Original Article

Highly expressed Claudin18.2 as a potential therapeutic target in advanced gastric signet-ring cell carcinoma (SRCC)

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Background: Advanced gastric signet-ring cell carcinoma (SRCC) is a specific type of malignant gastric cancer (GC) with distinct poorer survival. Claudin18.2 (CLDN18.2) is a promising neo-biomarker for the treatment of GC. Clinical trials of CLDN18.2-targeted antibody and T cell-based immunotherapy providing promising prospects for the treatment of GC. The effect of antibody therapy depended on the expression rate of CLDN18.2 has been found in clinical trials. This study aimed to determine the prevalence and the therapeutic value of CLDN18.2 in advanced gastric SRCC.

Methods: Expression of CLDN18.2 in 105 formalin-fixed, paraffin-embedded (FFPE) tumor tissues was detected by immunohistochemistry (IHC) and evaluated according to FAST criteria. Next-generation sequencing (NGS) using 416 pan-cancer genes panel was performed to characterize the genomic landscape in 61 advanced gastric SRCC patients. Fisher's exact test was used to determine gene differences in different CLDN18.2 expression levels.

Results: A total number of 105 advanced gastric SRCC samples were analyzed, of which 95.2% (100/105) were positive stained. Moderate-to-strong CLDN18.2 expression was observed in 64.8% (68/105) of all samples. In particularly, 21.0% (22/105) samples had positive staining in more than 90% tumor cells. No significance was found between CLDN18.2 expression and overall survival (OS). NGS results showed that single nucleotide variations (SNVs) could be frequently found in TP53 (26.2%), CDH1 (19.7%), MED12 (18.0%), PKHD1 (18.0%) and ARID1A (11.5%), besides, copy number variations (CNVs) were rich in NOTCH1 (18.0%) and FLT4 (9.8%) in SRCC samples. Moreover, SNVs in GRIN2A was found in 20% of the patients who had CLDN18.2 staining in <40% of tumor cells (P=0.043), indicating CLDN18.2 expression might be related to the aberration of GRIN2A in advanced gastric SRCC.

Conclusions: The highly expressed CLDN18.2 among advanced gastric SRCC patients that we found certified the value of CLDN18.2-targeted therapy in this specific type of GC. In addition, Analyses between CLDN18.2 expression and genetic abnormalities provided novel therapeutic options for advanced gastric SRCC.

Keywords: Gastric signet-ring cell carcinoma (gastric SRCC); CLDN18.2; next-generation sequencing (NGS)

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Introduction

Gastric signet-ring cell carcinoma (SRCC) is an exceptional subtype of gastric adenocarcinoma which has abundant intracellular mucin accumulation and the crescent-shaped nucleus are displaced toward one side of the tumor cells (1). According to World Health Organization (WHO) classification: gastric cancer (GC) pathologic specimen with at least 50% of signet-ring cell is defined as SRCC. In recent decades, the overall morbidity of GC decreased worldwide, however, the decline is mainly caused by the decrease of intestinal type, while for diffuse type, especially gastric SRCC, is on the rise (2-4). Usually, SRCC is more prone to invasion and metastasis and have unique chemosensitivities, which led to a worse prognosis (3,5,6). However, SRCC treatment regimen are still controversial as SRCC is not specifically identified in most studies. For diffuse type GC, targeted therapies including anti-HER2 (7) and anti-VEGFR2 (8) provide few benefit in overall survival (OS). Immunotherapy may be a promising treatment as PD-L1 is overexpressed in about 23% of gastric SRCC (5).

CLDN18.2 is a splice variant of the membrane epithelial tight junctions protein Claudin18 (CLDN18) and has been identified as a promising biomarker for targeted therapy (9). CLDN18.2 has a restricted expression profile in normal tissues, it physiologically expresses only in the tight junction supramolecular complex of gastric mucosa. While upon malignant transformation, the changes in cell polarity lead to the exposure of CLDN18.2 epitopes (10), which are suitable for targeted therapy. Prior evidence demonstrates that except for GC, CLDN18.2 is aberrantly expressed in various primary tumors and metastases, including pancreatic, biliary, ovarian, and lung adenocarcinomas (11-14), which makes CLDN18.2 a pan-cancer target. At present, antibody [zolbetuximab (10,15-18), formerly called IMAB362] and chimeric antigen receptor engineered T cells (CAR-T) (19,20) targeting CLDN18.2 has been applied in clinical trials with promising results achieved. Correlation between higher CLDN18.2 expression and better therapeutic benefits has been found through antibody-based clinical trials. Previous studies have reported a relatively high expression rate of CLDN18.2 in diffuse type GC (9,21), however, no study on advanced gastric SRCC has been conducted. Therefore, this study aimed to establish the prevalence of CLDN18.2 expression in advanced gastric SRCC, and to determine the therapeutic value of CLDN18.2 in this specific type of GC.

In this study, we identified, for the first time, that the expression rate of CLDN18.2 in advanced gastric SRCC patients was relatively high. The survival analyses based on CLDN18.2 expression were performed. We also conducted next-generation sequencing (NGS) in 61 advanced gastric SRCC samples, genetic abnormalities in advanced gastric SRCC were profiled and the relation between CLDN18.2 expression and GRIN2A mutation was discovered.

We present the following article in accordance with the MDAR reporting checklist. (available at: http://dx.doi.org/10.21037/jgo-20-344).

Methods

Tissue specimens and ethical statement

A total of 105 formalin-fixed, paraffin-embedded (FFPE) tissue specimens with histology of advanced gastric SRCC for the testing of CLDN18.2 expression were collected at Nanjing Drum Tower Hospital. Patients at stage III were all administrated first-line 5-FU-based adjuvant chemotherapy after D2 gastrectomy, while patients at stage IV were treated by first-line 5-FU-based palliative chemotherapy. None of the patients had radiotherapy, chemotherapy or other medical intervention before specimen collection. Samples from stage III patients were curative surgical specimens, while samples from stage IV patients were palliative surgical specimens or gastroscope specimens. OS data was available in 86 cases; 61 of the tissues were selected for NGS.

Immunohistochemistry (IHC) and histologic assessment

All tissue samples were stained using rabbit monoclonal anti-CLDN18.2 antibody (Abcam, 222512) at 1/800 dilution. This antibody was designed to recognize human CLDN18.2 aa 1–100. After diluted antibody was added to the whole tissue surface, the slides were incubated at 4 ℃ overnight. Slides were then rewarmed at room temperature for 10 minutes and sufficiently washed with PBS for three times. Goat Anti-Rabbit IgG H&L (HRP) (Abcam, 205718) were used as secondary antibody, when the slides were dry, secondary antibody were dropped to cover the whole tissue and incubate in a 37 ℃ incubator for 30 minutes. Nuclear were stained with hematoxylin; 3% hydrogen peroxide were used to block endogenous peroxidases. Each patient's slides were tested twice.

To determine CLDN18.2 expression status, tissue samples were analyzed according to the intensity of staining and the percentage of stained tumor cells. The intensity

was classified into 4 grades: no membrane or cytoplasmic reactivity as 0, weak membrane or cytoplasmic reactivity as 1+, moderate membrane or cytoplasmic reactivity as 2+, and strong membrane or cytoplasmic reactivity as 3+. Samples showing any specific staining with ≥1+ intensity were defined as CLDN18.2 positive. According to FAST criterion, more than 40% of tumor tissues specific staining with ≥2+ intensity were defined as moderate-to-strong expression (21). Percentage of overall CLDN18.2 positive cells was considered by the estimated number of CLDN18.2 positive cells divided by the estimated overall number of tumor cells in each sample.

