

Recurrent amplification of *MYC* and *TNFRSF11B* in 8q24 is associated with poor survival in patients with gastric cancer

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Abstract

Background Gastric cancer (GC) is an aggressive malignancy whose mechanisms of development and progression are poorly understood. The identification of prognosis-related genomic loci and genes may suffer from the relatively small case numbers and a lack of systematic validation in previous studies.

Methods Array-based comparative genomic hybridization (aCGH) coupled with patient clinical information was applied to identify prognosis-related loci and genes with high-frequency recurrent gains in 129 GC patients. The candidate loci and genes were then validated using an independent cohort of 384 patients through branched DNA signal amplification analysis (QuantiGene assays).

Results In the 129 patients, a copy number gain of three chromosome regions—namely, 8q22 (including *ESRP1* and *CCNE2*), 8q24 (including *MYC* and *TNFRSF11B*), and 20q11-q13 (including *SRC*, *MMP9*, and *CSE1L*)—conferred poor survival for patients. In addition, the correlation between the branched DNA signal amplification analysis results and the aCGH results was analyzed in 73 of these 129 patients, and *MYC*, *TNFRSF11B*, *ESRP1*, *CSE1L*, and *MMP9* were found to be well correlated. Further validation using an independent cohort ($n = 384$) verified that only *MYC* and *TNFRSF11B* within 8q24 are related to survival. Patients with gains in both *MYC* and *TNFRSF11B* had poorer survival than those with no gains, particularly those with noncardia GC. Gains in both of these genes were also a significant independent prognostic indicator.

Conclusions Our results revealed that copy number gains in *MYC* and *TNFRSF11B* located at 8q24 are associated with survival in GC, particularly noncardia GC.

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Introduction

Gastric cancer (GC) is one of the commonest causes of cancer-related death worldwide, and develops through a multistage process that involves the accumulation of genetic and epigenetic alterations [1, 2]. Many factors can affect the prognosis of GC patients. DNA copy number amplifications are essential drivers of tumorigenesis and malignant progression of solid tumors, including GC [3–5].

High-throughput, genome-wide profiling of DNA copy number variations (CNVs) has led to the discovery of regions that are recurrently amplified in GC. In addition, these regions house key oncogenes involved in GC progression, including *MYC* in 8q, *SRC* and *MMP9* in 20q, *ERBB2* in 17q, *EGFR* in 7p, *FGFR1* in 8p, and *FGFR2* in 10q [6–11]. The focal amplification of these regions and an increased frequency of their amplification throughout the genome are also associated with invasive clinicopathological characteristics and poor disease prognosis. For example, a gain of 1q32.3 has been significantly correlated with lymph node status [12]. The degree of genetic change at the DNA copy number level was found to be well correlated with the tumor stage in diffuse-type GC [13]. Moreover, some genomic imbalance may also be related to the different locations of GC [9, 12, 14, 15], such as cytogenetic abnormalities of 5p+ and 18q–, which were identified to result in significant differences between esophagogastric junction and distal gastric tumors [16]. However, the detection of the amplification of key regions was found to be inconsistent in a previous study, in which the number of cases was relatively small and no systematic validation was performed [10, 17].

To validate the copy number alterations of multiple genes within these key regions, a high-throughput quantitative method, such as branched DNA signal amplification technology (QuantiGene assay), has been used owing to its specificity and detection sensitivity, which demonstrates excellent correlation with traditional quantitative PCR and was adopted in the FDA-launched MicroArray Quality Control project [18]. This assay can be performed on 96-well plates to quantify DNA targets through the multiplexing of three to 80 DNA targets. Using this technology, researchers have found that the CNV of a specific region associated with pancreatic cancer risk is likely due to an effect on the long-term regulation of *CDKN2B* [19]. Moreover, the QuantiGene assay may be more significant in cancer research owing to its high accuracy and convenience for use with many types of samples, including blood, tissues, and, most importantly, formalin-fixed paraffin-embedded (FFPE) slides, which are easily accessed in a clinical setting [20].

In this study, we used a high-resolution array-based comparative genomic hybridization (aCGH) coupled with

patient clinical information to identify prognosis-related chromosome loci and genes that may functionally contribute to tumor progression in a training cohort of 129 GC patients. Then, using an independent validation cohort of 384 patients, the relationship between the copy number gains of selected cancer-related genes within candidate genomic loci detected by the QuantiGene assay from FFPE samples and patient survival was further verified.

Materials and methods

Patients and tissue specimens

All of the patients with GC included in this study received their diagnosis and were surgically treated at Peking University Cancer Hospital between 1999 and 2007 and were followed up to January 2013. The training cohort comprised 129 patients, including 94 males and 35 females (mean age 60 years, range 34–81 years). A total of 129 frozen cancer tissues and 22 matched noncancerous mucosa samples were subjected to aCGH. The validation cohort comprised 384 patients not included in the training cohort, including 264 males and 120 females (mean age 58.4 years, range 22–81 years). This investigation was performed after approval by the Ethics Committee of Peking University. General informed consent was obtained from each patient.

