 patients in the TCGA cohort, 280 (64.1%) were male, and the median (IQR) age was 67.6 (15.3) years. *MUC16* was one of the most frequently mutated genes in the TCGA cohort, accounting for 168 of 437 patients (38.4%). Gastric cancer samples with *MUC16* mutations had higher TMLs than samples without *MUC16* mutation (**Figure 1**A). Of the GC samples with *MUC16* mutations, 73 of 165 (44.2%) also harbored mutations in genes related to maintenance of genomic integrity, DNA replication proofreading, and MMR, such as *BRCA1/2*, *POLE*, and *MLH3* (Figure 1B). The mutational associations between *MUC16* and its family members are shown in Figure 1B.

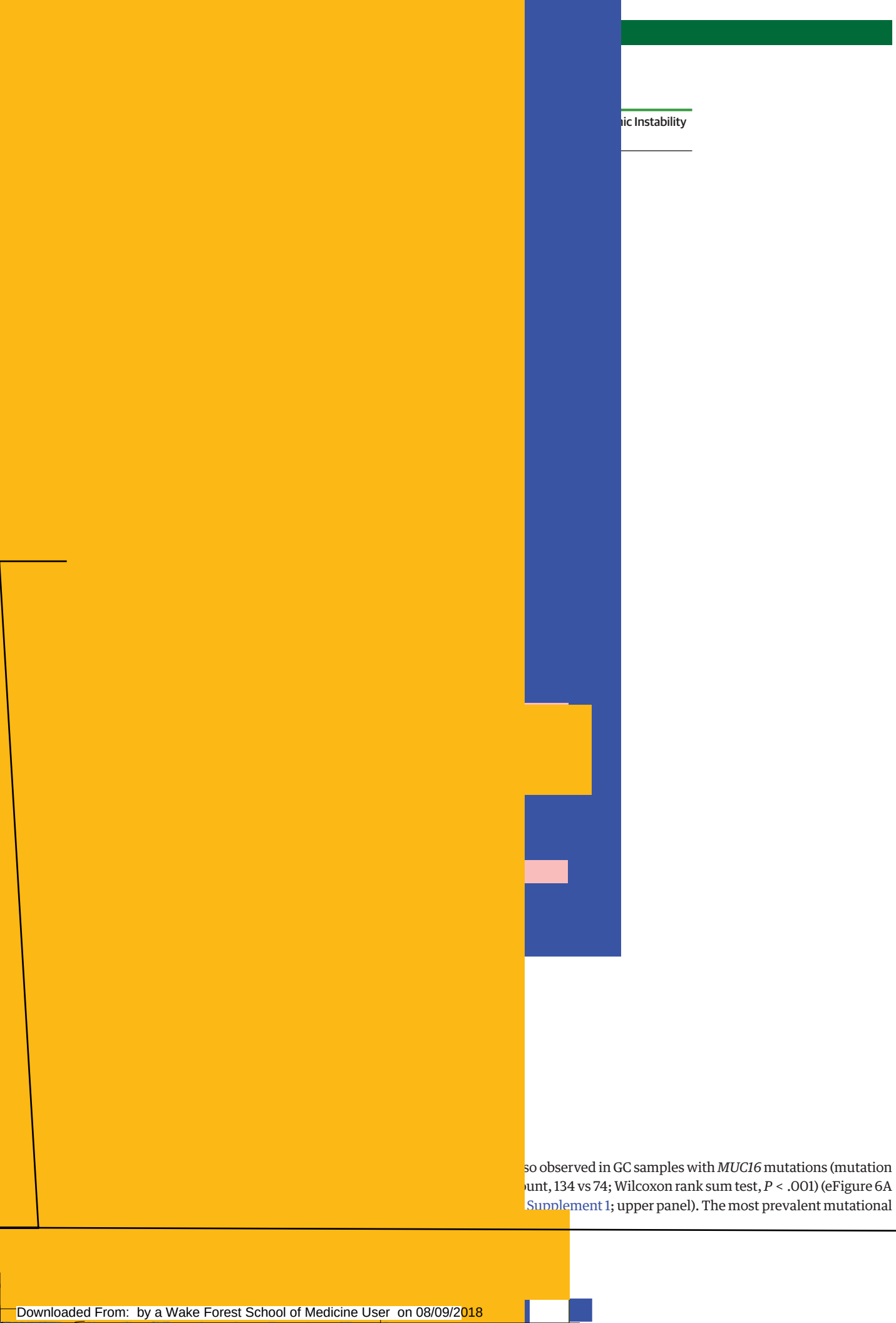
MUC16 Mutation Association With TML

Gastric cancer samples with *MUC16* mutations had a significantly higher mutation rate (**Figure 2**A; Wilcoxon rank sum test, $P < .001$). Tumor mutation load is largely attributed to genomic instability, which is prevalent in GC. In these samples, we found 6 mutational signatures (eFigure 1 in Supplement 1), including those related to genomic instability. The numbers of somatic mutations attributed to each mutational signature varied considerably in each sample. Underlying associations with these 6 mutational signatures included defects in DNA proofreading owing to recurrent somatic mutations in *POLE*[13] (signature 10, 8256 of 171 732 [4.8%]), overactivity of mRNA-editing enzyme APOBEC (signature 2, 18 669 of 171 732 [10.9%]), reflux of gastric acid (signature 17, 11 267 of 171 732 [6.6%]),[21] age-related accumulation of C>T at cytosine-phosphate-guanine dinucleotide (signature 1, 71 816 of 171 732 [41.8%]) and defective MMR (signature 15, 41 769 of 171 732 [24.3%] and signature 21, 19 954 of 171 732 [11.6%]). Signature 21 significantly co-occurred with signature 15 (Fisher exact test, odds ratio [OR], 186; 95% CI, 45.8-1596.3; $P < .001$). Tumors with MSI-H and a substantial presence of signatures 15 or 21 