Mutational analysis of driver genes with tumor suppressive and oncogenic roles in gastric cancer

Tianfang Wang¹, Yining Liu² and Min Zhao¹

¹ School of Science and Engineering, Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore DC, Australia
² The School of Public Health, Institute for Chemical Carcinogenesis, Guangzhou Medical University, Guangzhou, China

ABSTRACT

Gastric cancer (GC) is a complex disease with heterogeneous genetic mechanisms. Genomic mutational profiling of gastric cancer not only expands our knowledge about cancer progression at a fundamental genetic level, but also could provide guidance on new treatment decisions, currently based on tumor histology. The fact that precise medicine-based treatment is successful in a subset of tumors indicates the need for better identification of clinically related molecular tumor phenotypes, especially with regard to those driver mutations on tumor suppressor genes (TSGs) and oncogenes (ONGs). We surveyed 313 TSGs and 160 ONGs associated with 48 protein coding and 19 miRNA genes with both TSG and ONG roles. Using public cancer mutational profiles, we confirmed the dual roles of CDKN1A and CDKN1B. In addition to the widely recognized alterations, we identified another 82 frequently mutated genes in public gastric cancer cohort. In summary, these driver mutation profiles of individual GC will form the basis of personalized treatment of gastric cancer, leading to substantial therapeutic improvements.

INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer worldwide and, although rates have been declining by approximately 2% per year, it is responsible for the second highest rate of cancer-related morbidity and mortality (Bertuccio et al., 2009; Bosetti et al., 2013; Peleteiro et al., 2014). The clinical outcomes for patients with advanced gastric cancer are poor, despite the significant efforts that have been devoted to the development of therapeutic treatments (De Martel, Forman & Plummer, 2013; Karimi et al., 2014). Studies investigating molecular and biochemical changes in GC tissues/cells indicate that the development of GC is a complex process involving function-altering mutations of oncogenes (ONGs) and tumor suppressor genes (TSGs) (Brabek et al., 2010; Gan et al., 2015).

Proto-oncogenes are a group of genes that, when mutated, cause normal cells to become cancerous; the mutated version of a proto-oncogene is called an oncogene (Adamson, 1987; Weinstein & Joe, 2006). Usually, proto-oncogenes encode proteins that stimulate cell
division, inhibit cell differentiation, and inhibit cell death, while oncogenes increase production of these proteins and are considered as major molecular targets for anti-cancer drugs (Druker, 2002; Luo, Solimini & Elledge, 2009; Weinstein, 2002). TSGs represent are guardian genes that play important roles in controlling cell growth processes such as cell-cycle checkpoints and inducing apoptosis in normal cells (Macleod, 2000; Weinberg, 1991). In many tumors, TSGs are often inactivated, removing the restriction on cell proliferation and resulting in the progression of tumor cells (Greenblatt et al., 1994; Narla et al., 2001; Tamura, 2006). A number of ONGs and TSGs have been characterized in GC. For example, frequent mutation of p53 has been observed (Tamura et al., 1991) and E-cadherin activation, induced by mutations increase gastric carcinogenesis (Tamura et al., 1996). Promoter hyper-methylation of TSGs has been described in GC including APC, CHFR, COX2, DAP-kinase, DCC, E-cadherin, GSTP1, HRK, hMLH1, LOX, MGMT, P14, P15, P16, PTEN, RASSF1A, RUNX3, 14-3-3 sigma, THBS1, TIMP-3, and TSLC1 (Byun et al., 2001; Fleisher et al., 1999; Honda et al., 2004; Leung et al., 2001; Li et al., 2002; Obata et al., 2003; Sato et al., 2002; Satoh et al., 2002; Suzuki et al., 1999; Tamura et al., 2001; Tamura et al., 2000; Toyota et al., 1999; Tsuchiya et al., 2000).

Based on GC-implicated genes, we utilized an integrative analysis to identify potential TSGs and ONGs in GC. Additional driver gene prediction further prioritized the highly frequently mutated genes in The Cancer Genome Atlas (TCGA) gastric cancer dataset. Our study produced an up-to-date literature-based survey dedicated for TSGs and ONGs in gastric cancer and provides an important resource for large-scale advanced genetic screen and indication for experimental validation. We also identified 48 protein-coding genes and 19 miRNAs with both TSG and ONG roles. Those coding and non-coding dual role drivers primarily function as regulators in cell proliferation, which implies the potential reverse effect on those genes. The adoption of treatments, tailored according to the suppressive or oncogenic functions of these dual role genes, would involve a paradigm shift in cancer therapy but could lead to improvements in treatment.

**MATERIALS & METHODS**

**Gene list related to gastric cancer**

To systematically study the GC-related genes, we downloaded all 1,815 literature-based GC-related genes were downloaded from GCGene for further analysis (Zhao et al., 2016a). The GCGene was constructed by performing an extensive data integration and literature search followed by manual assembly of the data. To provide a more reliable gene list, we also download 683 genes with two or more PubMed abstracts, which represent reliable gene list related from GCGene. The full GC-related gene list provided a basis for integration. By subtraction of those well-studied 683 genes, we also collected 1,132 genes with a single reference, which will help identify unexplored genes and pathways.