After gastrectomy, resected specimens were processed routinely for macroscopic pathological assessment, and tissues were sampled and snap-frozen in liquid nitrogen. Fresh human tissues were stored at -80°C and fixed with 10 % formalin in phosphate-buffered saline. To ensure the quality of the tissues, routine histological evaluation was performed for each sample. In brief, the deep-frozen GC tissues and noncancerous mucosa tissue samples were cryosectioned and stained with hematoxylin–eosin, and the tumor tissues containing more than 80 % tumor cells qualified for aCGH. To prepare the FFPE slides, the adjacent noncancerous mucosa tissues were removed, and only tumor tissues were used for the QuantiGene assay.

The depth of tumor invasion, histological grade, lymph node metastasis, liver metastasis, and vascular invasion were obtained from the histopathology reports. The GC stage was classified according to the tumor–node–metastasis (TNM) classification (seventh edition) recommended by the American Joint Committee on Cancer [21].

DNA-based microarray-CGH and data analysis

The DNA from the frozen tissues was isolated according to the standard phenol/chloroform protocol. The extracted DNA was assessed for genomic DNA concentration and

purity using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Agarose gel electrophoresis was used to assess the integrity of the genomic DNA, and aCGH was performed using the human genome comparative genomic hybridization microarray kit 244A (Agilent Technologies, Santa Clara, CA, USA). The experiments were performed as recommended by the manufacturer with some modifications [22]. Briefly, DNA (1.5 µg) samples of the cells and control (a pool of normal human blood DNA samples) were differentially labeled with Cy5 and Cy3-dUTP, respectively, purified, and then hybridized to an Agilent human genome comparative genomic hybridization 244K microarray. The gene copy number alterations were measured through log ratios using the BioDiscovery Nexus Copy Number software application (<http://www.biodiscovery.com/software/nexus-copy-number/>) and a program written in R (<http://www.r-project.org/>), which detected the same chromosomal aberrations. The gain and amplified segments were analyzed as follows: a segment presenting a recurrent gain was defined as a continuous genome region that satisfied the criteria log ratio of 0.2 or greater and frequency of 30 % or greater; a log ratio greater than 0.5 was regarded to define amplification. The segments that exhibited loss and deletion were analyzed as follows: a segment that presented recurrent loss was defined by a continuous genome region that satisfied the criteria log ratio of -0.2 or lower and frequency of 30 % or greater; a log ratio less than -0.7 was regarded to define deletion.

QuantiGene assay for quantification of DNA in FFPE tissues

Branched DNA signal amplification technology (QuantiGene assay) is a sandwich nucleic acid hybridization assay that provides a unique approach for DNA detection and quantification by amplifying the reporter signal rather than the target sequence. First, tissue homogenates from FFPE slides were prepared according to the procedure described by the manufacturer of the QuantiGene sample processing kit for FFPE tissues (Panomics, Fremont, CA, USA). Briefly, deparaffinized 5-µm-thick sections were incubated with 200 µl of homogenizing solution supplemented with 2 µl of proteinase K (50 µg/µl) overnight at 65 °C. The tissue homogenate was then separated from the debris by brief centrifugation and transferred to a new tube. The target gene DNA in the tissue homogenates was hybridized to the probe set (Table S1). Standard probe design software was used to design specific oligonucleotide probe sets for the target genes in the QuantiGene Plex 2.0 reagent system (Panomics), which provides a 400-fold signal amplification. The QuantiGene Plex 2.0 reagent system was used according to the manufacturer's recommended protocols

(Panomics). Briefly, the oligonucleotide probe set was mixed with the sample, and the mixture was added to an assay well in a 96-well plate. The target and control DNA (pool of normal human blood DNA samples) was captured during an overnight incubation at 54 °C (QuantiGene Plex 2.0). Any unbound material was removed by three washes with 200 µl of wash buffer. This step was followed by the sequential hybridization of DNA amplifier molecules, and preamplifier hybridization, amplifier hybridization, and label probe hybridization were then performed. Finally, streptavidin–phycoerythrin working reagent was added to the wells, and the plate was prepared for analysis. Chr12_p, Chr13_q, Chr16_q, SULT1A1, and RPLP0 were chosen as internal controls for the QuantiGene assay. The gene copy numbers for the cancer samples were calculated by dividing the sample values by the control values: no gain was regarded as a copy number of 2 or less, gain was regarded as a copy number greater than 2 but not greater than 4, and high copy number gain was regarded as a copy number greater than 4.

Statistical analysis

All of the analyses were performed using the program SPSS (version 16.0; SPSS, Chicago, IL, USA). Overall survival was plotted and calculated using the Kaplan–Meier method, and differences between groups were compared by the log-rank test. The Cox proportional hazards model (backward, stepwise) was used to estimate the influence of each variable on survival. The chi-squared test was performed to analyze the correlations between gene co-gain and clinicopathological variables. The Pearson test was used to analyze correlations between the aCGH and QuantiGene results. *P* values less than 0.05 (two-sided) were considered statistically significant.