The pathological diagnosis of SRCC in our study were confirmed by two independent pathologists. The interpretation of IHC results were performed according to the FAST criterion.

NGS and data processing

61 out of 105 samples which had enough tumor tissue were selected for NGS. 15 had CLDN18.2 positive staining in <40% of tumor cells, 46 had CLDN18.2 positive staining in ≥40% of tumor cells. Genomic DNA from FFPE tissue specimens were extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, Catalog no.56404) according to the manufacturer's protocols. Hybridization-based enrichment was carried out with GeneseeqOne^{TM} pan-cancer gene panel (416 cancer-relevant genes). Captured libraries by Dynabeads M-270 (Life Technologies, MA, USA) were amplified in KAPA HiFi HotStart ReadyMix (KAPA Biosystems, MA, USA) and quantified by qPCR using the KAPA Library Quantification kit (KAPA Biosystems, MA, USA) for sequencing. The libraries were paired-end sequenced on Illumina HiSeq4000 NGS platforms (Illumina, CA, USA) according to the manufacturer's instructions. VarScan2 was employed for the detection of single nucleotide variations (SNVs). Copy number variations (CNVs) were detected by ADTEx.

Statistical analysis

SPSS was used for all statistical analysis. GraphPad Prism 7.0 (GraphPad Software) was used for presenting the statistical result graphs. Survival analysis were obtained using the Kaplan-Meier method and compared with the log-rank test. Fisher's exact test were used to determine NGS results. A P value of less than 0.05 was considered significant.

Ethical statement

The study was approved by the Ethics Committee of Nanjing Drum Tower Hospital (No. 2016-196-01). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Because of the retrospective nature of the study, the requirement for informed consent was waived.

Results

CLDN18.2 highly expressed in advanced gastric SRCC

Tissue samples from 105 advanced gastric SRCC patients were collected and stained with anti-CLDN18.2 antibody. Positive staining was detected in 95.2% (100/105) of all SRCC tumor tissues, which is much higher than general gastric adenocarcinoma (77%) (9). In accordance with the FAST clinical trial (NCT01630083), ≥2+ membrane staining intensity in ≥40% tumor cells was defined as moderate-to-strong CLDN18.2 expression. In our study, moderate-to-strong CLDN18.2 expression was observed in 64.8% (68/105) of all samples (*Table 1*). In particularly, 21.0% (22/105) samples had CLDN18.2 staining in ≥90% tumor cells (*Table 1*). There is no connection of CLDN18.2 expression with gender or stage (*Table 1* and Table S1). However, staining intensity ≥2+ in ≥90% of tumor cells often occurred in younger patients (<60 years old) (P=0.042).

Micrographs of representative stained tissues were shown in *Figure 1A* according to the 0–3+ staining intensity classification, of all samples, 5 (4.8%) were 0+, 22 (21.0%) were 1+, 33 (31.4%) were 2+ and 45 (42.9%) were 3+. The percentage distributions of overall CLDN18.2 positive cells in the 105 samples were as follows: 24 (22.9%) had 0–25% CLDN18.2 positive tumor cells; 21 (20.0%) had 26–50%; 16 (15.2%) had 51–75% and 44 (41.9%) had 76–100% (*Figure 1B*). The increased proportion of 3+ samples were observed as the percentage level went up (*Figure 1B*), which indicated that there might be a positive correlation between staining intensity and the percentage of positive cells.

Relationship of OS and CLDN18.2 expression in advanced gastric SRCC patients

OS was analyzed by the Kaplan-Meier method in 86 cases whose survival time were available. Different grouping patterns were set up according to previous studies (9,21) by different percentage of overall CLDN18.2 positive cells. The correlation between OS and CLDN18.2 expression

Table 1 CLDN18.2 expression in advanced gastric SRCC

Factors	Total cases (%)	Staining intensity ≥2+ in ≥40% of cells		Staining intensity ≥2+ in ≥90% of cells	
		N (%)	P value	N (%)	P value
All samples	105 (100.0)	68 (64.8)	–	22 (21.0)	–
Gender					
Male	71 (67.6)	50 (70.4)	0.079	17 (23.9)	0.276
Female	34 (32.4)	18 (52.9)		5 (14.7)	
Age					
≥60	38 (36.2)	22 (57.9)	0.267	4 (10.5)	0.042*
<60	67 (63.8)	46 (68.7)		18 (26.9)	
Stage					
III	92 (87.6)	59 (64.1)	0.719	19 (20.7)	0.841
IV	13 (12.4)	9 (69.2)		3 (23.1)	

*, represent for P<0.05. SRCC, signet-ring cell carcinoma.

were shown in *Figure 2*. There were no significant differences of OS by different CLDN18.2 expression levels in advanced gastric SRCC (log-rank test). This result indicated that CLDN18.2 expression was not a prognostic risk factor in advanced gastric SRCC patients.

GRIN2A mutation was related to CLDN18.2 expression

To explore the relation between CLDN18.2 expression and genetic abnormalities, 61 specimens were examined using a gene panel that covers entire exons in 416 cancer-relevant genes (Table S2). Genetic aberrations identified in all 61 samples revealed several common SNVs and CNVs in advanced gastric SRCC (Table S3 and Table S4). Briefly, the top 5 genes with the highest SNVs rate were *TP53* (26.2%), *CDH1* (19.7%), *MED12* (18.0%), *PKHD1* (18.0%) and *ARID1A* (11.5%). The top 2 genes with the highest CNVs rate were *NOTCH1* (18.0%) and *FLT4* (9.8%). Furthermore, specimens for NGS were divided into two groups based on CLDN18.2 expression. Of them, 15 were CLDN18.2 expressed in <40% of tumor cells, 46 were CLDN18.2 expressed in ⩾ 40% of tumor cells. After the analyses of genetic aberrations in these two groups, one gene named *GRIN2A* were found significantly different between the two groups (*Figure 3*, Table S5 and Table S6). Three (20.0%) of the 15 samples that had CLDN18.2 expression in <40% of tumor cells harboring GRIN2A mutation, while only 1 (2.2%) of the 46 moderate-to-strong CLDN18.2 expression samples had GRIN2A mutation (P=0.043), indicating GRIN2A variation was more likely to occur in patients who had lower CLDN18.2 staining. Since anti-CLDN18.2 antibody was not effective enough in CLDN18.2 low expression patients, mutant GRIN2A might be a potential therapeutic target in those patients. Other detected SNVs (MED12, TOP2A, EZH2, RNF43, WRN, STAG2, CDK6, CYLD and GATA6) or CNVs (CDK12, CCNE1, ERBB3, FGFR4 and MED12) were not statistically significant (*Figure 3*, Table S5 and Table S6).

Discussion

In this study, we first tested CLDN18.2 expression in 105 FFPE tumor tissues from advanced SRCC patients by IHC method. The results demonstrated that CLDN18.2 was highly expressed in gastric SRCC, which made a promising prospect for the clinical use of zolbetuximab in advanced gastric SRCC. Survival analyses based on different grouping patterns showed no significance between CLDN18.2 expression and OS. In addition, NGS found that GRIN2A mutation was related to CLDN18.2 expression level.

