Original Article

Mutational characteristics of young and elderly gastric cancer: a comparative study

Long Li[1#], Yin Li[2#], Li Lin[3#], Yanling Liu[4#], Linshan Duan[4], Dan Wang[1], Shuyu Cheng[1], Guoyan Liu[1,4,5]

[1]Medical College of Xiamen University, Xiamen University, Xiamen, China; [2]Xiamen International Travel Healthcare Center, Xiamen, China; [3]Department of Gastrointestinal Surgery and Xiamen City Key Laboratory of Gastrointestinal Cancer, Zhongshan Hospital, Xiamen University, China; [4]School of Pharmaceutical Sciences, Xiamen University, Xiamen, China; [5]Department of Gastrointestinal Surgery, Zhongshan Hospital of Xiamen University, Xiamen, China

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[#]These authors contributed equally to this work.

Correspondence to: Guoyan Liu. School of Pharmaceutical Sciences, Xiamen University, Xiang'an South Road, Xiamen 361102, China. Email: liuguoyan@xmu.edu.cn.

Background: Young gastric cancer (YGC) has been indicated as having a worse prognosis than in elderly gastric cancer (EGC). It has been reported that YGC and EGC patients show different genomic profiles; however, there has been no comparative study conducted to reveal their mutational characteristics.

Methods: Firstly, we divided and analyzed the mutational landscape and 50 cancer-related genes characters of YGC (n=18) and EGC (n=18) patients from The Cancer Genome Atlas-Stomach Adenocarcinoma (TCGA-STAD). A total of 8 gastric cancer samples including 4 YGC and 4 EGC patients were collected to detect 50 cancer-related genes by multiplex polymerase chain reaction (PCR) next generation sequencing. The R/maftools package was used to describe the mutational characteristics.

Results: Our results showed that the EGC group harbored more mutations than the YGC group. In 50 cancer-related genes in our cohort, the YGC group tended to be different from the EGC group using multiplex PCR next generation sequencing. In the YGC group, candidate mutations were identified within the following genes: *IDH2*, *PDGFRA*, *KRAS*, *FLT3*, *FGFR2*, and *FGFR3*. The YGC group showed less tumor mutational burden (TMB) level then EGC.

Conclusions: The YGC group tended to be more sensitive to molecularly targeted therapy because of it having more somatic mutations in 50 cancer-related genes using targeted next-generation sequencing.

Keywords: Young gastric cancer (YGC); elderly gastric cancer (EGC); targeted next-generation sequencing; tumor mutation burden (TMB); molecularly targeted therapy

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Introduction

Gastric cancer (GC) is a common malignant cancer worldwide, and is especially prevalent in Asia regions including China, Japan, and Korea. Young gastric cancer (YGC, under 40 years old) has shown a poorer rate of diagnosis than elderly gastric cancer (EGC, above 70 years old). Clinically, YGC is associated with delayed diagnosis and being more aggressive. Generally, GC is considered to be associated with age, with its incidence peaking among those older than 50 years (1). In the last decades, characteristics of this neoplasm in young adults had been reported (2-6). The proportion of YGCs has varied from 6% to 8% (5-8). Most comparative studies

of the clinicopathological characteristics between YGC and EGC have been conducted in Japan (9-12), and the different features of GCs between YGCs and EGCs has been demonstrated in a Japanese study (11).

It has been reported that YGC and EGC patients show different genomic profiles (13). Molecularly targeted therapy targeting sensitizing mutations has been a successful strategy for the clinical treatment of cancer (14). For example, the presence of epidermal growth factor receptor (*EGFR*)-positive mutations in lung cancer patients is the gold-standard biomarker for the first-line *EGFR* tyrosine kinase inhibitor (TKI) therapy. The rate of *EGFR* mutations is high in the Chinese cancer population (15,16). The purpose of the present study was to analyze the mutational landscape of GC, compare the molecular features of young patients with those of elderly patients, and to profile the point mutation frequency of 50 GC-associated genes using targeted next-generation sequencing, a more sensitive mutation detection technology.