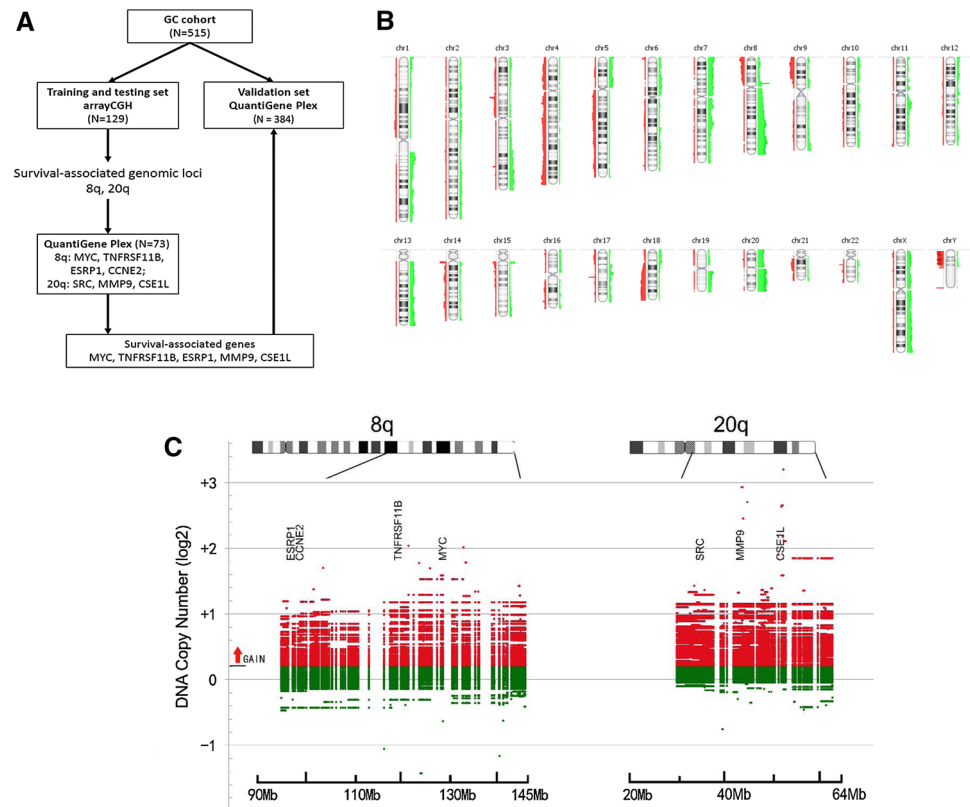
Results

Genomic alterations in GCs detected by aCGH and identification of candidate survival-related loci and genes

We applied a genome-wide search for prognosis-related genomic loci and genes based on the results from two cohorts—namely, a training cohort comprising 129 patients and a validation cohort comprising 384 patients. The overall study design is shown in Fig. 1a.

First, 129 GC and 22 noncancerous gastric mucosa samples were profiled by aCGH. The clinical and pathological characteristics of the patients included in this training cohort are shown in Table 1. Chromosomal CNVs were observed in all 129 cancer patients, and the ideogram of the

Fig. 1 The study design and the results of the copy number variation analysis. **a** Overview of the study design. **b** Ideogram of genomic variations in 129 gastric carcinomas. Summary of chromosomal variations in 129 cases of gastric carcinoma. *Red bars* indicate deletion and *green bars* indicate amplification. **c** Summary of specific regions of 8q and 20q gain in 129 cases. Each dot represents a single gene, and *red dots* represent gene gain (\log_2 ratio greater than 0.2). *CGH* comparative genomic hybridization, *GC* gastric cancer



GC chromosomal abnormalities is shown in Fig. 1b. The regions that presented gains of at least 30 % in all of the cases were mainly localized on 8p, 8q, 20p, 20q, 7q, 13q, 1q, 19q, 3p, and 3q in order of decreasing frequency, whereas the 22 adjacent noncancerous mucosa samples were almost free of genomic alterations. Representative amplified regions and genes are presented in Table S2. Because DNA copy number gain and amplification are essential drivers of tumorigenesis, particularly in epithelial cancers, such as GC, our study mainly focused on the association between gene gain/amplification and survival. Representative deleted regions and genes are presented in Table S3.

The analysis of the regions that presented gains revealed that the copy number gains of three regions—namely, 8q22, 8q24, and 20q11-q13—were greater than 50 % in all of the cases. Sixty cancer-related genes located at 8q22 and 8q24 and 70 cancer-related genes located at 20q11-q13 were selected for the Kaplan–Meier survival analysis. Our results suggested that almost all of gene gains in 20q11-q13, 8q22, and 8q24 are correlated with high risk in GC patients ($P < 0.05$ for all except two genes, for which $P = 0.06$). Some well-recognized oncogenes, such as *MYC* (chromosome 8, gain of 62 % in all of the cases) and *SRC* (chromosome 20, gain of 55.8 % in all of the cases), which are also well known as amplification targets at chromosomes 8 and 20 (Fig. 1c), were affected by genomic CNVs.