CLDN18.2 had been identified as a highly selective cell lineage marker since its expression in normal tissues was strictly confined to differentiated epithelial of the gastric mucosa. Besides, the retained expression of CLDN18.2 had been found in a significant proportion of primary GCs (any positive 77%, ≥2+ in ≥60% of cells 56%) and its metastases (lymph node 66%, ovarian 96%), which made CLDN18.2 become one of the most notable targets in GC studies (9).

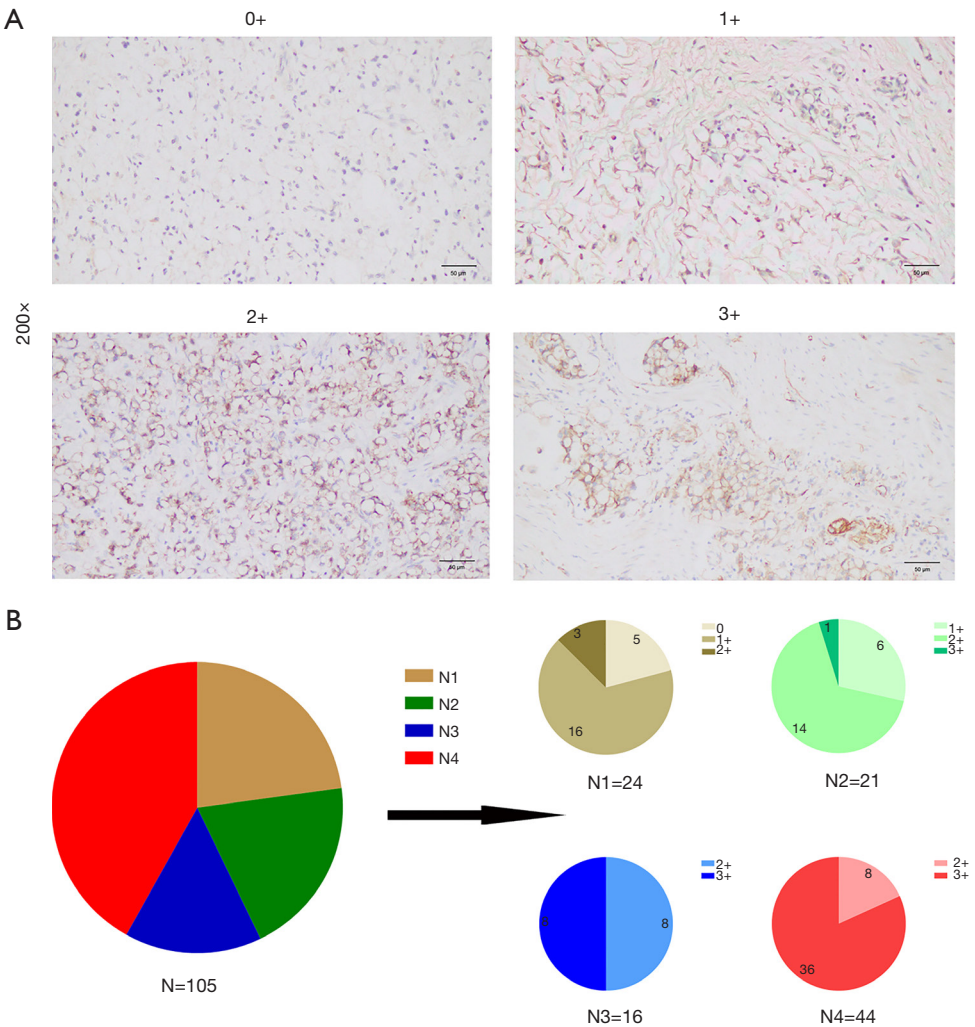

Figure 1 Expression of CLDN18.2 in advanced gastric SRCC. (A) Micrographs of representative stained tissues: 0+, 1+, 2+ and 3+ staining intensity. The magnification was 200×. (B) The graph depicted the distribution of CLDN18.2 staining percentages and intensities in tumor cells from advanced gastric SRCC patient samples. N, total number of cases; N1, number of cases that had 0–25% CLDN18.2 positive tumor cells; N2, number of cases that had 26–50% CLDN18.2 positive tumor cells; N3, number of cases that had 51–75% CLDN18.2 positive tumor cells; N4, number of cases that had 76–100% CLDN18.2 positive tumor cells. SRCC, signet-ring cell carcinoma.

Zolbetuximab (IMAB362) was a first-in-class monoclonal antibody specific targeted to CLDN18.2, recently, a multicentre phase II clinical study of zolbetuximab found that all responders had ≥70% CLDN18.2 expression in tumor cells, which suggested the correlation between higher CLDN18.2 expression and better therapeutic benefit (17). Besides, another phase II trial (FAST; NCT01630083) had shown that patients with ≥2+ membrane staining intensity in ≥40% tumor cells could get benefits from zolbetuximab therapy (15). In this study, high expression rate of CLDN18.2 in advanced gastric SRCC patients had been found for the first time. Significant difference of CLDN18.2 expression between primary GCs and advanced gastric SRCC (77% *vs.* 95.24%) hinted that CLDN18.2-based targeted therapy had great potential in the treatment of advanced gastric SRCC. Moreover, since PD-L1 overexpressed in gastric SRCC (5), antibodies targeting PD-1/PD-L1 combined with CLDN18.2 antibody zolbetuximab might be an effective treatment for advanced gastric SRCC.

In the survival analyses, 4 grouping patterns were set up according to the percentage of overall CLDN18.2 positive