Although some articles have reported the gene mutation characteristics of gastric cancer in the TCGA database, they mainly compared the relationship between the ACRG classification and the TCGA classification in evaluating the prognosis. In this paper, the mutation status and 50 cancer-related gene signatures of YGC (n=18) and EGC (n=18) patients in the TCGA-STAD database were classified and analyzed. The two have different focuses. We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-934/rc).

Methods

Patient collection

A total of 8 GC patients were recruited from the Zhongshan Hospital of Xiamen University. Of these, 4 participants (50%) were 40 years of age or younger. This study was approved by the Ethics Committee of Zhongshan Hospital of Xiamen University (No. MULAC20180085), and performed in accordance with the principles of the Declaration of Helsinki (as revised in 2013). After the patients had provided informed consent to participate in the study, tumor tissues and their matched control samples were obtained for targeted next-generation sequencing. All samples were subjected to pathology review for histological subtyping and detailed clinical characteristics. The clinical characteristics of the 8 GCs are shown in *Table 1*.

PCR and barcoding

The 50 cancer-related genes included well-known oncogenes and tumor suppressor genes [as listed in the Candidate Cancer Gene Database: http://ccgd-starrlab.oit.umn.edu/search.html]. Multiplexed panel sequencing across 50 cancer-related genes (shown in Table S1) was performed on germline DNA. Genes were selected based on implication in cancers identified through literature review (17). Amplification of 50 cancer-related genes was performed using a single multiplex PCR amplification.

Sequencing and data analysis

The barcoded PCR products were mixed and cleaned using AMPure beads (Beckman Coulter, Brea, CA, USA). The pooled amplicons were then mixed 7:3 with PhiX (Illumina, San Diego, CA, USA), denatured and clustered at 6 pM on Illumina Miseq 500 cycle flow-cell and sequenced (250 cycles forward, 10 cycles barcode, 250 cycles reverse). Raw trace files were processed with trimmomatic (version 0.36) (18) in paired-end mode to remove adapter sequences, and to filter out pairs with a sequence <100 nt to exclude short read artefacts. Local alignments of reads to the hg19 genome were performed using Burrows-Wheeler Aligner (BWA; version 0.7.17) (19) in paired-end mode. Sequence alignment map (SAM) files were converted to binary alignment map (BAM) files, sorted, and indexed using samtools (version 1.4) (20). Mutations were called using samtools/bcftools to generate the vcf files.

TCGA WES somatic mutations

Confident somatic mutation calls derived from the whole-exome sequencing (WES) data of the stomach adenocarcinoma (STAD) cohorts were directly downloaded from the Cancer Genome Atlas Genomic Data Commons (TCGA GDC) data portal using R/Bioconductor package 'TCGAbiolinks' (21). The somatic mutations were annotated with oncotator (22) using the same settings as in our analysis.

Statistical analysis

The R/maftools package was used to describe the mutational characteristics. Mutations were called using samtools/bcftools to generate the vcf files. The somatic mutations were annotated with oncotator using the same settings as in our analysis.