Both *MYC* and *SRC* gains were correlated with poor prognosis in GC patients ($P = 0.012$ and $P = 0.035$, respectively) (Fig. 2a, e). Moreover, the amplification of some other important genes (Table S1), such as *TNFRSF11B*, *ESRP1*, *CCNE2*, *MMP9*, *CSE1L*, *BCL2L1*, *PLAGL2*, *E2F1*, *CD40*, *CEBPB*, *BCAS4*, *BMP7*, *PTK6*, *SAMD10*, *SNORA72*, *STK3*, *COX6C*, *YWHAZ*, *SAMD12*, *MAPK15*, *EPPK1*, and *PARP10*, located at the three regions was also associated with poor survival (the survival plots of the first five genes are shown in Fig. 2).

Analysis of seven candidate genes located at 20q11-q13, 8q22, and 8q24 by the QuantiGene assay

Within 20q11-q13, 8q22, and 8q24, we selected seven candidate genes that are generally related to tumorigenesis, as determined in previous studies, for further testing: *ESRP1* and *CCNE2* at 8q22, *TNFRSF11B* and *MYC* at 8q24, and *SRC*, *MMP9*, and *CSE1L* at 20q11-q13. Branched DNA signal amplification, which is also known as the QuantiGene assay, which exhibits high accuracy and precision for the detection of CNVs in FFPE slides, was applied to analyze these genes in 73 of the 129 GC patients included in the training cohort (the characteristics of this test cohort are shown in Table 1). The correlations between the QuantiGene assay results and the aCGH results were

Table 1 Clinical and pathological characteristics of patients in three cohorts

	Training cohort (aCGH assay, frozen tissues, <i>n</i> = 129)	Test cohort (QGP assay, matched FFPE slides, <i>n</i> = 73)	Validation cohort (QGP assay, independent FFPE slides, <i>n</i> = 384)
Mean age at resection ± SD (years)	60.5 ± 10.6	61.1 ± 11.0	58.4 ± 12.2
Sex			
Male	94 (72.9 %)	55 (75.3 %)	264 (68.8 %)
Female	35 (27.1 %)	18 (24.7 %)	120 (31.3 %)
Median survivor follow-up (months) ^a	50 (37.75–61)	48 (30.5–61)	72.16 (59.3–88.46)
Differentiation			
Well to moderately differentiated	39 (30.2 %)	19 (26.0 %)	99 (25.8 %)
Poorly differentiated	90 (69.8 %)	54 (74.0 %)	273 (71.1 %)
Not recorded ^b	0	0	12 (3.1 %)
Location			
Cardia	39 (30.2 %)	21 (28.8 %)	112 (29.2 %)
Noncardia	90 (69.8 %)	52 (71.2 %)	272 (70.8 %)
TNM stage			
I	4 (3.1 %)	2 (2.7 %)	33 (8.6 %)
II	35 (27.1 %)	17 (23.3 %)	89 (23.2 %)
III	73 (56.6 %)	43 (58.9 %)	262 (68.2 %)
IV	17 (13.2 %)	11 (15.1 %)	0
T stage			
1	1 (0.8 %)	0	20 (5.2 %)
2	16 (12.4 %)	12 (16.4 %)	32 (8.3 %)
3	46 (35.7 %)	22 (30.1 %)	119 (31.0 %)
4	66 (51.2 %)	39 (53.4 %)	213 (55.5 %)
N stage			
0	30 (23.3 %)	18 (24.7 %)	92 (24.0 %)
1	18 (14.0 %)	8 (11.0 %)	51 (13.3 %)
2	30 (23.3 %)	19 (26.0 %)	86 (22.4 %)
3	51 (39.5 %)	28 (38.4 %)	155 (40.4 %)
M stage			
0	112 (86.8 %)	62 (84.9 %)	384 (100 %)
1	17 (13.2 %)	11 (15.1 %)	0
Vascular invasion			
V(–)	50 (38.8 %)	27 (37.0 %)	145 (37.8 %)
V(+)	72 (55.8 %)	42 (57.5 %)	215 (56.0 %)
Not recorded ^b	7 (5.4 %)	4 (5.5 %)	24 (6.3 %)

aCGH array-based comparative genomic hybridization, FFPE formalin-fixed paraffin-embedded, QGP QuantiGene Plex, SD standard deviation