**Data source for driver identification in gastric cancer**

To identify the driver genes in gastric cancer, we used three bioinformatic databases: (i) TSGene (Zhao et al., 2016b), including 1,207 known human TSGs curated from literatures; (ii) ONGene (Liu, Sun & Zhao, 2016), the ONG list with 803 human genes from 8,849
PubMed abstracts; and (iii) DriverDB 2.0 (Chung et al., 2016), a comprehensive cancer driver genes database constructed by integrating 15 published bioinformatics driver identification algorithms.

For TSGene and ONGene, we downloaded all the genes including protein-coding and non-coding genes from corresponding websites. For DriverDB 2.0, we focused on the TCGA GC dataset and predicted putative driver genes in all the GC samples by using the integrated 15 driver identification tools. To obtain a reliable driver gene list, we required that any driver gene should be supported by at least two tools based on the TCGA mutational data.

Pathway and mutational analysis
To assess the function of all the identified GC-related driver genes, we conducted functional enrichment tests using the online tool ToppFunc (Chen et al., 2009). ToppFunc adopts a hypergeometric model in order to measure whether an input gene list has a different annotation frequency to the one that would occur randomly. We conducted chromosome cytoband-based enrichment analysis to identify the genomic regions where the input genes were significantly enriched using all the genes in these regions as background. Similar processes were used to identify enriched gene ontology terms, KEGG and wiki pathways. In these enrichment analyses, all the human genes in ToppFunc were used as background to calculate statistical significance. In addition, the Benjamini–Hochberg method was implemented in the ToppFunc to further exclude false negative results.

Finally, a p-value <0.01 was adopted as the cutoff for enriched pathways in KEGG and gene ontology (biological process) and we only considered those representative pathways with two or more genes. For the enrichment analysis on miRNAs, we adopted an online server miEAA (Watanabe et al., 2007). MiEAA offers both over-representation analysis and set enrichment analysis, which is similar to gene set enrichment analysis implemented in ToppFunc.

Throughout the study, the GC-related mutational analyses were conducted using the cBio portal (Cerami et al., 2012). We selected the Stomach Adenocarcinoma (TCGA) dataset. In total, there are 393 tumor samples with single nucleotide mutations, INDELs, or copy number variation (CNV) data. Abnormal gene expression and protein expression were not included. For the DNA copy-number data, the putative discrete values were calculated for all genes, e.g., “deeply deleted” or “amplified”. For single-nucleotide variations and INDELs, we excluded those mutations without any functional effect, such as synonymous mutations.

RESULTS & DISCUSSION
Identification of dual role protein-coding genes as TSGs and ONGs in gastric cancer
To survey how many TSGs and ONGs are involved in the development of gastric cancers, we used the data from three large-scale literature mining databases. The GCGene is the database developed to curate the gastric cancer-related genes from literature. The TSGene and ONGene were the databases to collect known critical cancer genes with literature. By
overlapping the protein-coding genes from GCGene to TSGene and ONGene, we identified 313 TSGs, 160 ONGs (Fig. 1A) and 48 dual role genes with both TSGs and ONGs effects in other cancers (Table S1).

Many genes could function as both TSG and ONG depending on the cancer type, stage of development, or interaction partners (Zhao et al., 2016b). For example, as an oncogene, SIRT1 can promote cancer progression by negative control of the TGF-beta signaling pathway (Lamouille & Derynck, 2009). However, SIRT1 can also interact with...
promyelocytic leukemia protein to express its tumor suppressor property by stabilizing TP53 and inducing cell senescence. To provide a global functional distribution of the 48 dual role genes in GC, we performed a functional enrichment analysis of gene ontology, KEGG pathway, genomic location, and protein family (Figs. 1B–1C, Table S2). We found 35 genes are active regulators in cell proliferation (GO:0042127, corrected P-value = 2.89E–22). More interesting, there are 32 genes involving in “response to endogenous stimulus” (GO:0009719, corrected P-value = 6.70E–18). Consistently, the subcellular localization also mainly group into nuclear chromosome (11 genes, GO:0000228, corrected P-value = 9.13E–06) and the plasma membrane region (12 genes, GO:0098590, corrected P-value = 1.57E–04). Those genes located on chromosome or chromatin are mainly from transcription factor complex. For example, we found five p53-like transcription factors: RUNX1, RUNX3, STAT3, TP63, and TP73 (InterPro domain IPR008967, corrected P-value = 3.65E–05). By mapping to the pathways (Fig. 1C), we were able to locate those genes in the critical cancer pathways. The dual role genes are associated with cancer pathways (corrected P-value = 5.75E–20), microRNA (corrected P-value = 2.72E–10) and transcriptional regulation (corrected P-value = 9.64E–07). Cell cycle (corrected P-value = 1.22E–08) and response to DNA damage (corrected P-value = 1.24E–07) may be controlled by the dual role genes which are also competing in the androgen receptor (corrected P-value = 3.74E–09), TGF-beta (corrected P-value = 1.84E–08) and p53 signaling (corrected P-value = 4.60E–08) pathways. In summary, our integrative analysis revealed that those genes with both TSG and ONG roles may group into two main functional clusters in gastric cancer: transcriptional regulation inside nuclear, and response to endogenous signals in plasma membrane region. The competition of these genes in critical pathways, such as the cell cycle p53 signaling pathway, may be critical for GC progression.