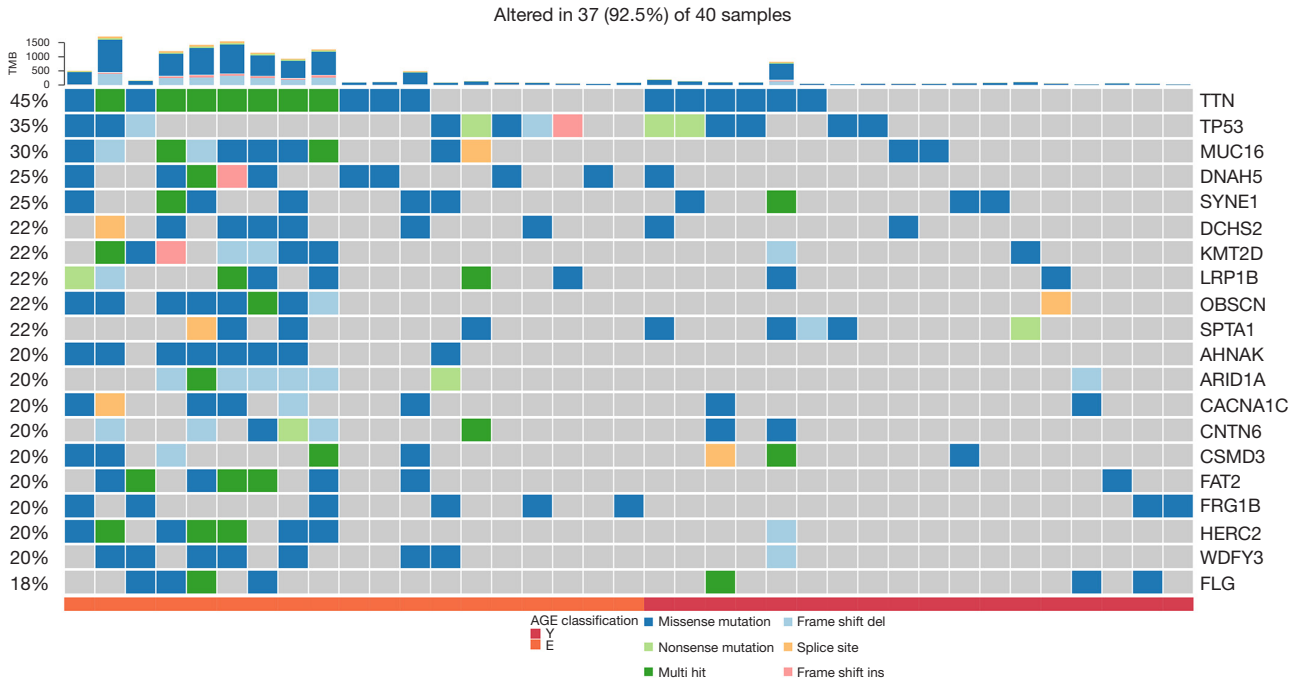

Figure 1 Distribution of top 20 genes aberrances, stratified by subgroups. Subgroups were defined as EGC group (orange block, n=20) and YGC group (red block, n=20). EGC, early gastric cancer; YGC, young gastric cancer.

Results

The mutational landscape of YGC and EGC based on TCGA-STAD database

To declare the molecular difference between YGC and EGC groups, a distribution of gene aberrances, stratified by subgroups in *Figure 1* was made based on TCGA-STAD database. The 40 patients were equally divided into young (n=20) and old (n=20) according to their age, forming a cohort to be analyzed. The mean age of the YGC group was 42.5 years old (30–46 years) and that of the EGC was 86.2 years old (83–90 years). Tumor mutation burden (TMB) was shown to be associated with clinical benefit of anti-programmed cell death protein 1 (PD-1) therapy. From the results, we observed that the EGC group harbored higher TMB level than the YGC group. As an oncogenic gene, *TP53* showed the same mutation rate in YGC (4/18) and EGC (4/18). Most genes (19 out of 20) showed more somatic variation in the EGC group than the YGC group.

The comparation of 50 cancer-related genes in YGC and EGC form TCGA-STAD

In this study, the 50 cancer-related genes included well-known oncogenes and tumor suppressor genes [as listed in the Candidate Cancer Gene Database (23), such as *CCND1*, *CCNE1*, *CDK6*, *CDKN2A*, *EGFR*, *ERBB2*, *FGFR2*, *KRAS*, *MET*, *MYC*, and *PTEN*. Genetic alterations involving the phosphatidylinositol-3 kinase/AKT signaling pathway also occur in GCs, particularly in advanced and dedifferentiating tumors. The distribution of gene aberrances, stratified by subgroups in *Figure 2* was made based on TCGA-STAD database. The EGC subgroup (n=20) harbored 35 missense mutations, and only in *ALK*, *FBXW7*, *GNA11*, *PTPN11*, and *FGFR3*; the YGC subgroup (n=20) had 23 missense mutations, and only in *CDH1*, *FGFR2*, *CTNNB1*, and *ATM*. Taken together, these data indicated that there is no obvious somatic mutations between YGC and EGC from TCGA-STAD database.