^a The interquartile range is given in parentheses

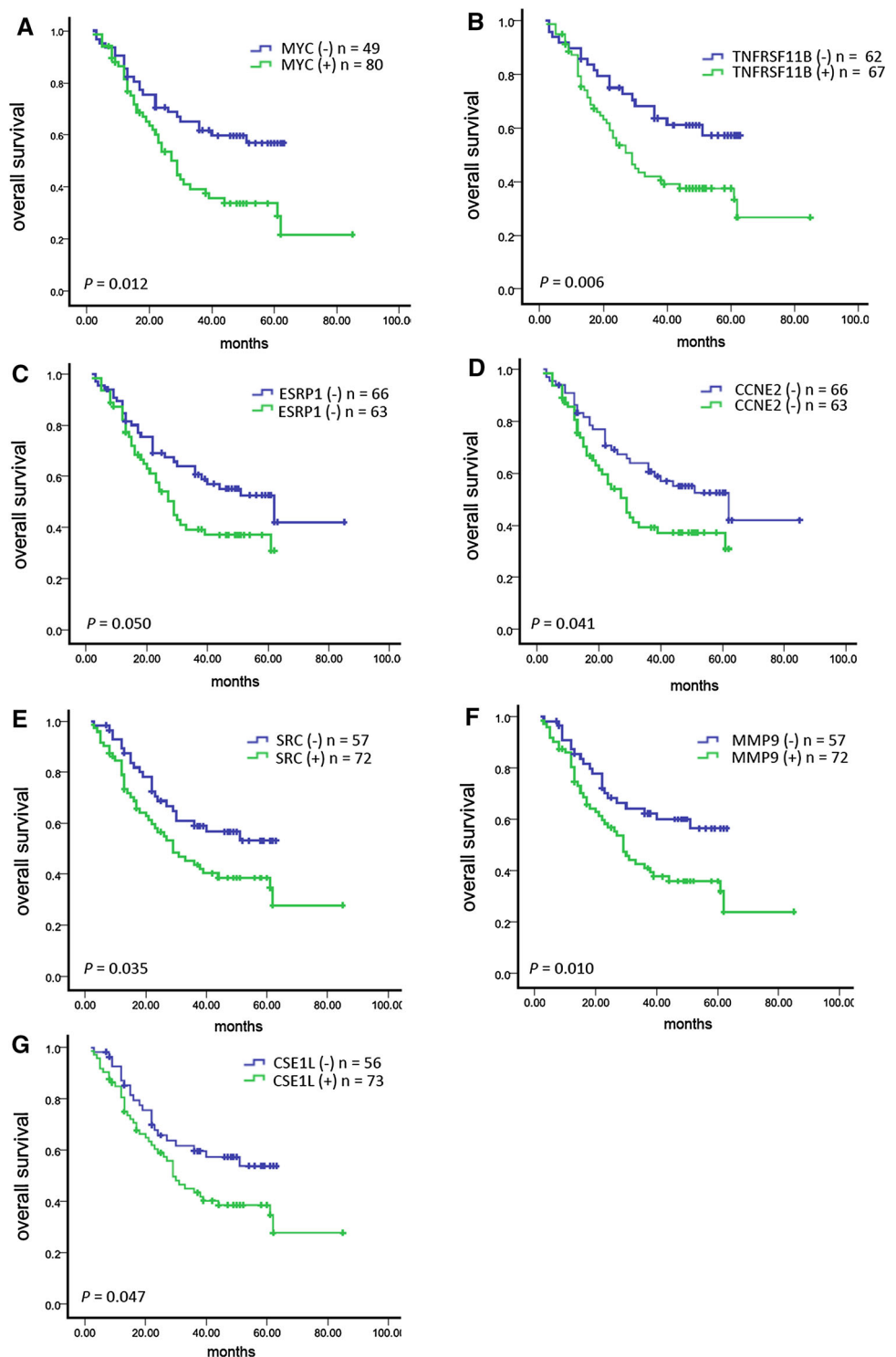
^b Data incomplete

analyzed. Our results suggested that there was a significant correlation between the aCGH results and the QuantiGene assay results for the *MYC*, *CSE1L*, *TNFRSF11B*, *ESRP1*, and *MMP9* genes ($P = 0.012$, $P < 0.001$, $P = 0.001$, $P = 0.004$, and $P = 0.005$, respectively; Table 2). The other two genes—namely, *CCNE2* and *SRC*—showed poor correlations ($P > 0.05$; Table 2) and were removed from further validation.

Independent cohort validation of *MYC* and *TNFRSF11B* at 8q24

An independent validation cohort was used to validate the association between gains of *MYC*, *TNFRSF11B*, *ESRP1*, *MMP9*, and *CSE1L* and patient prognosis. The results including stage IV GC are shown in Fig. S1 and Table S4. To remove any excess statistical confounding factors,

Fig. 2 The survival curves of seven survival-associated genes at 20q and 8q in the training cohort of 129 gastric cancer patients: **a** *MYC*; **b** *TNFRSF11B*; **c** *ESRP1*; **d** *CCNE2*; **e** *SRC*; **f** *MMP9*; **g** *CSE1L*. Plus sign gene gain (\log_2 ratio greater than 0.2), minus sign no gain



patients with TNM stage IV GC were excluded from the validation cohort (the characteristics of this group are shown in Table 1). The general conditions of the patients in the training and validation cohorts are comparable, with the exception of the TNM stage (Table S5). Patients with a gain of either *MYC* or *TNFRSF11B* had a shorter median

overall survival than those without gain (*MYC*, median survival 24.92 months vs 48.78 months, $P = 0.016$; *TNFRSF11B*, median survival 26.66 months vs 45.07 months, $P = 0.030$; Fig. 3a, b), whereas there was no significant difference in survival between the patients with a gain of *MMP9*, *CSE1L*, or *ESRP1* and those without