The majority of genes are unique to some pathways (Fig. 1C). However, several genes, including CDKN1A and CDKN1B, are involved in multiple oncogenic pathways (Fig. 1C). Due to their dual functions in these critical oncogenic pathways, we need to be cautious about drawing conclusions concerning their effects on GC cells. By overlapping those genes with TCGA somatic mutation data, we investigated the potential functions of the 48 genes with dual roles in TCGA GC samples. A few genes did not have dual roles according to their mutational pattern (Fig. 2). For example, SALL4, JUP, NOTCH1 and CDK6 are all with frequent amplification in multiple cancer samples, which may indicate their oncogenic roles in GC. In contrast, more copy number deletions were observed in CDH1, RHOA, PLK1, and MST1R, which may imply a TSG role in GC. For the two genes with broad effects, CDKN1A and CDKN1B, both gene copy gain and loss were found, which means they may have dual roles in GC. The mutational pattern also confirmed some of the genes, such as CDKN1A and CDKN1B, may have dual roles in the TCGA GC cohort.

The functional and mutational features of protein-coding driver TSGs and ONGs in TCGA GC cohort

To explore the driver TSGs and ONGs, we utilized 15 driver mutational detection tools to identify the protein-coding drivers in the TCGA GC cohort. For a driver gene, we required two positive results from two or more detection tools. In total, we found 874 genes
Figure 2  Mutational profile for the 48 genes with both tumor suppressor and oncogenes roles. The sample-based mutational profile for the 48 genes with both tumor suppressor and oncogenes roles in TCGA stomach dataset. Each gene is depicted in each row across multiple samples (each sample for a single column).
with driver mutations in the TCGA data. By overlapping with the gastric cancer-related
genes in GCGene, we identified those driver genes with and without TSG and ONG roles.
We found 30, 18, 6, and 84 driver genes as TSGs, ONGs, dual role, and non-TSG-ONG
respectively (Fig. 3A). We considered that those 138 driver genes were well-studied in
gastric cancer. However, based on the number of literature evidence in GCGene database,
we found there are only 56 driver genes with two or more literature evidences (Fig. 3B).
All the six genes with dual roles in cancers are supported by at least two references, which
confirmed their important roles in GC. By using TCGA GC mutation data, we explored
the mutational frequency of the six genes (Fig. 3C). In total, there were 105 instances
with at least one somatic mutation (copy number variation included) in 393 sequenced
patients (27%). Some of the six genes had only one function based on the mutational
pattern. For example, STAT3 is amplified in the majority of mutated samples suggesting
that the activation of STAT3 signaling genes supports GC cell survival (Kanda et al., 2004).
Similarly, PLK1 and RHOA were deleted in a number of patients and behave more like
TSGs. However, the remaining three genes (PTPN11, VIM, and CDH1) are more likely to
have dual roles with both mutations for gain-of-function and loss-of-function.

To discover some novel driver genes not extensively studied in GC, we focused on 82
predicted driver genes with one reference in the GCGene database (Figs. 3A, 3B). There
were 12 TSG and 7 ONG driver genes associated with a single PubMed abstract. Among
the 82 non-TSG-ONG driver genes (Fig. 3A), 63 are not well studied in GC (Fig. 3B
and Table S3). To validate the functions of these genes in GC it is important to check
over the mutational frequency, we investigated the 82 putative drivers in the TCGA GC
mutational data (Fig. 4). All of the seven ONG driver genes are highly mutated with a
highest mutational frequency of 14% on GLI3 and the lowest mutational frequency 4%
on SMO. Only the ONG NEDD9 had sporadic deletions, which is not consistent with
its oncogenic role. For 12 of the TSG driver genes, we found sporadic amplifications on
AXIN1, CSMD1, LRP1B, NEDD4L, NF1, PARK2, and RHOBTB2. Furthermore, two of
the TSG driver genes (SOCS3, PTPRT) have concordant amplifications which implies an
ONG role in the TCGA GC cohort. For the remaining driver genes, which have no TSG
and ONG functions, the majority have both amplifications and deep deletions in multiple
cancer samples.

MicroRNA TSGs and OGCs in gastric cancer
Recent studies have reported that some micro-RNAs (miR), single-stranded, small
noncoding RNA genes, can function as TSGs and ONGs. Evidence from our GCGene
shows that there are 111 miRNAs related to GC. By intersecting with those curated TSG
and ONG miRNAs, we found 55 TSGs, 14 ONGs and 19 dual role miRNAs (Fig. 5A).
Among these genes, there are 29 TSGs, 9 ONGs and 12 dual role miRNAs with two or
more references in GC (Fig. 5B). Some of the dual role miRNAs are confirmed as having
dual roles in gastric cancer. For example, miR-223 is overexpressed in metastatic GC cells
and stimulates non-metastatic GC cells migration and invasion by directly targeting its
3′-untranslated regions of EPB41L3 (Li et al., 2011). In addition, miR-223 functions as an
oncogene in human GC by targeting FBXW7/hCdc4 (Li et al., 2012) and targets oncogene
Figure 3 Overlapping analysis of GC-implicated genes, tumor suppressors, oncogenes and predicted driver genes. (A) The overlapping of coding GC-implicated genes, tumor suppressors, oncogenes and predicted driver genes from Driver DB 2.0. (B) The overlapping of reliable GC-implicated genes with two or more literature evidence, tumor suppressors, oncogenes and predicted driver genes from Driver DB 2.0. (C) The sample-based mutational profile for the six driver genes with both tumor suppressor and oncogenes roles in TCGA stomach dataset.