had greater TML compared with tumors without these features, whereas for TML of tumors with MSI-H, the presence of signatures 15 and 21 was comparable (eFigure 2 in Supplement 1; median TML, 5.51 [95% CI, 2.54 to 7.78] vs 5.74 [95% CI, −0.22 to 7.23]; Kruskal-Wallis rank sum test, $P = .18$). Mutational activities of signatures 15 and 21 were significantly higher in MSI-H tumors than either MSI-low or MS-stable tumors (eFigure 3 in Supplement 1; MSI-H: 344.3 vs 7.8, MS-stable: 108.4 vs 2.7; Wilcoxon rank sum test, both $P < .001$). The mutational activity attributable to each mutational signature in each GC sample and variation of these mutational activities is shown in eFigure 4 in Supplement 1. A heat map depicting these 6 mutational signatures and Catalogue of Somatic Mutations in Cancer signatures is shown in eFigure 5 in Supplement 1.

To rule out the possibility that associations between *MUC16* mutations and TML were affected by these confounding factors, we included all mutational signatures (except signature 10) and mutations in *BRCA1/2* and *POLE* in the multivariate model. Four GC samples showed a significant presence of signature 10 and 2 samples harbored somatic mutations in *POLE*. Associations between *MUC16* mutations and TML remained statistically significant (OR, 1.87; 95% CI, 1.49-2.36; Wilcoxon rank sum test, $P < .001$) (**Figure 3**A).

Survival in TCGA Cohort

In Kaplan-Meier survival analysis, the *MUC16* mutation was significantly associated with a better survival outcome in the TCGA cohort (Figure 2B; medl(med)B;allsurviv,lli5-
a



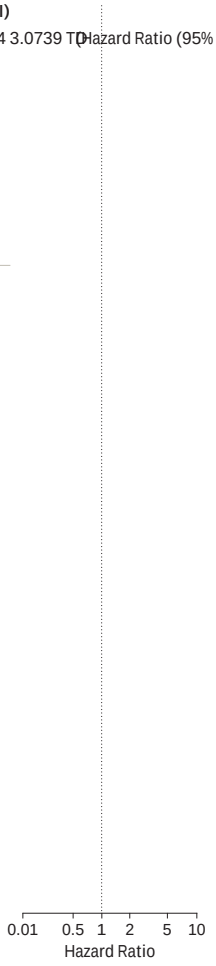
so observed in GC samples with *MUC16* mutations (mutation
unt, 134 vs 74; Wilcoxon rank sum test, $P < .001$) (eFigure 6A
Supplement 1; upper panel). The most prevalent mutational

Figure 3. Association of *MUC16* Mutation With Higher Tumor Mutation Load and Survival Outcome in The Cancer Genome Atlas Cohort of Gastric Cancer Samples