Table 2 Representative gene correlation analysis of the array-based comparative genomic hybridization (aCGH) and QuantiGene Plex (QGP) assay

Gene symbol	Gene name	<i>P</i>	Correlation coefficient for aCGH vs QGP results
<i>CSE1L</i>	CSE1 chromosome segregation 1-like	<0.001	0.427
<i>TNFRSF11B</i>	Tumor necrosis factor receptor superfamily, member 11b	0.001	0.397
<i>ESRP1</i>	Epithelial splicing regulatory protein 1	0.004	0.339
<i>MMP9</i>	Matrix metalloproteinase 9	0.005	0.322
<i>MYC</i>	v-myc avian myelocytomatosis viral oncogene homolog	0.012	0.306
<i>CCNE2</i>	Cyclin E2	0.129	0.196
<i>SRC</i>	v-src avian sarcoma viral oncogene homolog	0.682	0.052

gain ($P = 0.243$, $P = 0.327$, and $P = 0.668$, respectively; data not shown).

Moreover, the GC patients with a high copy number gain (copy number greater than 4) of *MYC* or *TNFRSF11B* exhibited even poorer overall survival than those with a copy number gain corresponding to a copy number greater than 2 but not greater than 4 and those with no gain (*MYC*, median survival 12.79 months vs 27.02 months vs 48.78 months, $P < 0.001$; *TNFRSF11B*, median survival 17.98 months vs 27.75 months vs 45.07 months, $P = 0.012$; Fig. 3c, d).

Association of 8q24 gain with survival and clinicopathological features

The survival-related genes *MYC* and *TNFRSF11B* are found within a wide range of amplified regions in 8q24, which spans approximately 20 million base pairs. There was a significant correlation between the copy number gains of *MYC* and *TNFRSF11B*, with a correlation coefficient of 0.328 ($P < 0.001$). In addition, 40.36 % of the patients had gains of both *MYC* and *TNFRSF11B*. We found that the patient group with gains of both *MYC* and *TNFRSF11B* was markedly segregated from the nongain group. The co-gain group had a significantly lower 5-year survival rate than the nongain group (median survival 24.92 months vs 51.94 months, $P = 0.008$; Fig. 3e).

The statistical analysis showed that the co-gain of *MYC* and *TNFRSF11B* was strongly associated with the depth of wall invasion, lymph node metastasis, and TNM stage in both the training cohort and the validation cohort

(Table 3). A multivariate Cox proportional hazards model (Table 4) using variables that were found to be associated with survival in our study revealed that a co-gain in *MYC* and *TNFRSF11B* was a significant independent prognostic indicator in both the training cohort and the validation cohort ($P = 0.004$ and $P = 0.032$, respectively).

Association of 8q24 gain with survival in GC at different locations

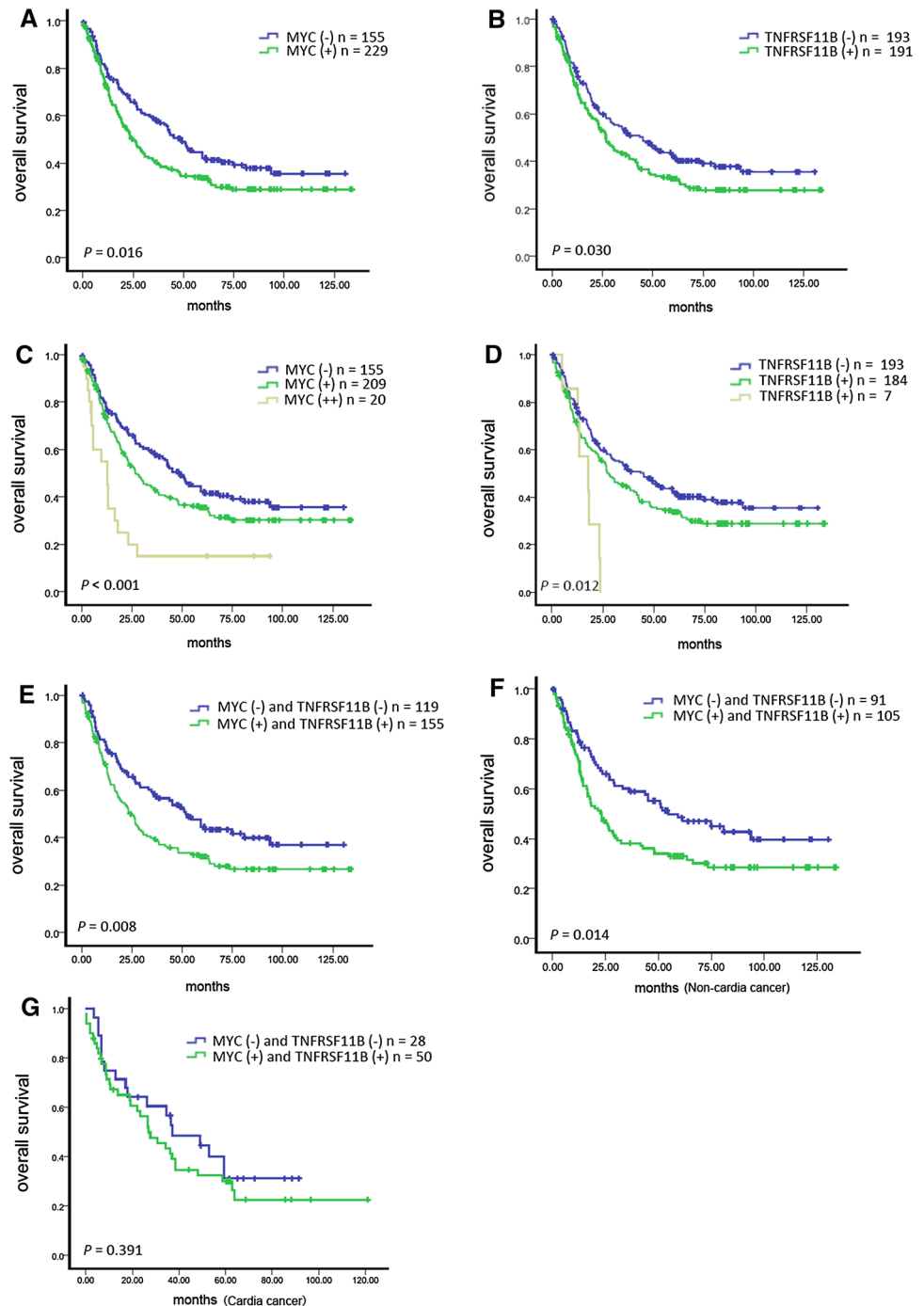
Moreover, the analysis of patients with noncardia GC revealed that the patients with a co-gain of *MYC* and *TNFRSF11B* had a shorter overall survival than those with a nongain gene signature (median survival 23.08 months vs 54.57 months, $P = 0.014$; Fig. 3f), whereas the analysis of the patients with cardia GC revealed that the overall survival did not differ significantly between those patients with a co-gain signature and those with a nongain signature (median survival 27.02 months vs 37.11 months, $P = 0.391$; Fig. 3g).