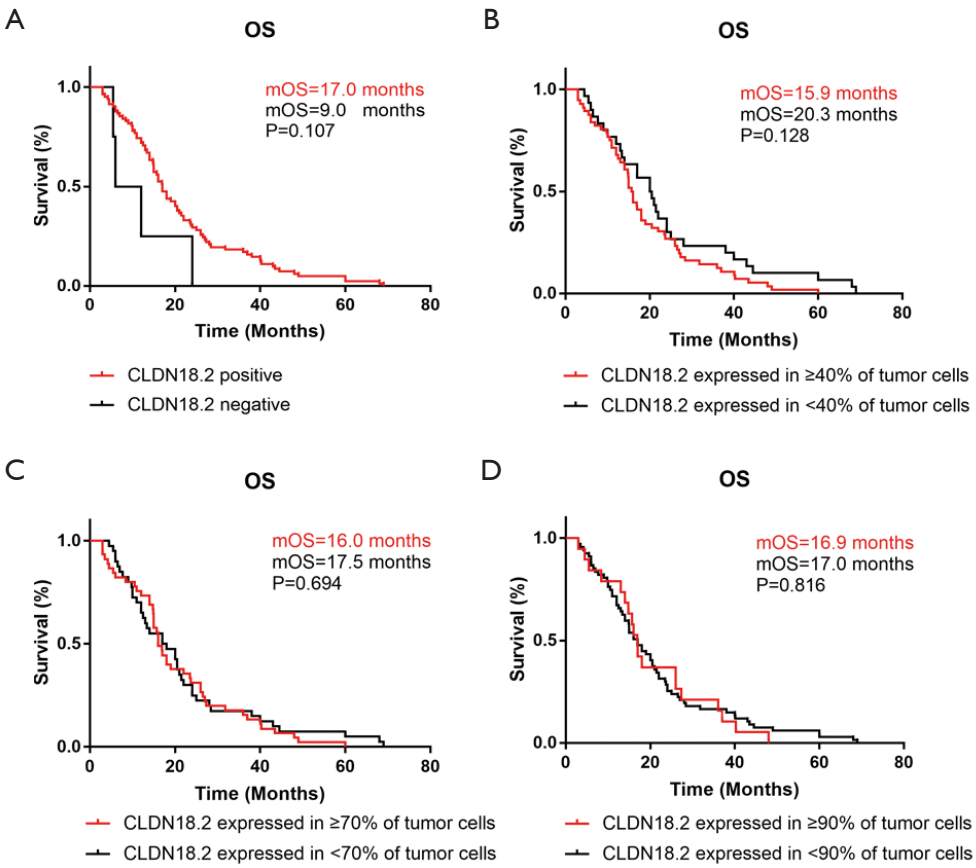

Figure 2 OS was irrelated with CLDN18.2 expression. (A) OS in CLDN18.2 positive patients and negative patients, median OS (mOS) was 17.0 months compared to 9.0 months, P=0.107. (B) mOS was 15.9 months in patients who had CLDN18.2 expression in ≥40% of tumor cells and 20.3 months in patients who had CLDN18.2 expression in <40% of tumor cells, P=0.128. (C) mOS was 16.0 months in patients who had CLDN18.2 expression in ≥70% of tumor cells and 17.5 months in patients who had CLDN18.2 expression in <70% of tumor cells, P=0.694. (D) mOS was 16.9 months in patients who had CLDN18.2 expression in ≥90% of tumor cells and 17.0 months in patients who had CLDN18.2 expression in <90% of tumor cells, P=0.816. OS, overall survival.

cells. However, no relationship had been found between OS and CLDN18.2 expression, which exactly indicated that the high expression of CLDN18.2 was irrelevant to a poor prognosis in advanced gastric SRCC. No survival analysis of CLDN18.2 expression in advanced gastric SRCC has been reported before, however, some previous studies on general GC suggested that as disease progressing, the expression of CLDN18.2 decreasing, which contributed to the increased invasive potential of the tumor cells (22,23). However, according to FAST clinical trial and other studies (9,21), no significant correlation between the expression of CLDN18.2 and the progression or prognosis of GC had been found, which consisted with our findings. According to TCGA database (Figure S1), no statistical difference in OS was discovered between CLDN18.2 high and low expression groups as well (P=0.14), however, disease free survival (DFS) was statistically significant in those two groups (P=0.0062), which indicating CLDN18.2 high expression might be related with the progression of the disease. Further studies were needed for clarifying the role of CLDN18.2 in the prognosis of GC.

NGS was performed to explore the genomic landscape for advanced gastric SRCC and to discover the correlated genes in different CLDN18.2 expression groups. Our results confirmed several common gene abnormalities in advanced gastric SRCC, such as *TP53*, *CDH1*, *MED12*, *PKHD1*, *ARID1A* and *NOTCH1*. We also found that CLDN18.2 expression might have some relation with

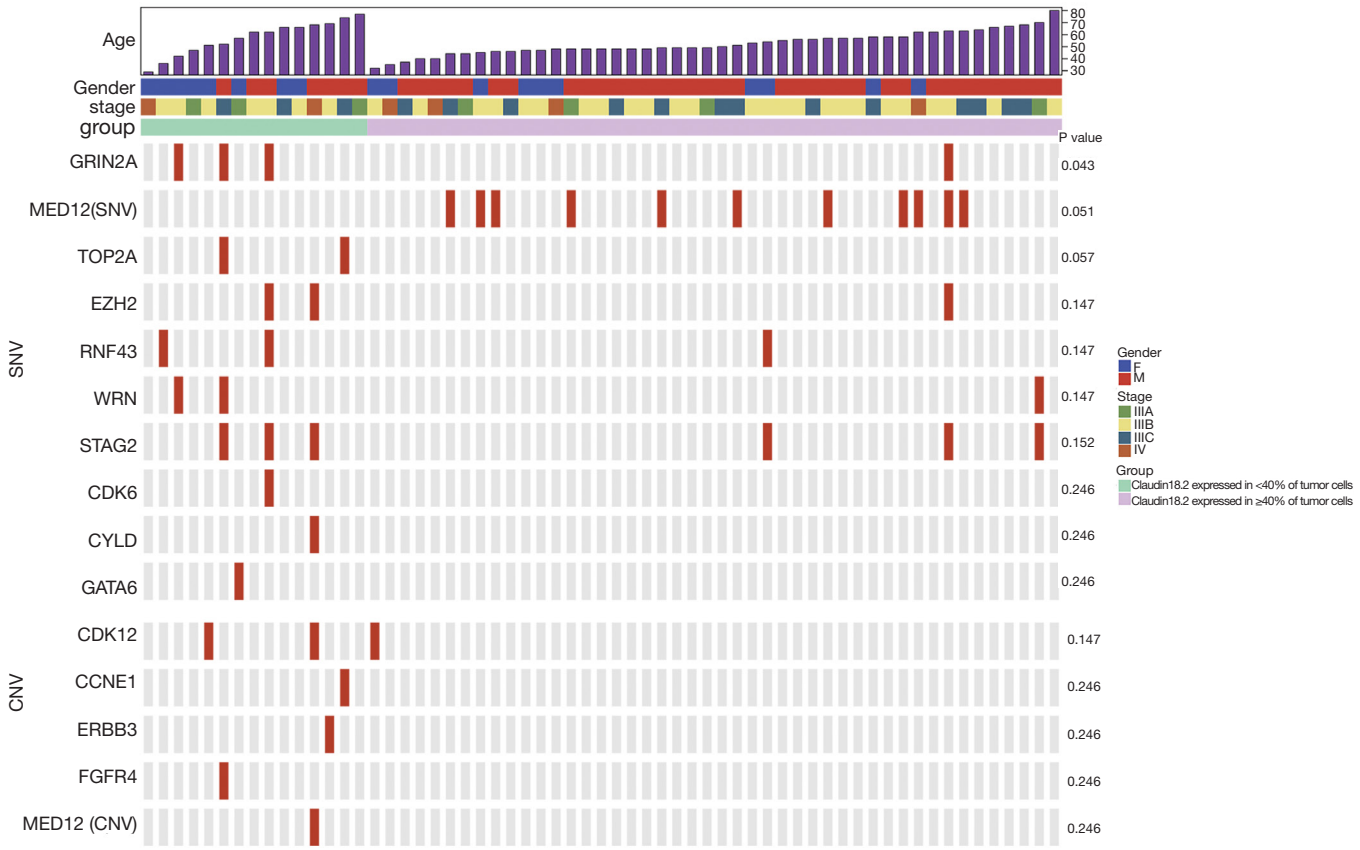

Figure 3 A representative genomic landscape in different CLDN18.2 expression levels. Each row represented one gene, and each column represented one sample. A P value of less than 0.05 was considered significant. SNV, single nucleotide variation; CNV, copy number variation.