STMN1 (Kang et al., 2012). However, the majority of the miRNAs with dual roles in other cancers are not associated with GC. For instance, miR-335 was only reported as TSGs to target Bcl-w and specificity protein 1 (Xu et al., 2012).

By running an enrichment analysis on the 19 identified dual role miRNAs using miEAA (Watanabe et al., 2007), we confirmed that these miRNAs are enriched in various cancers (Table 1). Among the 19, four (miR-20a, miR-18a, miR-16-1, miR-17) are located on chromosome 13 (Corrected $P$-value = 0.00376467) and three of these belong to the miR-17 family. Although the mir-17/92 cluster are known as oncogenes, recent studies suggest that their dual roles depend on the targeting genes (Xiang & Wu, 2010).

By subtracting those genes shown in Fig. 5B from those in Fig. 5A, we found there are seven dual role miRNAs with single literature evidence in GCGene, which may warrant further investigation. For example, the miR-150 functioned as ONG to target a TSG EGR2 (Wu et al., 2010) but there is no information concerning its TSG role in gastric cancer.

By overlapping to TCGA CNV data, we found there are four miRNAs with copy number
changes: miR-31, miR-182, miR-135a-1, and miR-150. We found that miR-31 has both amplification and deletion functions in different tumor samples and this suggests dual roles in gastric cancer. The miR-182 and miR-150 only have amplifications but miR-135a-1 was all deleted in 1.5% TCGA GC cohort. In summary, we found several potential dual role miRNAs in GC with different CNV pattern.

Figure 4  Mutational profile for the 82 driver genes. The sample-based mutational profile for the 82 driver genes with single literature evidence. Each gene is depicted in each row across multiple samples (each sample for a single column).
Figure 5 Overlapping analysis of GC-implicated miRNAs, tumor suppressors, and oncogenes. (A) The overlapping of GC-implicated miRNAs, tumor suppressors, and oncogenes. (B) The overlapping of reliable GC-implicated miRNAs with two or more literature evidence, tumor suppressors, and oncogenes. (C) The sample-based mutational profile for the four dual role, five oncogenic, and 19 tumor suppressive miRNAs with single literature evidence (each sample for a single column).
Table 1  Enrichment analysis of dual role miRNAs. The enrichment of 19 dual role miRNAs identified in different cancers.

| CANCERS                          | \( P \)-value | miRNAs/precursors                                                   |
|----------------------------------|----------------|---------------------------------------------------------------------|
| Neoplasms                        | 1.373E−06      | miR-31; miR-20A; miR-18A; miR-149; miR-34B; miR-200C; miR-27A; miR-182; miR-7-1; miR-335; miR-34A; miR-155; miR-16-1; miR-17; miR-150 |
| Stomach Neoplasms                | 1.373E−06      | miR-31; miR-20A; miR-18A; miR-149; miR-34B; miR-223; miR-200C; miR-27A; miR-182; miR-7-1; miR-335; miR-34A; miR-155; miR-16-1; miR-17; miR-107; miR-150 |
| Colorectal Neoplasms             | 1.66E−06       | miR-31; miR-20A; miR-18A; miR-149; miR-34B; miR-200C; miR-27A; miR-7-1; miR-335; miR-34A; miR-155; miR-16-1; miR-17; miR-107; miR-150 |
| Pancreatic Neoplasms             | 1.878E−06      | miR-31; miR-20A; miR-18A; miR-149; miR-200C; miR-27A; miR-182; miR-7-1; miR-335; miR-34A; miR-155; miR-16-1; miR-17; miR-107; miR-150 |
| Glioma                           | 5.232E−06      | miR-31; miR-20A; miR-18A; miR-149; miR-223; miR-200C; miR-27A; miR-182; miR-155; miR-16-1; miR-17 |
| Lung Neoplasms                   | 5.232E−06      | miR-31; miR-20A; miR-18A; miR-34B; miR-223; miR-200C; miR-27A; miR-182; miR-7-1; miR-335; miR-34A; miR-155; miR-17; miR-107; miR-150 |
| Urinary Bladder Neoplasms        | 5.232E−06      | miR-31; miR-20A; miR-18A; miR-149; miR-200C; miR-27A; miR-182; miR-7-1; miR-34A; miR-155; miR-17; miR-107 |
| Leukemia, Lymphocytic, Chronic, B-Cell | 3.762E−05   | miR-20A; miR-18A; miR-34B; miR-223; miR-34A; miR-155; miR-16-1; miR-17; miR-107 |
| Ovarian Neoplasms                | 4.527E−05      | miR-31; miR-20A; miR-18A; miR-34B; miR-223; miR-200C; miR-27A; miR-182; miR-335; miR-34A; miR-155; miR-16-1; miR-17 |
| Leukemia                         | 7.58E−05       | miR-31; miR-20A; miR-18A; miR-27A; miR-34A; miR-16-1; miR-17; miR-150 |
| Adenocarcinoma                   | 7.627E−05      | miR-31; miR-20A; miR-18A; miR-34B; miR-223; miR-200C; miR-182; miR-155; miR-16-1; miR-17 |
| Carcinoma, Non-Small-Cell Lung   | 7.627E−05      | miR-149; miR-34B; miR-223; miR-200C; miR-27A; miR-182; miR-7-1; miR-34A; miR-155; miR-16-1; miR-17; miR-150 |
| Carcinoma, Squamous Cell         | 8.583E−05      | miR-31; miR-20A; miR-18A; miR-34B; miR-223; miR-200C; miR-27A; miR-182; miR-34A; miR-155; miR-16-1 |