Discussion

In this study, the genomic CNVs of gastric carcinoma were well characterized by aCGH using a very large training cohort of 129 patients. Our results suggested that the regions that presented gain in GC were mainly focused on chromosome 8 (p11, q11-q24), chromosome 20 (p11-p13, q12-q13.33), chromosome 7 (q22), chromosome 13 (q32.1-q34), chromosome 1 (q21.1-q22), chromosome 19 (q13), and chromosome 3 (p22, q26.31), and particularly in the regions 20q13, 20q11, 8q24, and 20p12. Our results are partially consistent with the results from two previous studies that used aCGH. In those studies, which included 30 patients [10] and 43 patients [17], respectively, the results revealed that the commonest gains observed in GC were in 7q (35 %), 8q (35 %), 7p (28 %), 1q (26 %), 13q (26 %), and 20q (21 %). However, our results are more precise owing to the inclusion of a higher number of patient samples.

Although the association between copy number gain of genomic loci and genes and survival has been widely studied by aCGH in other cancer types, a similar study in GC has not been performed. For example, 8q gains were associated with tumor progression and poor prognosis in prostate cancer [23], [24], and a gain of 8q has been associated with metastases and poor survival in patients with clear cell renal cell carcinoma [25]. Moreover, some genes located in 8p have been associated with progression and patient survival in hepatocellular carcinoma [26] and breast cancer [27]. Our study provides the first investigation of the association of gene gain and survival of GC

Fig. 3 Survival analysis in the validation cohort of 384 gastric cancer patients. Two genes, *MYC* and *TNFRSF11B*, were validated to be associated with survival. **a, b** The association of *MYC* and *TNFRSF11B*, respectively, with survival. **c, d** The association of copy number (CN) variation status (high CN gain, CN greater than 4; gain, CN greater than 2 but not greater than 4; no gain, CN of 2 or less) of *MYC* and *TNFRSF11B*, respectively, with survival. **e** The association of concurrent gain and concurrent no gain of *MYC* and *TNFRSF11B* with survival. **f, g** Survival analysis in the cardia cancer subgroup and noncardia cancer subgroup, respectively, of the validation cohort. *Minus sign* no gain, *one plus sign* gene gain, *two plus signs* high CN gain



patients by aCGH using a larger sample size ($n = 129$). In addition, our aCGH results suggested that 20q11-13, 8q22, and 8q24 confer poor prognosis on GC patients. An association between gains of 8q and poor survival has also been reported in prostate cancer [23, 24], renal cell carcinoma [25], and breast cancer [27].