GRIN2A mutation. *GRIN2A* was the encoding gene of GluN2A, which was a subunit of the N-methyl-D-aspartate receptor (NMDAR) (24), and participated in the transport of calcium ions. *GRIN2A* was one of the key genes in epilepsy researches, however, only a few researches had been done on GRIN2A in cancer field. The existing studies had shown that GRIN2A mutation was frequently found in melanoma (25) and induced the loss of tumor suppressor function (26,27). Besides, NMDAR signal had been found to be related with brain metastasis of breast cancer (28). Here, in our study, we found for the first time that GRIN2A mutation had some potential relation with CLDN18.2 expression in advanced gastric SRCC. GRIN2A mutation was enriched in patients with lower CLDN18.2 expression, indicating NMDAR inhibitors were probably useful in those patients. However, supporting evidence seems relatively weak due to the small sample size of GRIN2A mutation in our study, further and more comprehensive researches were needed to clarify the relevant mechanism.

In summary, the objective of this study was to assess the expression status of CLDN18.2 in advanced gastric SRCC patients, and to determine the therapeutic value of CLDN18.2 in this particular GC subtype. The high expression rate of CLDN18.2 in advanced gastric SRCC that we found provided the basic information for the CLDN18.2-based targeted therapy in advanced gastric SRCC patients. NGS results revealed the relation between GRIN2A mutation and CLDN18.2 expression, which provided possible innovative targeted therapy direction in advanced gastric SRCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at: http://dx.doi.org/10.21037/jgo-20-344). The authors have no 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. All experimental methods were carried out in accordance with the approved guidelines. This study was conducted with the approval of the Ethics Committee of Nanjing Drum Tower Hospital (No. 2016-196-01). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Because of the retrospective nature of the study, the requirement for informed consent was waived.

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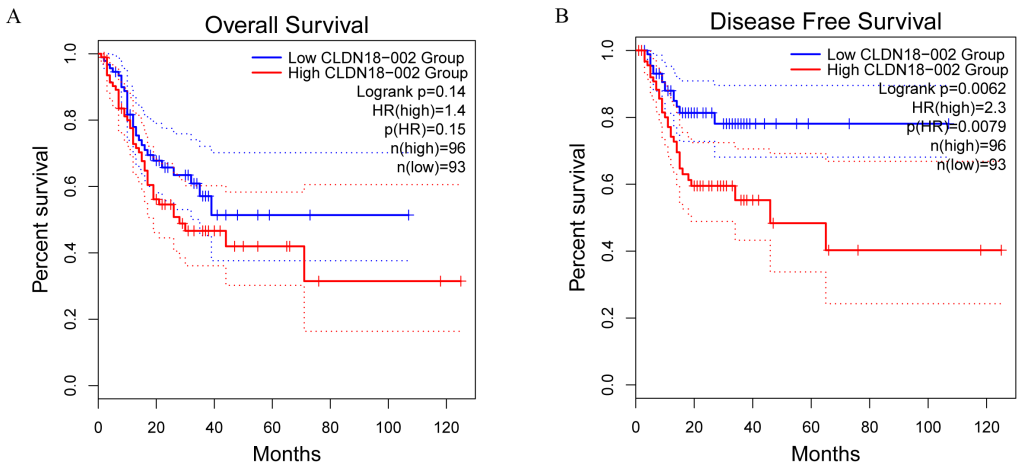

Figure S1 Relation of OS/DFS and CLDN18.2 expression in GC according to TCGA. (A) OS was irrelated with CLDN18.2 expression. (B) DFS was related with CLDN18.2 expression. HR, hazard ratio; n, number of cases.

Table S1 The relation between CLDN18.2 expression and TNM stage in stage III patients

Stage	TNM		Total cases (%)	CLDN18.2 expression			
				Staining intensity ≥2+ in ≥40% of cells (%)	P value	Staining intensity ≥2+ in ≥90% of cells (%)	P value
III	T	T3	62 (67.4)	40 (64.5)	0.674	15 (24.2)	0.108
		T4	30 (32.6)	18 (60.0)		3 (10.0)	
	N	N1	6 (6.5)	2 (33.3)	0.293	0 (0.0)	0.326
		N2	18 (19.6)	12 (66.7)		5 (27.8)	
		N3	68 (73.9)	44 (64.7)		13 (19.1)	

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Table S2 The summary information of the sequenced patients