As shown in Figs. 5A and 5B, there are 55 gastric cancer-related miRNAs with TSG functions. A few miRNAs have been suggested as potential TSGs via suppressing different oncogenes. For instance, the miR-148a (Zheng et al., 2011), miR-195 and miR-375 (Ding et al., 2010) may function as TSG in GC, and theirs anti-oncogenic activity may involve the direct targeting and inhibition of ONG ROCK1, CDK6, and JAK2. Some miRNAs may not function in cancer development, but cancer metastasis original from GC tissues. The interaction between Robo1 on the Slit2Slit-Robo1 pathway triggers tumor metastasis of GC, which can be suppressed by miR-218 (Tie et al., 2010). By subtracting those genes shown in Fig. 5B from Fig. 5A, we found there are 26 miRNA TSGs with single literature evidence in GCGene. Examples include miR-486 (targeting OLFM4) (Oh et al., 2011), miR-378 (suppressing VEGF) (Deng et al., 2013), and miR-101 (targeting EZH2, Cox-2, Mcl-1 and
Among the 26, there are 19 miRNAs with CNVs in TCGA GC cohort. For example, the miR-148b could have a TSG role by targeting CCKBR (Song et al., 2011), which was mutated in 1.5% of 393 TCGA patients. Although most of these CNVs for miR-148b are deletions, the amplifications were still observed in a few samples. A few of the miRNA TSGs were amplified in all the samples with mutations, such as miR-196b, miR-206, miR-let-7i, miR-100, and miR-29c, which either implies the potential oncogenic role or those amplifications may not have dosage effects on these miRNAs.

In general, miRNA ONGs are located in the amplified regions in human cancers and tend to cleave target mRNAs more frequently (Wang et al., 2010a). For example, the miR-21 (Zhang et al., 2012), negatively regulates the tumor suppressors PTEN which, in-turn, promote gastric tumor proliferation and invasion. All five ONGs with a single study recorded in GCGene have more amplifications than deletions in the TCGA cohort (Fig. 5C); this confirms their critical oncogenic roles in GC.

CONCLUSIONS

A better understanding of the molecular drivers and pathways of tumor formation has led to the development of targeted agents. In this study, we performed a systematic evaluation of cancer driver genes in GC by integrating literature and mutational data. We identified 313 TSGs and 160 ONGs implicated in GC. By applying driver mutation identification tools, we reduced the gene list to 30 TSGs and 18 ONGs.

As ONGs and TSGs normally perform their cellular functions jointly, different mechanisms have been conceived behind this experimentally based on one or only a few ONGs and TSGs, though controversies remain when considering multiple ONGs and TSGs at a time. Recent investigations have used bioinformatics analysis to compare the mutation patterns and network properties of ONGs and TSGs of different cancers. Distinct regulatory patterns of TSGs and ONGs by transcription factors have been found in ovarian cancer, which competitively acts upon apoptosis and the ErbB signalling pathway (Zhao, Sun & Zhao, 2012). The TSG and ONG miRNAs show distinct patterns in function, evolutionary rate, expression, chromosome distribution, molecule size, free energy, transcription factors, and targets, suggested by a large-scale survey of human miRNA (Wang et al., 2010a). However, we identified 48 coding genes and 19 miRNAs with both TSG and OCG roles. According to the mutation data, some of these genes may have only a single function, in contrast with their role in other cancer types. Interestingly, a few of the genes have mixed mutational patterns with both gain-of-function and loss-of-function. For the first time, we provide the dual role gene list in GC to support further large-scale genetic screen and our systematic evaluation provides a blueprint for the interplay of TSGs and ONGs in GC.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The authors received no funding for this work.
Competing Interests
Min Zhao is an Academic Editor for PeerJ. The authors declare there are no competing interests.

Author Contributions
• Tianfang Wang and Yining Liu performed the experiments, wrote the paper, reviewed drafts of the paper.
• Min Zhao conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Data Availability
The following information was supplied regarding data availability:
The raw data has been supplied as a Supplementary File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.3585#supplemental-information.