Targeted next-generation sequencing for mutations detection of 50 cancer-related genes in a Chinese cohort

The next-generation sequencing (NGS) offers single nucleotide level information on a different scale including whole-genome sequencing (WGS), WES, whole-transcriptome sequencing (WTS), and PCR-based targeting sequencing of multiple specific genomic regions. Whereas

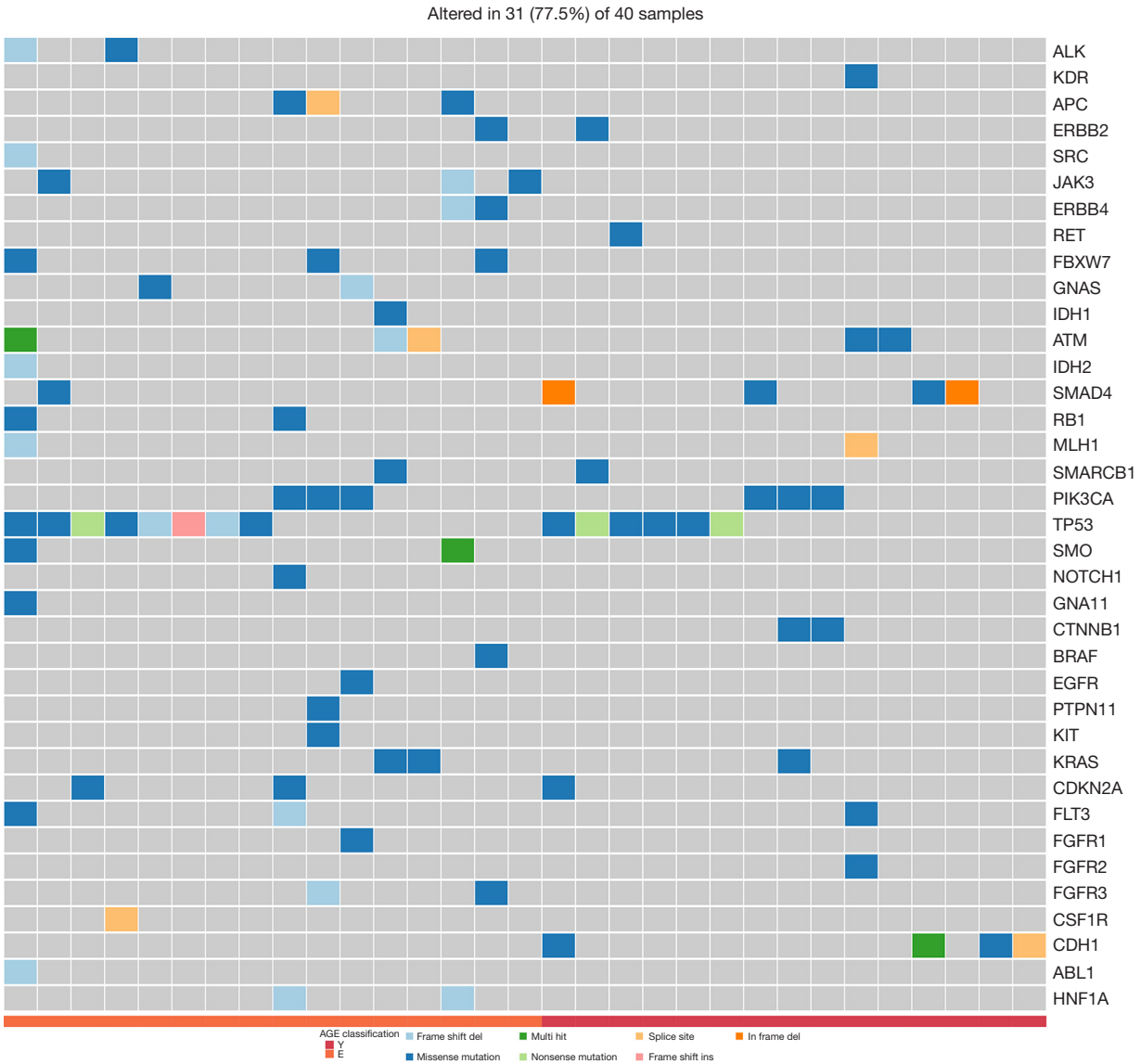

Figure 2 Distribution of 50 cancer-associated genes aberrances in EGC and YGC groups from TCGA/STAD. Subgroups were defined as EGC group (orange block, n=20) and YGC group (red block, n=20). EGC, early gastric cancer; YGC, young gastric cancer.

Table 1 Clinical characteristics of 8 the gastric cancer patients in this study

Patient	Gender	Type	Age (years)
1	Male	Diffuse	78
2	Female	Diffuse	77
3	Female	Diffuse	73
4	Male	Diffuse	75
5	Female	Diffuse	33
6	Male	Diffuse	19
7	Male	Diffuse	39
8	Male	Diffuse	34

largescale analyses are essential for discovery projects, targeted panels with a well-known gene list may offer further advances in the routine molecular diagnostics of cancer. We collected 8 GC samples, as shown in *Table 1*, as our cohort for comparative study of GCs. In this comparative study, such an approach may be helpful to expand the currently existing 50 cancer-related gene panels to enable simultaneous testing for multiple mutations. The distribution of gene aberrances, stratified by the subgroups shown in *Figure 3*, was made based on our cohort. The EGC subgroup (n=4, >70 years old) harbored 11 missense