Furthermore, the correlations between CNVs and prognosis were validated by the QuantiGene assay using a large independent cohort ($n = 384$). Because the treatment of

TNM stage IV GC is mostly palliative and prognosis-related factors are farraginous and confounding owing to a complicated clinical intervention rather than the genetic background, patients with this stage of GC were not included in the validation cohort. With the exception of the TNM stage, the other clinicopathological characteristics of the patients in the training and validation cohorts were comparable, similarly to the designs used in previous studies [28]. Our validation results verified that gains of

Table 3 Association of co-gain of *MYC* and *TNFRSF11B* with clinicopathological parameters in gastric cancer patients

Characteristic	Nongain signature	Co-gain signature	<i>P</i>
Original cohort			
Patients	20	21	
Age at surgery (years)	57.05 ± 9.71	62.43 ± 8.55	0.032
Sex			
Male	12	17	0.232
Female	8	4	
Tumor stage			
I and II	8	1	0.022
III and IV	12	20	
T stage			
1 and 2	8	1	0.022
3 and 4	12	20	
N stage			
Negative	34	27	0.023
Positive	84	129	
Differentiation			
Moderately differentiated	5	4	0.886
Poorly differentiated	15	17	
Vascular invasion			
V(-)	10	7	0.378
V(+)	8	14	
Location			
Cardia	5	7	0.764
Noncardia	15	14	
Validation cohort			
Patients	119	155	
Age at surgery (years)	57.14 ± 12.67	59.35 ± 11.32	0.130
Sex			
Male	80	111	0.434
Female	39	44	
Tumor stage			
I and II	46	37	0.008
III	73	118	
T stage			
1 and 2	24	11	0.001
3 and 4	95	144	
N stage			
Negative	34	27	0.028
Positive	85	128	
Differentiation			
Moderately differentiated	28	33	0.653
Poorly differentiated	87	117	
Vascular invasion			
V(-)	50	51	0.100
V(+)	61	95	
Location			
Cardia	28	50	0.098
Noncardia	91	103	

Table 4 Multivariate analysis of prognostic factors by the Cox proportional hazards model

Variables	Univariate analysis		Multivariate analysis	
	HR ^a	P	HR ^a	P
Training cohort				
Gender	0.7 (0.367–1.337)	0.28		
Age >60 years	1.041 (1.011–1.071)	0.007	1.042 (1.010–1.076)	0.01
<i>MYC</i> and <i>TNFRSF11B</i> , co-gain vs no gain	1.521 (1.137–2.034)	0.005	1.553 (1.150–2.096)	0.004
TNM stage, III and VI vs I and II	3.425 (1.652–7.101)	0.001	3.166 (1.516–6.612)	0.002
Vascular invasion, positive vs negative	2.191 (1.049–4.578)	0.037		
Differentiation, poorly vs well to moderately differentiated	0.946 (0.512–1.748)	0.861		
Location, noncardia vs cardia	0.691 (0.386–1.236)	0.213		
Validation cohort				
Gender	0.950 (0.684–1.320)	0.76		
Age >60 years	1.017 (1.003–1.030)	0.013		
<i>MYC</i> and <i>TNFRSF11B</i> , co-gain vs no gain	1.232 (1.056–1.437)	0.008	1.194 (1.015–1.405)	0.032
TNM stage, III vs I and II	3.617 (2.441–5.359)	<0.001	2.946 (1.921–4.519)	<0.001
Vascular invasion, positive vs negative	1.860 (1.339–2.585)	<0.001	1.373 (0.968–1.948)	0.075
Differentiation, poorly vs well to moderately differentiated	1.659 (1.137–2.421)	0.009		
Location, noncardia vs cardia	0.827 (0.598–1.145)	0.253		

HR hazard ratio

^a The 95 % confidence interval is given in *parentheses*.

TNFRSF11B and *MYC* located at 8q24 were associated with poor survival, whereas gains of *CSE1L*, *MMP9*, and *SRC* located at 20q13 were not. The co-gain of *TNFRSF11B* and *MYC* was strongly associated with TNM stage and was found to be a significant independent prognostic indicator. Among these regions, *MYC*, a well-known oncogene that is altered in some primary tumors [29, 30], was previously reported to be associated with late-onset, intestinal-type, advanced-stage tumor, and the presence of distant metastasis in GC [31, 32]. *TNFRSF11B*, another gene located at 8q24 and that encodes osteoprotegerin (OPG), is a member of the tumor necrosis factor (TNF) receptor superfamily. OPG has been identified to be a prognostic marker in various cancer types, including myeloma, breast cancer, prostate cancer [33–35], and GC [36]. A number of studies have suggested that OPG protects tumor cells from the effects of TNF-related apoptosis-inducing ligand (TRAIL) [37, 38] and may thereby provide a survival advantage to OPG-producing tumor cells and contribute to carcinogenesis and cancer cell survival by driving the expression of OPG through the Wnt/ β -catenin pathway [39].

Furthermore, the analysis of patients with noncardia GC revealed that those patients with a co-gain of *MYC* and *TNFRSF11B* at 8q24 had shorter overall survival than those without these alterations. A previous study also suggested that a genomic imbalance of 5p+ and 18q- may be related to the location of the GC, such as esophagogastric junction and distal gastric tumors [16]. These

abnormalities may distinguish distinct tumor subtypes with identical histological features.

In conclusion, through a global correlation analysis of aCGH data coupled with GC clinicopathological information from a large cohort and further validation using a large independent cohort, our study revealed that copy number gains of *MYC* and *TNFRSF11B* located at 8q24 are associated with survival in GC, particularly noncardia GC.

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