A *MUC16* mutation vs tumor mutation load

Variable	No.	Odds Ratio (95% CI)	*P* Value
Age	402	1.03 (1.02-1.04)	<.001
Sex			
F	145	1 [Reference]	
M	257	1.06 (0.85-1.31)	.62
Stage			
I	54	1 [Reference]	
II	128	1.15 (0.82-1.60)	.42
III	178	1.04 (0.76-1.43)	.80
IV	42	1.05 (0.69-1.62)	.81
POLE			
Wild-type	372	1 [Reference]	
Mutated	30	1.92 (1.22-3.03)	.005
BRCA 1/2			
Wild-type	353	1 [Reference]	
Mutated	49	1.79 (1.26-2.56)	.001
Signature 1			
0	59	1 [Reference]	
1	343	0.97 (0.70-1.33)	.85
Signature 17			
0	348	1 [Reference]	
1	54	2.51 (1.77-3.54)	<.001
Signature 2			
0	148	1 [Reference]	
1	254	1.21 (0.91-1.60)	.17
Signature 15			
0	323	1 [Reference]	
1	79	15.30 (9.85-23.60)	<.001
Signature 21			
0	358	1 [Reference]	
1	44	1.23 (0.79-1.91)	.36
MUC16			
Wild-type	250	1 [Reference]	
Mutated	152	1.87 (1.49-2.36)	<.001

Variable	Hazard Ratio (95% CI)
Age	1.03 O202.010.0034 3.0739 TD
Sex	1

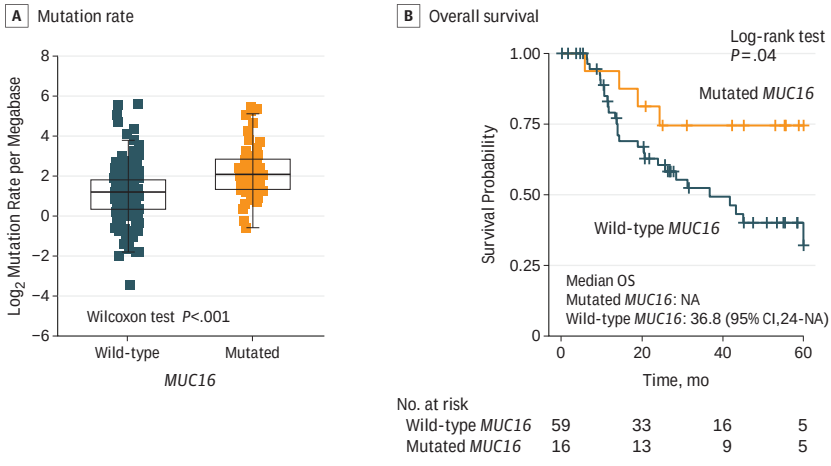


signatures included signature 1, which accounted for 11 401 of 30 115 total mutations (37.9%), and signature 2, which accounted for 7628 of 30 115 (25.3%). Mismatch repair signature 15 contributed to 4363 of 30 115) total mutations (14.5%) and MMR signature 21 contributed to 2158 of 30 115 (7.2%) (eFigure 6B and C in Supplement 1). Associations of mutations among the mucin gene family and *BRCA1/2*, *POLE*, and *MLH3* are shown in the middle panel of eFigure 6A in Supplement 1. As in TCGA cohort, GC samples with *MUC16* mutations had significantly more mutations than those without *MUC16* mutation (TML, 2.1 vs 1.2 per megabase; $\log_2$ transformation of mutation count per megabase; Wilcoxon rank sum test, $P < .001$) (**Figure** A). The association of *MUC16* mutations with higher TML remained statistically significant after controlling for age, sex, TNM stage, mutational signatures, and mutations in *BRCA1/2* and *POLE* in the multivariate model (OR, 1.69; 95% CI, 1.25-2.29; $P < .001$) (**Figure** A). In Kaplan-Meier survival analyses, *MUC16* mutations were significantly associated with better survival outcomes (Figure 4B; median overall survival, not calculable [the median overall survival of patients with GC and *MUC16* mutations could not be calculated because more than half the patients in the group were alive] vs 36.8 months; log-rank test, $P = .04$). This association remained statistically significant after controlling for confounding factors such as age, sex, TNM stage, and mutational signatures (hazard ratio, 0.26 [95% CI, 0.07-1.02]; $P = .05$) (Figure 5B).