Group	Patients number	SNVs	CNVs	Age	Gender	Stage
Claudin18.2 expressed in <40% of tumor cells	F1512239113	SF3B1	None	62	M	IIIB
	F1512239126	RAD50, CDK6, MDM2, STAG2, ROS1, PIK3CA, ALK, RHOA, PHOX2B, PKD1, GRIN2A, EZH2, JAK1, RNF43	TNFRSF14	62	M	IIIB
	F1512239103	PRSS3, TSC2, TOP2A, PKHD1, EPHA3, BRCA1, PDGFRA	CCNE1	74	M	IIIC
	F1512289348	PARP2, TUBB2B	None	29	F	IV
	F1512239125	MAP3K1, KDR, STAG2, AXL, CREBBP, CYLD, RRM1, EZH2	MED12, IKBKE, XPC, CDK12, FGFR1, CSF3, CASC3, GSDMA, CDC6, GSDMB, ORMDL3, LRRC3C, RAPGEFL1, MSL1, WIPF2, PSMD3, NR1D1, ZPBP2, RARA, MED24, GRB7, ERBB2	68	M	IV
	F1512239107	TP53	CDK12	51	F	IIIB
	F1512239097	BRIP1, WISP3, TOP2A, PKHD1, CDH1, STAG2, MTOR, ATRX, ROS1, ARID1A, KDM5A, TOP2A, FANCA, EPHA3, GRIN2A, TTF1, SMARCA4, NSD1, KDM5A, PALLD, EPCAM, PMS1, PCDH11Y, WRN	GATA2, ERCC2, FGFR4, NOTCH1, FLT4, HNF1A, CDKN2B, CDKN2A, RECQL4	52	M	IIIC
	F1512239098	NKX2-4, TP53	ERBB3, WT1	69	M	IIIB
	F1512289328	AXL, GATA6, IGF1R	None	57	F	IIIA
	F1512289347	MET, CYP2D6, GSTM5, ERBB2	None	66	F	IIIC
	F16020411111	GRM3	None	66	F	IIIB
	F16020411108	TP53, PKHD1, ATRX, ERBB3, TGFBR2, MLH3, ERBB2, RNF43, AKT1	NOTCH1, FLT4, RECQL4	36	F	IIIB
	F1512239112	CDH1, ARID1A	None	47	F	IIIA
	F1512289322	IL7R, DDR2, WRN, RICTOR, RUNX1, TP53, GRIN2A	None	42	F	IIIB
	F1512239111	FGFR2, PRDM12, RAD51, MPL, MUTYH, PCDH11X	None	77	M	IIIA
Claudin18.2 expressed in ≥40% of tumor cells	F1512239099	TUBB3, TP53, PKHD1, ERBB3	MUTYH, HNF1A, XPC, IKBKE	53	F	IIIB
	F1512289323	CDH1	CSF3, CASC3, GSDMA, CDC6, GSDMB, ORMDL3, LRRC3C, RAPGEFL1, MSL1, PSMD3, WIPF2, NR1D1, ZPBP2, RARA, MED24, GRB7, MIEN1, ERBB2, IKZF3	58	M	IIIB
	F1512239101	MED12, ADH1C	CDK6	44	M	IIIC
	F1512239104	TP53, ATRX, PTCH1, BRCA1, POLE, FLCN, SF3B1	None	56	M	IIIB
	F1512289326	TP53, PKHD1, TTF1, BLM, KIT, PIK3CA, EPAS1, RET, PDE11A, PARP1, KMT2B, FLCN, JAK2, CYP2B6, PRF1, MPL, RICTOR, ABCB4, CTCF, ZNF217, GNAQ, PRKACA, UGT1A1, UGT1A10, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9	None	55	M	IIIB
	F1512239110	None	FLT1, PAN3, FLT3, CCND1	56	M	IIIC
	F1512239114	PDGFRB, NOTCH2, BRCA2, JAK1, BARD1, PRDM12, PRSS3, GATA2, HGF, CYP2B6, ATM, FLT3, RARA, ERCC4	None	37	M	IIIC
	F1512239115	WAS, ASXL1, VHL, AKT2, PMS2, PKHD1, MED12, ARID1A, FANCD2, CHD4, TGFBR2, SMARCA4, SETD2, PDE11A, PARP1, CSF1R, SDHB, PARK2, TSC2, SMO, RHOA	None	57	M	IIIB
	F1512289330	MEGF9, WAS, UGT1A1, CDKN1C, MED12, TTF1, FGFR2, NF1, PAK3	WRN	49	M	IIIC
	F1512239116	TP53, PTEN	FGFR1	50	M	IIIC
	F1512289331	NF1, TUBB4A, ESR1, GSTP1, ATRX	None	58	F	IIIC
	F1512289332	MTOR, FANCA, PKD1, MED12, PKHD1, POLD1, FANCG, SGK1, NAT1, CDKN1A, NAT2, NBN, SRY, PTPN11, AKT1, FGFR4, THADA, NTRK1, BTK, ATM, RB1, IKBKE, ROS1, AXL, TEK, NOTCH2, MLH3, PTEN, SETD2, KDM5A, JAK1, PDGFRA, BARD1, CDK12, PDE11A, PMS1, POLE, POLH, ERBB2, SDHB, DPYD, JAK3, TSC2, AXIN2, ERCC2, RAC1, DENND1A, KDR	None	46	M	IIIB
	F1512239119	PKHD1, RRM1, PTPN11, STAT3, FANCF, GATA3, MET, IGF1R, PAK3, RB1, CYP2B6, JAK2, GATA2, JAK3, RAD51, PMS1, PARP1, PDE11A, PALB2, BARD1, PALLD, ERCC3, BRCA1, KDM5A, BRCA2, CREBBP, LHCGR, NSD1, PTCH1, APC, TET2, GRM3, SMARCA4, EZH2, NOTCH2, GNAS, NF1, TEK, KIT, EPCAM, FGFR2, BLM, IL7R, KMT2A, GRIN2A, PDGFRB, CDH1, MED12, KDR, PKD1, STAG2, FANCA, FAT1, AMER1, ROS1, ARID2, FANCD2, SETD2	TNFAIP3, EGFR	63	M	IIIB
	F1512289335	MED12, TEK	None	48	M	IIIA
	F1512289336	CHD4, PDK1, NRAS	None	48	M	IIIB
	F1512289337	TP53, KDR, PIK3CA, CDK12, FGFR3	NOTCH1, FLT4, HNF1A, RPTOR, FGFR3	66	M	IIIB
	F1512239123	PRDM12, TTF1, BLM, NF1	FLT4, RPTOR, GATA2, NOTCH1	68	M	IIIC
	F1512239127	PTPRD, CDH1	FGFR1, SMO, MYD88	48	M	IIIB
	F1512239128	TUBB4A, TSHR, CYP2D6	None	47	F	IIIB
	F1512239129	MED12, FANCD2, PCDH11Y	NOTCH1, BAP1	51	M	IIIC
	F1512289343	ATM, GNAS, BRCA1	CCND1	64	M	IIIC
	F1512289344	None	CDKN2B, CDKN2A, CDK6, CCND1	62	M	IIIB
	F1512239131	PIK3CA, RECQL4	MET	49	M	IIIB
	F1512289345	BCL2L11, KRAS, CDH1, MED12, AMER1	None	62	F	IV
	F1512289349	NTRK1, CDH1	PRDM1	48	M	IIIC
	F1512239134	None	NOTCH1, FLT4, HNF1A, RECQL4, RPTOR, SMO, BAP1, ERCC2, FLCN, FANCA, CREBBP, TSC2	48	M	IIIB
	F16020411109	MED12, STK11, XPC	NOTCH1	63	M	IIIC
	F1512239108	None	CDK12, MDM2	32	F	IIIB
	F1512289333	JAK1, DDR2, ABCB4, MYCN, MGMT, PDCD1, ERCC2, PAK3, QKI, RAD51, POLE, RET, FANCD2, PTCH1, BRCA2, APC, FGFR2, CHD4, BLM, PDGFRB, FAT1, ATRX, ARID2, AMER1, TOP1, FGFR1, NF2, TSC1, RAD51C, EGFR, TP53, PKHD1, CDH1, MED12, KDR, FANCA	CCND1	45	F	IIIB
	F1512239117	STK11, ALK, WRN, EPCAM, CDK12, PDGFRA, TET2, PTEN, EPAS1, PIK3CA, EPHA3, NF1, CHD4, PDGFRB, ARID2, FLT4, ARID1A, PIK3R1, AMER1, ATRX, FAT1, MTOR, STAG2, FGFR4, PKHD1, MSH6, MUTYH, BRIP1, PIK3C3, MYC, CYP2C19, PRDM1	CDKN2B, CDKN2A, NOTCH1	70	M	IIIA
	F1512239100	None	WT1	80	M	IIIB
	F1512239102	ROS1, ARID1A, CDKN1B	None	49	M	IIIB
	F1512289324	TP53	NOTCH1	40	M	IIIB
	F1512239106	MED12, GSTM5, ATRX, TOP1	WT1, TNFRSF14, EXT2	58	M	IIIB
	F1512239109	PRDM12	IKBKE, CDKN2B, CDKN2A	57	M	IIIB
	F1512289329	TP53, CDH1, ARID1A, ETV1, FLT4, PTCH1	NOTCH1	57	M	IIIB
	F1512239120	PRSS3	None	49	M	IIIA
	F1512239121	ARIH1	XPC	40	M	IV
	F1512239122	TP53, AXL, RHOA	None	44	M	IIIA
	F1512239124	RNF43, STAG2, TGFBR2, ALK, ERBB2	None	54	F	IIIB
	F1512289338	CDH1, ROS1, GNAS, ATR, SMAD4, PKD1, LHCGR	None	48	M	IIIB
	F1512239132	CDH1, LZTR1	None	67	M	IIIC
	F1512239133	ERBB3, TP53, HNF1A, PKHD1, EPAS1, BARD1	EXT2, TSC1, MITF, NSD1	46	M	IIIC
	F16020411106	ARID1A, ERBB3, TP53, GNAS, CREBBP, DNMT3A, EPHA3	KRAS	35	F	IV
	F1512239135	ERBB3, TP53, CDH1, PTEN, LHCGR, GSTM5, HDAC2, GATA1	None	47	F	IIIB
	F16020411107	RAD50, SOX21, ARAF	NOTCH1, FLT4, FGFR3, RECQL4, TNFRSF14	48	F	IV

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Table S3 Genes list and mutation rate of SNV