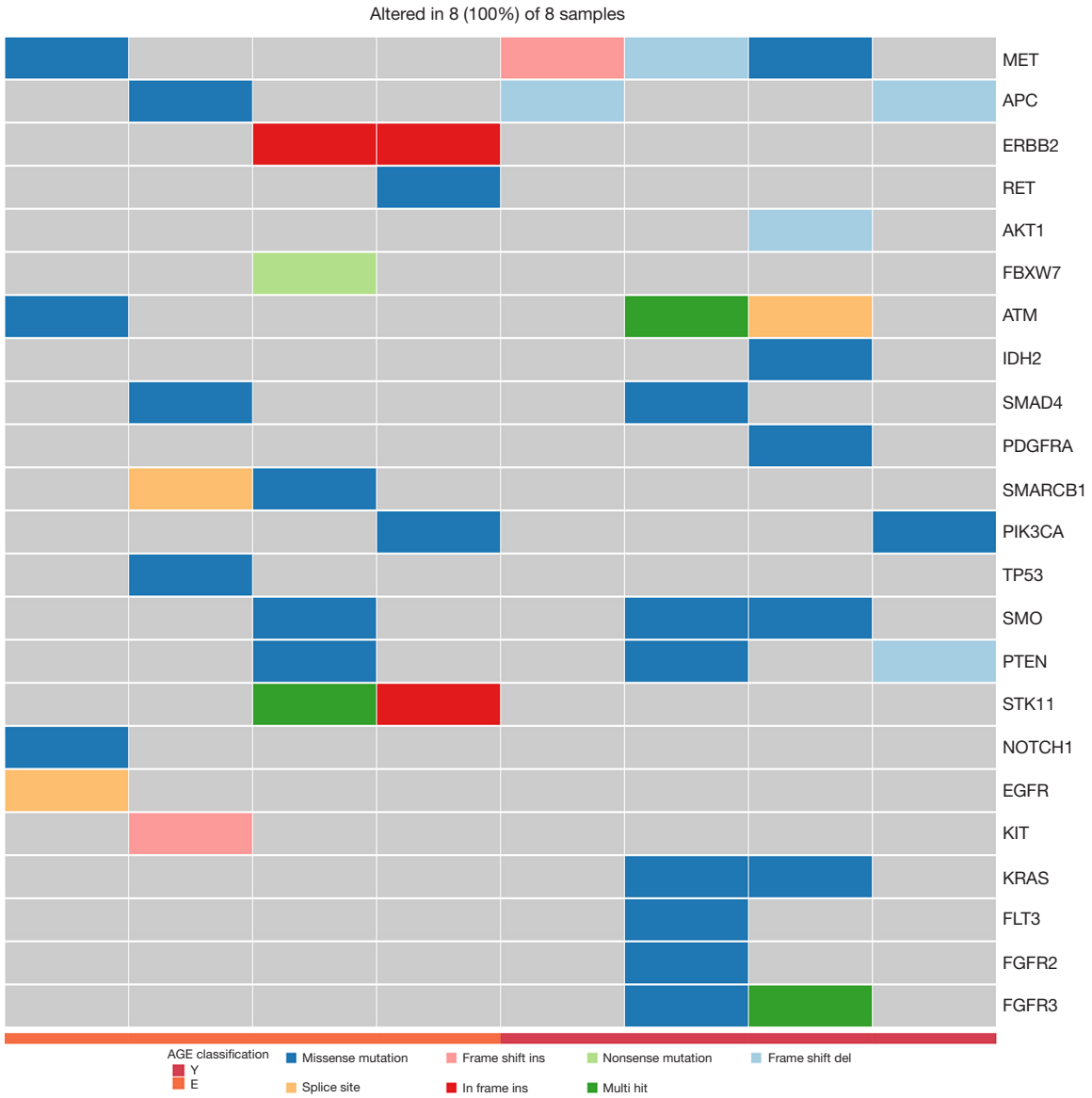

Figure 3 Distribution of 50 cancer-associated genes aberrances in EGC and YGC groups from a Chinese cohort using targeted next-generation sequencing. Subgroups were defined as EGC group (orange block, n=4) and YGC group (red block, n=4). EGC, early gastric cancer; YGC, young gastric cancer.

mutations, and in *ALK*, *FBXW7*, *GNA11*, *PTPN11*, and *FGFR3*; the YGC subgroup (n=4, <40 years old) had 13 missense mutations, and only in *CDH1*, *FGFR2*, *CTNNB1*, and *ATM*. These alterations will affect many genes, and these affected genes will become the main carcinogens, including several therapeutic targets, such as *ERBB2*, *FGFR2*, and *MET*.

Discussion

A controversial issue emerged when the prognosis of YGC patients was mentioned, and it is generally believed that the EGC population has a better prognosis (11). However, it has been reported that compared with EGC, YGC has a better prognosis, which may be related to the better overall

physical condition of young people. After all, the prognosis of the elderly may be affected by other common diseases of the elderly. In this comparative study, we find that the YGC group harbored more variation in 50 cancer-related genes than EGC using targeted NGS, while not in the TCGA-STAD database using WES; this may be the result of more reads and the depth of targeted NGS compared to WES. The detection of some mutations in YGC will further indicate the clinical benefit of YGC using target therapy. Indeed, due to the inclusion of additional mutations, such as those of *KRAS* and *FGFR2*, more YGCs were identified by the 50 cancer-related genes panel to carry at least one-point mutation.

This study had some limitations. First, the sample size of the research cohort was small and, therefore, the results should be interpreted carefully. Second, our cohort had no objective response or even progression-free survival analysis for molecularly targeted therapy, which increased the bias of the study. Third, the frequency of other 50 cancer-related genes mutations were not explored, which meant that some useful information was missing. Finally, only patients who was young or elderly were involved in this study and its analysis, leading to population bias.

Conclusions

In summary, by using PCR-based targeted NGS mutation detection, the present study demonstrated a higher proportion of 50 cancer-related genes mutation in YGC participants than EGC participants in our cohort. Through investigation of TMB's role in the predication of clinical response of checkpoint inhibitor therapy, a higher TMB level was revealed in EGC than YGC patients from TCGA-STAD database. The comparation of mutational characteristics in YGC and EGC will deepen the understanding of GC development and therapy for precision medicine.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-934/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-934/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-21-934/coif). All authors report they received technical support from Shanghai Tongshu Biotechnology Co., Ltd. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethics Committee of Zhongshan Hospital of Xiamen University (No. MULAC20180085), and performed in accordance with the principles of the Declaration of Helsinki (as revised in 2013). Informed consent was taken from all the patients.