Significantly Mutated Genes and Pathways Associated With *MUC16* Mutations

In this analysis, we excluded GC samples with significant MMR signatures and mutations in *BRCA1/2* and *POLE* (see Methods). We performed SMG and gene set enrichment analyses for GC samples with and without *MUC16* mutations, respectively. The SMG mutational landscapes of these 2 groups (eFigure 7 in Supplement 1) exhibited differential mutations in *RPL22* (8 of 165 [4.8%] vs 4 of 428 [0.9%]; 2-sided $P = .005$),

Figure 4. Association of *MUC16* Mutation With Tumor Mutation Load and Prognosis in the Asian Cohort



A, Mutation rates of gastric cancer stratified by *MUC16* mutation status. B, Kaplan-Meier survival analysis stratified by *MUC16* mutation status. NA indicates not available.

Figure 5. Association of *MUC16* Mutation With Tumor Mutation Load and Survival Outcome in the Asian Cohort

A *MUC16* mutation vs tumor mutation load

Variable	No.	Odds Ratio (95% CI)	*P* Value
Age	226	1.02 (1.01-1.03)	<.001
Sex			
F	85	1 [Reference]	
M	141	1.36 (1.05-1.77)	.02
Stage			
Early	43	1 [Reference]	
Late	183	0.69 (0.50-0.94)	.02
POLE			
Wild-type	216	1 [Reference]	
Mutated	10	2.87 (1.59-5.21)	<.001
BRCA 1/2			
Wild-type	212	1 [Reference]	
Mutated	14	3.29 (1.90-5.69)	<.001
Signature 1			
0	42	1 [Reference]	
1	184	1.15 (0.83-1.59)	.40
Signature 17			
0	208	1 [Reference]	
1	18	2.30 (1.37-3.83)	.002
Signature 2			
0	63	1 [Reference]	
1	163	1.10 (0.79-1.53)	.56
Signature 15			
0	208	1 [Reference]	
1	18	5.20 (2.90-9.27)	<.001
Signature 21			
0	219	1 [Reference]	
1	7	1.35 (0.62-2.93)	.44
MUC16			
Wild-type	174	1 [Reference]	
Mutated	52	1.69 (1.25-2.29)	<.001

0.05 0.2 1 2 5 20 50
Odds Ratio

B *MUC16* mutation vs survival

Variable	No.	Hazard Ratio (95% CI)	*P* Value
Age	75	1.00 (0.96-1.04)	.93
Sex			
F	33	1 [Reference]	
M	42	2.37 (0.98-5.72)	.06
Stage			
Early	26	1 [Reference]	
Late	49	3.32 (1.20-9.12)	.02
POLE			
Wild-type	70	1 [Reference]	
Mutated	5	2.96 (0.78-11.26)	.11
BRCA 1/2			
Wild-type	69	1 [Reference]	
Mutated	6	0.57 (0.06-5.66)	.63
Signature 1			
0	10	1 [Reference]	
1	65	1.20 (0.35-4.16)	.77
Signature 17			
0	70	1 [Reference]	
1	5	0.40 (0.04-3.80)	.43
Signature 2			
0	13	1 [Reference]	
1	62	0.59 (0.16-2.19)	.43
Signature 15			
0	69	1 [Reference]	
1	6	2.82 (0.16-50.83)	.48
Signature 21			
0	73	1 [Reference]	
1	2	3.47 (0.17-69.08)	.41
MUC16			
Wild-type	59	1 [Reference]	
Mutated	16	0.26 (0.07-1.02)	.05

0.05 0.2 1 2 5 20 50
Hazard Ratio

Data are adjusted for age, sex, TNM stage, mutational signature, and mutations in *BRCA1/2* and *POLE*. Square data markers indicate estimated odds ratios (A) and hazard ratios (B). Error bars represent 95% CIs.