SNV_genes_list	SNV_number	SNV_rate
TP53	16	26.2%
CDH1	12	19.7%
MED12	11	18.0%
PKHD1	11	18.0%
ARID1A	7	11.5%
ATRX	7	11.5%
STAG2	6	9.8%
ROS1	6	9.8%
NF1	5	8.2%
ERBB3	5	8.2%
KDR	5	8.2%
PIK3CA	5	8.2%
GRIN2A	4	6.6%
AXL	4	6.6%
EPHA3	4	6.6%
ERBB2	4	6.6%
AMER1	4	6.6%
BARD1	4	6.6%
BLM	4	6.6%
CHD4	4	6.6%
FANCD2	4	6.6%
GNAS	4	6.6%
PDE11A	4	6.6%
PDGFRB	4	6.6%
PTCH1	4	6.6%
PTEN	4	6.6%
BRCA1	4	6.6%
FANCA	4	6.6%
FGFR2	4	6.6%
JAK1	4	6.6%
PKD1	4	6.6%
PRDM12	4	6.6%
TTF1	4	6.6%
EZH2	3	4.9%
RNF43	3	4.9%
WRN	3	4.9%
ARID2	3	4.9%
ATM	3	4.9%
BRCA2	3	4.9%
CDK12	3	4.9%
CYP2B6	3	4.9%
EPAS1	3	4.9%
FAT1	3	4.9%
LHCGR	3	4.9%
NOTCH2	3	4.9%
PAK3	3	4.9%
PARP1	3	4.9%
POLE	3	4.9%
SETD2	3	4.9%
TEK	3	4.9%
ALK	3	4.9%
CREBBP	3	4.9%
EPCAM	3	4.9%
GSTM5	3	4.9%
KDM5A	3	4.9%
MTOR	3	4.9%
PDGFRA	3	4.9%
PMS1	3	4.9%
PRSS3	3	4.9%
RAD51	3	4.9%
RHOA	3	4.9%
SMARCA4	3	4.9%
TGFBR2	3	4.9%
TSC2	3	4.9%
TOP2A	2	3.3%
AKT1	2	3.3%
BRIP1	2	3.3%
CYP2D6	2	3.3%
DDR2	2	3.3%
GRM3	2	3.3%
IGF1R	2	3.3%
IL7R	2	3.3%
MET	2	3.3%
MLH3	2	3.3%
MPL	2	3.3%
MUTYH	2	3.3%
NSD1	2	3.3%
PALLD	2	3.3%
PCDH11Y	2	3.3%
RAD50	2	3.3%
RICTOR	2	3.3%
RRM1	2	3.3%
SF3B1	2	3.3%
ABCB4	2	3.3%
APC	2	3.3%
ERCC2	2	3.3%
FGFR4	2	3.3%
FLCN	2	3.3%
FLT4	2	3.3%
GATA2	2	3.3%
JAK2	2	3.3%
JAK3	2	3.3%
KIT	2	3.3%
NTRK1	2	3.3%
PTPN11	2	3.3%
RB1	2	3.3%
RET	2	3.3%
SDHB	2	3.3%
STK11	2	3.3%
TET2	2	3.3%
TOP1	2	3.3%
TUBB4A	2	3.3%
UGT1A1	2	3.3%
WAS	2	3.3%
CDK6	1	1.6%
CYLD	1	1.6%
GATA6	1	1.6%
MAP3K1	1	1.6%
MDM2	1	1.6%
NKX2-4	1	1.6%
PARP2	1	1.6%
PCDH11X	1	1.6%
PHOX2B	1	1.6%
RUNX1	1	1.6%
TUBB2B	1	1.6%
WISP3	1	1.6%
ADH1C	1	1.6%
AKT2	1	1.6%
ARAF	1	1.6%
ARIH1	1	1.6%
ASXL1	1	1.6%
ATR	1	1.6%
AXIN2	1	1.6%
BCL2L11	1	1.6%
BTK	1	1.6%
CDKN1A	1	1.6%
CDKN1B	1	1.6%
CDKN1C	1	1.6%
CSF1R	1	1.6%
CTCF	1	1.6%
CYP2C19	1	1.6%
DENND1A	1	1.6%
DNMT3A	1	1.6%
DPYD	1	1.6%
EGFR	1	1.6%
ERCC3	1	1.6%
ERCC4	1	1.6%
ESR1	1	1.6%
ETV1	1	1.6%
FANCF	1	1.6%
FANCG	1	1.6%
FGFR1	1	1.6%
FGFR3	1	1.6%
FLT3	1	1.6%
GATA1	1	1.6%
GATA3	1	1.6%
GNAQ	1	1.6%
GSTP1	1	1.6%
HDAC2	1	1.6%
HGF	1	1.6%
HNF1A	1	1.6%
IKBKE	1	1.6%
KMT2A	1	1.6%
KMT2B	1	1.6%
KRAS	1	1.6%
LZTR1	1	1.6%
MEGF9	1	1.6%
MGMT	1	1.6%
MSH6	1	1.6%
MYC	1	1.6%
MYCN	1	1.6%
NAT1	1	1.6%
NAT2	1	1.6%
NBN	1	1.6%
NF2	1	1.6%
NRAS	1	1.6%
PALB2	1	1.6%
PARK2	1	1.6%
PDCD1	1	1.6%
PDK1	1	1.6%
PIK3C3	1	1.6%
PIK3R1	1	1.6%
PMS2	1	1.6%
POLD1	1	1.6%
POLH	1	1.6%
PRDM1	1	1.6%
PRF1	1	1.6%
PRKACA	1	1.6%
PTPRD	1	1.6%
QKI	1	1.6%
RAC1	1	1.6%
RAD51C	1	1.6%
RARA	1	1.6%
RECQL4	1	1.6%
SGK1	1	1.6%
SMAD4	1	1.6%
SMO	1	1.6%
SOX21	1	1.6%
SRY	1	1.6%
STAT3	1	1.6%
THADA	1	1.6%
TSC1	1	1.6%
TSHR	1	1.6%
TUBB3	1	1.6%
UGT1A10	1	1.6%
UGT1A3	1	1.6%
UGT1A4	1	1.6%
UGT1A5	1	1.6%
UGT1A6	1	1.6%
UGT1A7	1	1.6%
UGT1A8	1	1.6%
UGT1A9	1	1.6%
VHL	1	1.6%
XPC	1	1.6%
ZNF217	1	1.6%

Table S4 Genes list and mutation rate of CNV

CNV_genes_list	CNV_number	CNV_rate
NOTCH1	11	18.0%
FLT4	6	9.8%
RECQL4	4	6.6%
CCND1	4	6.6%
CDKN2A	4	6.6%
CDKN2B	4	6.6%
HNF1A	4	6.6%
CDK12	3	4.9%
RPTOR	3	4.9%
FGFR1	3	4.9%
IKBKE	3	4.9%
TNFRSF14	3	4.9%
WT1	3	4.9%
XPC	3	4.9%
CASC3	2	3.3%
CDC6	2	3.3%
CSF3	2	3.3%
ERBB2	2	3.3%
ERCC2	2	3.3%
GATA2	2	3.3%
GRB7	2	3.3%
GSDMA	2	3.3%
GSDMB	2	3.3%
LRRC3C	2	3.3%
MED24	2	3.3%
MSL1	2	3.3%
NR1D1	2	3.3%
ORMDL3	2	3.3%
PSMD3	2	3.3%
RAPGEFL1	2	3.3%
RARA	2	3.3%
WIPF2	2	3.3%
ZPBP2	2	3.3%
BAP1	2	3.3%
CDK6	2	3.3%
EXT2	2	3.3%
FGFR3	2	3.3%
SMO	2	3.3%
CCNE1	1	1.6%
ERBB3	1	1.6%
FGFR4	1	1.6%
MED12	1	1.6%
CREBBP	1	1.6%
EGFR	1	1.6%
FANCA	1	1.6%
FLCN	1	1.6%
FLT1	1	1.6%
FLT3	1	1.6%
IKZF3	1	1.6%
KRAS	1	1.6%
MDM2	1	1.6%
MET	1	1.6%
MIEN1	1	1.6%
MITF	1	1.6%
MUTYH	1	1.6%
MYD88	1	1.6%
NSD1	1	1.6%
PAN3	1	1.6%
PRDM1	1	1.6%
TNFAIP3	1	1.6%
TSC1	1	1.6%
TSC2	1	1.6%
WRN	1	1.6%