REFERENCES
Adamson ED. 1987. Oncogenes in development. Development 99:449–471.
Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, Malvezzi M, La Vecchia C. 2009. Recent patterns in gastric cancer: a global overview. International Journal of Cancer 125:666–673 DOI 10.1002/ijc.24290.
Bosetti C, Bertuccio P, Malvezzi M, Levi F, Chatenoud L, Negri E, La Vecchia C. 2013. Cancer mortality in Europe, 2005–2009, and an overview of trends since 1980. Annals of Oncology 24:2657–2671 DOI 10.1093/annonc/mdt301.
Brabek J, Mierke CT, Rosel D, Vesely P, Fabry B. 2010. The role of the tissue microenvironment in the regulation of cancer cell motility and invasion. Cell Communication and Signaling 8:22 DOI 10.1186/1478-811x-8-22.
Byun DS, Lee MG, Chae KS, Ryu BG, Chi SG. 2001. Frequent epigenetic inactivation of RASSF1A by aberrant promoter hypermethylation in human gastric adenocarcinoma. Cancer Research 61:7034–7038.
Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. 2012. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discovery 2:401–404 DOI 10.1158/2159-8290.CD-12-0095.
Chen J, Bardes EE, Aronow BJ, Jegga AG. 2009. ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. Nucleic Acids Research 37:W305–W311 DOI 10.1093/nar/gkp427.
Chung IF, Chen CY, Su SC, Li CY, Wu KJ, Wang HW, Cheng WC. 2016. DriverDBv2: a database for human cancer driver gene research. Nucleic Acids Research 44:D975–D979 DOI 10.1093/nar/gkv1314.
Deng H, Guo Y, Song H, Xiao B, Sun W, Liu Z, Yu X, Xia T, Cui L, Guo J. 2013. MicroRNA-195 and microRNA-378 mediate tumor growth suppression by epigenetical regulation in gastric cancer. *Gene* 518:351–359 DOI 10.1016/j.gene.2012.12.103.

De Martel C, Forman D, Plummer M. 2013. Gastric cancer: epidemiology and risk factors. *Gastroenterology Clinics of North America* 42:219–240 DOI 10.1016/j.gtc.2013.01.003.

Ding L, Xu Y, Zhang W, Deng Y, Si M, Du Y, Yao H, Liu X, Ke Y, Si J, Zhou T. 2010. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. *Cell Research* 20:784–793 DOI 10.1038/cr.2010.79.

Druker BJ. 2002. Perspectives on the development of a molecularly targeted agent. *Cancer Cell* 1:31–36 DOI 10.1016/S1535-6108(02)00025-9.

Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, Zou TT, Abraham JM, Kong D, Smolinski KN, Shi YQ, Rhyu MG, Powell SM, James SP, Wilson KT, Herman JG, Meltzer SJ. 1999. Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. *Cancer Research* 59:1090–1095.

Gan L, Xu M, Zhang Y, Zhang X, Guo W. 2015. Focusing on long noncoding RNA dysregulation in gastric cancer. *Tumour Biology* 36:129–141 DOI 10.1007/s13277-014-2894-9.

Greenblatt MS, Bennett WP, Hollstein M, Harris CC. 1994. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Research* 54:4855–4878.

Honda T, Tamura G, Waki T, Kawata S, Nishizuka S, Motoyama T. 2004. Promoter hypermethylation of the Chfr gene in neoplastic and non-neoplastic gastric epithelia. *British Journal of Cancer* 90:2013–2016 DOI 10.1038/sj.bjc.6601849.

Kanda N, Seno H, Konda Y, Marusawa H, Kanai M, Nakajima T, Kawashima T, Nanakin A, Sawabu T, Uenoyma Y, Sekikawa A, Kawada M, Suzuki K, Kayahara T, Fukui H, Sawada M, Chiba T. 2004. STAT3 is constitutively activated and supports cell survival in association with survivin expression in gastric cancer cells. *Oncogene* 23:4921–4929 DOI 10.1038/sj.onc.1207606.

Kang W, Tong JH, Chan AW, Lung RW, Chau SL, Wong QW, Wong N, Yu J, Cheng AS, To KF. 2012. Stathmin1 plays oncogenic role and is a target of microRNA-223 in gastric cancer. *PLOS ONE* 7:e33919 DOI 10.1371/journal.pone.0033919.

Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. 2014. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiology, Biomarkers & Prevention* 23:700–713 DOI 10.1158/1055-9965.epi-13-1057.

Lamouille S, Derynck R. 2009. Oncogene and tumour suppressor: the two faces of SnoN. *EMBO Journal* 28:3459–3460 DOI 10.1038/emboj.2009.311.

Leung WK, Yu J, Ng EK, To KF, Ma PK, Lee TL, Go MY, Chung SC, Sung JJ. 2001. Concurrent hypermethylation of multiple tumor-related genes in gastric carcinoma and adjacent normal tissues. *Cancer* 91:2294–2301 DOI 10.1002/1097-0142(20010615)91:12<2294::AID-CNCR1261>3.0.CO;2-G.
Li J, Guo Y, Liang X, Sun M, Wang G, De W, Wu W. 2012. MicroRNA-223 functions as an oncogene in human gastric cancer by targeting FBXW7/hCdc4. *Journal of Cancer Research and Clinical Oncology* **138**:763–774 DOI 10.1007/s00432-012-1154-x.

Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. 2002. Causal relationship between the loss of RUNX3 expression and gastric cancer. *Cell* **109**:113–124 DOI 10.1016/S0092-8674(02)00690-6.

Li X, Zhang Y, Zhang H, Liu X, Gong T, Li M, Sun L, Ji G, Shi Y, Han Z, Han S, Nie Y, Chen X, Zhao Q, Ding J, Wu K, Daiming F. 2011. miRNA-223 promotes gastric cancer invasion and metastasis by targeting tumor suppressor EPB41L3. *Molecular Cancer Research* **9**:824–833 DOI 10.1158/1541-7786.mcr-10-0529.

Liu Y, Sun J, Zhao M. 2016. ONGene: a literature-based database for human oncogenes. *Journal of Genetics and Genomics* **4**:1 DOI 10.1016/j.jgg.2016.12.004.

Luo J, Solimini NL, Elledge SJ. 2009. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* **136**:823–837 DOI 10.1016/j.cell.2009.02.024.

Macleod K. 2000. Tumor suppressor genes. *Current Opinion in Genetics & Development* **10**:81–93 DOI 10.1016/S0959-437X(99)00041-6.

Narla G, Heath KE, Reeves HL, Li D, Giono LE, Kimmelman AC, Glucksman MJ, Narla J, Eng FJ, Chan AM, Ferrari AC, Martignetti JA, Friedman SL. 2001. KLF6, a candidate tumor suppressor gene mutated in prostate cancer. *Science* **294**:2563–2566 DOI 10.1126/science.1066326.

Obata T, Toyota M, Satoh A, Sasaki Y, Ogi K, Akino K, Suzuki H, Murai M, Kikuchi T, Mita H, Itoh F, Issa JP, Tokino T, Imai K. 2003. Identification of HRK as a target of epigenetic inactivation in colorectal and gastric cancer. *Clinical Cancer Research* **9**:6410–6418.

Oh HK, Tan AI, Das K, Ooi CH, Deng NT, Tan IB, Beillard E, Lee J, Ramnarayanan K, Rha SY, Palanisamy N, Voorhoeve PM, Tan P. 2011. Genomic loss of miR-486 regulates tumor progression and the OLFM4 antiapoptotic factor in gastric cancer. *Clinical Cancer Research* **17**:2657–2667 DOI 10.1158/1078-0432.ccr-10-3152.

Peleteiro B, Severo M, La Vecchia C, Lunet N. 2014. Model-based patterns in stomach cancer mortality worldwide. *European Journal of Cancer Prevention* **23**:524–531 DOI 10.1097/CEJ.0b013e328364f2b6.

Sato K, Tamura G, Tsuchiya T, Endoh Y, Sakata K, Motoyama T, Usuba O, Kimura W, Terashima M, Nishizuka S, Zou T, Meltzer SJ. 2002. Analysis of genetic and epigenetic alterations of the PTEN gene in gastric cancer. *Virchows Archiv* **440**:160–165 DOI 10.1007/s004280100499.

Satoh A, Toyota M, Itoh F, Kikuchi T, Obata T, Sasaki Y, Suzuki H, Yawata A, Kusano M, Fujita M, Hosokawa M, Yanagihara K, Tokino T, Imai K. 2002. DNA methylation and histone deacetylation associated with silencing DAP kinase gene expression in colorectal and gastric cancers. *British Journal of Cancer* **86**:1817–1823 DOI 10.1038/sj.bjc.6600319.
Song YX, Yue ZY, Wang ZN, Xu YY, Luo Y, Xu HM, Zhang X, Jiang L, Xing CZ, Zhang Y. 2011. MicroRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation. *Molecular Cancer* 10:1 DOI 10.1186/1476-4598-10-1.

Suzuki H, Itoh F, Toyota M, Kikuchi T, Kakiuchi H, Hinoda Y, Imai K. 1999. Distinct methylation pattern and microsatellite instability in sporadic gastric cancer. *International Journal of Cancer* 83:309–313 DOI 10.1002/(SICI)1097-0215(19991029)83:3<309::AID-IJC4>3.0.CO;2-Z.

Tamura G. 2006. Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer. *World Journal of Gastroenterology* 12:192–198 DOI 10.3748/wjg.v12.i2.192.

Tamura G, Kihana T, Nomura K, Terada M, Sugimura T, Hirohashi S. 1991. Detection of frequent p53 gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation polymorphism analysis. *Cancer Research* 51:3056–3058.

Tamura G, Sakata K, Nishizuka S, Maesawa C, Suzuki Y, Iwaya T, Terashima M, Saito K, Satodate R. 1996. Inactivation of the E-cadherin gene in primary gastric carcinomas and gastric carcinoma cell lines. *Japanese Journal of Cancer Research* 87:1153–1159 DOI 10.1111/j.1349-7006.1996.tb03125.x.

Tamura G, Sato K, Akiyama S, Tsuchiya T, Endoh Y, Usuba O, Kimura W, Nishizuka S, Motoyama T. 2001. Molecular characterization of undifferentiated-type gastric carcinoma. *Laboratory Investigation* 81:593–598 DOI 10.1038/labinvest.3780268.

Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, Kong D, Smolinski KN, Wilson KT, James SP, Silverberg SG, Nishizuka S, Terashima M, Meltzer SJ. 2000. Distinct methylation patterns of two APC gene promoters in normal and cancerous gastric epithelia. *Oncogene* 19:3642–3646 DOI 10.1038/sj.onc.1203704.

Tie J, Pan Y, Zhao L, Wu K, Liu J, Sun S, Guo X, Wang B, Gang Y, Zhang Y, Li Q, Qiao T, Zhao Q, Nie Y, Fan D. 2010. MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo1 receptor. *PLOS Genetics* 6:e1000879 DOI 10.1371/journal.pgen.1000879.

Toyota M, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. 1999. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Research* 59:5438–5442.

Tsuchiya T, Tamura G, Sato K, Endoh Y, Sakata K, Jin Z, Motoyama T, Usuba O, Kimura W, Nishizuka S, Wilson KT, James SP, Yin J, Fleisher AS, Zou T, Silverberg SG, Kong D, Meltzer SJ. 2000. Distinct methylation patterns of two APC gene promoters in normal and cancerous gastric epithelia. *Oncogene* 19:3642–3646 DOI 10.1038/sj.onc.1203704.

Wang D, Qiu C, Zhang H, Wang J, Cui Q, Yin Y. 2010a. Human microRNA oncogenes and tumor suppressors show significantly different biological patterns: from functions to targets. *PLOS ONE* 5:e13067 DOI 10.1371/journal.pone.0013067.
Wang HJ, Ruan HJ, He XJ, Ma YY, Jiang XT, Xia YJ, Ye ZY, Tao HQ. 2010b. MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. *European Journal of Cancer* **46**:2295–2303 DOI 10.1016/j.ejca.2010.05.012.

Watanabe Y, Toyota M, Kondo Y, Suzuki H, Imai T, Ohe-Toyota M, Maruyama R, Nojima M, Sasaki Y, Sekido Y, Hiratsuka H, Shinomura Y, Imai K, Itoh F, Tokino T. 2007. PRDM5 identified as a target of epigenetic silencing in colorectal and gastric cancer. *Clinical Cancer Research* **13**:4786–4794 DOI 10.1158/1078-0432.CCR-07-0305.

Weinberg RA. 1991. Tumor suppressor genes. *Science* **254**:1138–1146 DOI 10.1126/science.1659741.

Weinstein IB. 2002. Cancer addiction to oncogenes—the Achilles heal of cancer. *Science* **297**:63–64 DOI 10.1126/science.1073096.

Weinstein IB, Joe AK. 2006. Mechanisms of disease: oncogene addiction—a rationale for molecular targeting in cancer therapy. *Nature Clinical Practice Oncology* **3**:448–457 DOI 10.1038/nccopnc0558.

Wu Q, Jin H, Yang Z, Luo G, Lu Y, Li K, Ren G, Su T, Pan Y, Feng B, Xue Z, Wang X, Fan D. 2010. MiR-150 promotes gastric cancer proliferation by negatively regulating the pro-apoptotic gene EGR2. *Biochemical and Biophysical Research Communications* **392**:340–345 DOI 10.1016/j.bbrc.2009.12.182.

Xiang J, Wu J. 2010. Feud or Friend? The role of the miR-17-92 cluster in tumorigenesis. *Current Genomics* **11**:129–135 DOI 10.2174/138920210790886853.

Xu Y, Zhao F, Wang Z, Song Y, Luo Y, Zhang X, Jiang L, Sun Z, Miao Z, Xu H. 2012. MicroRNA-335 acts as a metastasis suppressor in gastric cancer by targeting Bcl-w and specificity protein 1. *Oncogene* **31**:1398–1407 DOI 10.1038/onc.2011.340.

Zhang BG, Li JF, Yu BQ, Zhu ZG, Liu BY, Yan M. 2012. microRNA-21 promotes tumor proliferation and invasion in gastric cancer by targeting PTEN. *Oncology Reports* **27**:1019–1026 DOI 10.3892/or.2012.1645.

Zhao M, Chen L, Liu Y, Qu H. 2016a. GCGene: a gene resource for gastric cancer with literature evidence. *Oncotarget* **7**:33983–33993 DOI 10.18632/oncotarget.9030.

Zhao M, Kim P, Mitra R, Zhao J, Zhao Z. 2016b. TSGene 20: an updated literature-based knowledgebase for tumor suppressor genes. *Nucleic Acids Research* **44**:D1023–D1031 DOI 10.1093/nar/gkv1268.

Zhao M, Sun J, Zhao Z. 2012. Distinct and competitive regulatory patterns of tumor suppressor genes and oncogenes in ovarian cancer. *PLOS ONE* **7**:e44175 DOI 10.1371/journal.pone.0044175.

Zheng B, Liang L, Wang C, Huang S, Cao X, Zha R, Liu L, Jia D, Tian Q, Wu J, Ye Y, Wang Q, Long Z, Zhou Y, Du C, He X, Shi Y. 2011. MicroRNA-148a suppresses tumor cell invasion and metastasis by downregulating ROCK1 in gastric cancer. *Clinical Cancer Research* **17**:7574–7583 DOI 10.1158/1078-0432.ccr-11-1714.