ACVR2A (10 of 165 [6.1%] vs 6 of 428 [1.4%]; *P* = .003), *APC* (21 of 165 [12.7%] vs 30 of 428 [7%]; *P* = .03), *CDH1* (9 of 165 [5.5%] vs 53 of 428 [12.4%]; *P* = .02) and *ELF3* (0 of 165 [0%] vs 12 of 428 [2.8%]; *P* = .02) (eFigure 8 in Supplement 1). Although

mutation frequency for *B2M* was not statistically significant in GC samples with and without *MUC16* mutations (4 of 165 [2.4%] vs 3 of 428 [0.7%]; $P = .10$), it was significant by the MutSigCV algorithm in the *MUC16* mutant group. It was not significant in the *MUC16* wild-type group (eFigure 7 in Supplement 1). *B2M* was associated with antigen presentation and cytolytic activity, and previously its mutation was associated with resistance to immune checkpoint blockade in melanoma.[22] Signaling pathways involved in the immune system, cell cycle checkpoints, antigen processing, and DNA replication and repair were significantly altered in GC samples with *MUC16* mutations compared with those without *MUC16* mutations (normalized enrichment score, 1.70 [95% CI, 1.57-1.79] and 2.04 [95% CI, 1.90-2.18]; adjusted $P < .001$) (eFigure 9 in Supplement 1). Results of differential gene expression analysis are shown in the eTable in Supplement 2.

Discussion

We analyzed 437 GC samples from the TCGA cohort and 256 GC samples from an Asian cohort for validation. *MUC16* was frequently mutated in GC, and its mutation was associated with higher TML and better survival outcome. The association of *MUC16* mutation with TML was independent of a significant presence of mutational signatures and of mutations in *BRCA1/2* and *POLE*. Gastric cancer samples with *MUC16* mutations were characterized by upregulation of signaling pathways involved in immune response, antigen processing, cell cycle checkpoints, and DNA replication and repair.

MUC16 is frequently mutated in multiple types of human cancer. Owing to its large size, it was often excluded from lists of significantly mutated genes.[14] Nonetheless, *MUC16* is known to modulate immune response to cancer.[6] Our gene set enrichment analyses also indicated that immune response, cell cycle checkpoints, and DNA replication and repair were significantly altered in GC samples with *MUC16* mutations. Therefore, therapeutic regimens to abrogate immune inhibition, such as immune checkpoint blockade, may be beneficial for patients with GC who have *MUC16* mutations. Gastric cancer may develop other strategies to survive host immune attack, such as loss of antigen presentation via *B2M* mutation (eFigure 7 in Supplement 1), which has been associated with acquired resistance to anti–programmed death 1 immunotherapy in patients with melanoma.[22]

Limitations

Our study has several limitations. First, somatic mutation data of the Asian cohort were aggregated from 4 previous studies,[4,8-10] and the tools used in analyzing sequencing data may have been different between these studies. This difference in sequencing could introduce bias in the final mutation list. Second, the number of samples with follow-up data in the Asian cohort was limited, which limits the ability to adjust for confounding factors. In the Asian cohort, TML was significantly lower in the TCGA cohort (1.4 vs 2.2 $\log_2$ transformation of mutation count per megabase; Wilcoxon rank sum test, $P < .001$). The proportion of GC samples with significant presence of signatures 15 and 21 (associated with MMR) is significantly lower than the TCGA cohort (signature 15: 7.8% vs 20.1%; signatures 21: 3.1% vs 11%; χ^2 test, both $P < .001$). This is probably because there was a higher proportion of MSI-H samples in the TCGA cohort than in the Asian cohort (22% vs 10%; χ^2 test, $P < .001$).

MUC16 is frequently mutated in many other human cancer types (the eTable in Supplement 1). MUC16 or CA-125 has been implicated in pancreatic, breast, lung, and bladder cancers. For instance, MUC16 is involved in inhibiting anticancer immune responses by binding to natural killer cells and acting as a barrier between natural killer cells and targeted cancer cells, thus preventing direct interaction between the natural killer cells and their targets.[7] However, the mechanisms underlying the association between *MUC16* mutations with better prognosis and higher TML are still unclear. The full implication of MUC16 or CA-125 in GC diagnosis and monitoring remains elusive and requires in-depth studies.

Conclusions

In 2 independent genomic data sets from TCGA and Asian cohorts, *MUC16* mutations were associated with higher TML and improved outcome in patients with GC. This finding may have implications for prognostic prediction and therapeutic guidance for GC.

ARTICLE INFORMATION

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Author Contributions: Drs Li and Chen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Li, Zhang, Chen.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Li, Zhang, Chen.
Critical revision of the manuscript for important intellectual content: Li, Pasche, Zhang.
Statistical analysis: Li.
Obtained funding: Chen.
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Supervision: Zhang, Chen.

approval of the manuscript; and decision to submit the manuscript for publication.

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