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Supplementary

Table S1 50 cancer-related gene list in this study

Symbol	Approved name	HGNC ID	Location
ALK	ALK receptor tyrosine kinase	427	2p23.2-p23.1
HRAS	HRas proto-oncogene, GTPase	5173	11p15.5
MET	MET proto-oncogene, receptor tyrosine kinase	7029	7q31
KDR	kinase insert domain receptor	6307	4q12
APC	APC regulator of WNT signaling pathway	583	5q22.2
JAK2	Janus kinase 2	6192	9p24.1
ERBB2	erb-b2 receptor tyrosine kinase 2	3430	17q12
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	11283	20q11.23
JAK3	Janus kinase 3	6193	19p13.11
ERBB4	erb-b2 receptor tyrosine kinase 4	3432	2q34
RET	ret proto-oncogene	9967	10q11.21
AKT1	AKT serine/threonine kinase 1	391	14q32.33
GNAQ	G protein subunit alpha q	4390	9q21.2
FBXW7	F-box and WD repeat domain containing 7	16712	4q31.3
GNAS	GNAS complex locus	4392	20q13.32
IDH1	isocitrate dehydrogenase (NADP(+)) 1, cytosolic	5382	2q34
ATM	ATM serine/threonine kinase	795	11q22.3
IDH2	isocitrate dehydrogenase (NADP(+)) 2, mitochondrial	5383	15q26.1
SMAD4	SMAD family member 4	6770	18q21.2
NRAS	NRAS proto-oncogene, GTPase	7989	1p13.2
RB1	RB transcriptional corepressor 1	9884	13q14.2
MLH1	mutL homolog 1	7127	3p22.2
PDGFRA	platelet derived growth factor receptor alpha	8803	4q12
SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1	11103	22q11.23
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	8975	3q26.32
TP53	tumor protein p53	11998	17p13.1
SMO	smoothened, frizzled class receptor	11119	7q32.1
VHL	von Hippel-Lindau tumor suppressor	12687	3p25.3
PTEN	phosphatase and tensin homolog	9588	10q23.31
STK11	serine/threonine kinase 11	11389	19p13.3
NOTCH1	notch receptor 1	7881	9q34.3
GNA11	G protein subunit alpha 11	4379	19p13.3
CTNNB1	catenin beta 1	2514	3p22.1
EZH2	enhancer of zeste 2 polycomb repressive complex 2 subunit	3527	7q36.1

Table S1 *(continued)*

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Table S1 *(continued)*

Symbol	Approved name	HGNC ID	Location
BRAF	B-Raf proto-oncogene, serine/threonine kinase	1097	7q34
EGFR	epidermal growth factor receptor	3236	7p11.2
PTPN11	protein tyrosine phosphatase non-receptor type 11	9644	12q24.13
KIT	KIT proto-oncogene, receptor tyrosine kinase	6342	4q12
KRAS	KRAS proto-oncogene, GTPase	6407	12p12.1
CDKN2A	cyclin dependent kinase inhibitor 2A	1787	9p21.3
MPL	MPL proto-oncogene, thrombopoietin receptor	7217	1p34.2
FLT3	fms related tyrosine kinase 3	3765	13q12.2
NPM1	nucleophosmin 1	7910	5q35.1
FGFR1	fibroblast growth factor receptor 1	3688	8p11.23
FGFR2	fibroblast growth factor receptor 2	3689	10q26.13
FGFR3	fibroblast growth factor receptor 3	3690	4p16.3
CSF1R	colony stimulating factor 1 receptor	2433	5q32
CDH1	cadherin 1	1748	16q22.1
ABL1	ABL proto-oncogene 1, non-receptor tyrosine kinase	76	9q34.12
HNF1A	HNF1 homeobox A	11621	12q24.31

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