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Table S5 Relation of SNVs and CLDN18.2 expression

All_genes_list	SNV_and_A	SNV_and_B	WT_and_A	WT_and_B	Fisher.test
GRIN2A	3	1	12	45	0.043
MED12	0	11	15	35	0.051
TOP2A	2	0	13	46	0.057
EZH2	2	1	13	45	0.147
RNF43	2	1	13	45	0.147
WRN	2	1	13	45	0.147
STAG2	3	3	12	43	0.152
CDK6	1	0	14	46	0.246
CYLD	1	0	14	46	0.246
GATA6	1	0	14	46	0.246
MAP3K1	1	0	14	46	0.246
MDM2	1	0	14	46	0.246
NKX2-4	1	0	14	46	0.246
PARP2	1	0	14	46	0.246
PCDH11X	1	0	14	46	0.246
PHOX2B	1	0	14	46	0.246
RUNX1	1	0	14	46	0.246
TUBB2B	1	0	14	46	0.246
WISP3	1	0	14	46	0.246
AXL	2	2	13	44	0.251
EPHA3	2	2	13	44	0.251
ERBB2	2	2	13	44	0.251
NF1	0	5	15	41	0.321
AKT1	1	1	14	45	0.434
BRIP1	1	1	14	45	0.434
CYP2D6	1	1	14	45	0.434
DDR2	1	1	14	45	0.434
GRM3	1	1	14	45	0.434
IGF1R	1	1	14	45	0.434
IL7R	1	1	14	45	0.434
MET	1	1	14	45	0.434
MLH3	1	1	14	45	0.434
MPL	1	1	14	45	0.434
MUTYH	1	1	14	45	0.434
NSD1	1	1	14	45	0.434
PALLD	1	1	14	45	0.434
PCDH11Y	1	1	14	45	0.434
RAD50	1	1	14	45	0.434
RICTOR	1	1	14	45	0.434
RRM1	1	1	14	45	0.434
SF3B1	1	1	14	45	0.434
AMER1	0	4	15	42	0.564
BARD1	0	4	15	42	0.564
BLM	0	4	15	42	0.564
CHD4	0	4	15	42	0.564
FANCD2	0	4	15	42	0.564
GNAS	0	4	15	42	0.564
PDE11A	0	4	15	42	0.564
PDGFRB	0	4	15	42	0.564
PTCH1	0	4	15	42	0.564
PTEN	0	4	15	42	0.564
ARID2	0	3	15	43	0.569
ATM	0	3	15	43	0.569
BRCA2	0	3	15	43	0.569
CDK12	0	3	15	43	0.569
CYP2B6	0	3	15	43	0.569
EPAS1	0	3	15	43	0.569
FAT1	0	3	15	43	0.569
LHCGR	0	3	15	43	0.569
NOTCH2	0	3	15	43	0.569
PAK3	0	3	15	43	0.569
PARP1	0	3	15	43	0.569
POLE	0	3	15	43	0.569
SETD2	0	3	15	43	0.569
TEK	0	3	15	43	0.569
ROS1	2	4	13	42	0.630
CDH1	2	10	13	36	0.712
ABCB4	0	2	15	44	1.000
ADH1C	0	1	15	45	1.000
AKT2	0	1	15	45	1.000
ALK	1	2	14	44	1.000
APC	0	2	15	44	1.000
ARAF	0	1	15	45	1.000
ARID1A	2	5	13	41	1.000
ARIH1	0	1	15	45	1.000
ASXL1	0	1	15	45	1.000
ATR	0	1	15	45	1.000
ATRX	2	5	13	41	1.000
AXIN2	0	1	15	45	1.000
BCL2L11	0	1	15	45	1.000
BRCA1	1	3	14	43	1.000
BTK	0	1	15	45	1.000
CDKN1A	0	1	15	45	1.000
CDKN1B	0	1	15	45	1.000
CDKN1C	0	1	15	45	1.000
CREBBP	1	2	14	44	1.000
CSF1R	0	1	15	45	1.000
CTCF	0	1	15	45	1.000
CYP2C19	0	1	15	45	1.000
DENND1A	0	1	15	45	1.000
DNMT3A	0	1	15	45	1.000
DPYD	0	1	15	45	1.000
EGFR	0	1	15	45	1.000
EPCAM	1	2	14	44	1.000
ERBB3	1	4	14	42	1.000
ERCC2	0	2	15	44	1.000
ERCC3	0	1	15	45	1.000
ERCC4	0	1	15	45	1.000
ESR1	0	1	15	45	1.000
ETV1	0	1	15	45	1.000
FANCA	1	3	14	43	1.000
FANCF	0	1	15	45	1.000
FANCG	0	1	15	45	1.000
FGFR1	0	1	15	45	1.000
FGFR2	1	3	14	43	1.000
FGFR3	0	1	15	45	1.000
FGFR4	0	2	15	44	1.000
FLCN	0	2	15	44	1.000
FLT3	0	1	15	45	1.000
FLT4	0	2	15	44	1.000
GATA1	0	1	15	45	1.000
GATA2	0	2	15	44	1.000
GATA3	0	1	15	45	1.000
GNAQ	0	1	15	45	1.000
GSTM5	1	2	14	44	1.000
GSTP1	0	1	15	45	1.000
HDAC2	0	1	15	45	1.000
HGF	0	1	15	45	1.000
HNF1A	0	1	15	45	1.000
IKBKE	0	1	15	45	1.000
JAK1	1	3	14	43	1.000
JAK2	0	2	15	44	1.000
JAK3	0	2	15	44	1.000
KDM5A	1	2	14	44	1.000
KDR	1	4	14	42	1.000
KIT	0	2	15	44	1.000
KMT2A	0	1	15	45	1.000
KMT2B	0	1	15	45	1.000
KRAS	0	1	15	45	1.000
LZTR1	0	1	15	45	1.000
MEGF9	0	1	15	45	1.000
MGMT	0	1	15	45	1.000
MSH6	0	1	15	45	1.000
MTOR	1	2	14	44	1.000
MYC	0	1	15	45	1.000
MYCN	0	1	15	45	1.000
NAT1	0	1	15	45	1.000
NAT2	0	1	15	45	1.000
NBN	0	1	15	45	1.000
NF2	0	1	15	45	1.000
NRAS	0	1	15	45	1.000
NTRK1	0	2	15	44	1.000
PALB2	0	1	15	45	1.000
PARK2	0	1	15	45	1.000
PDCD1	0	1	15	45	1.000
PDGFRA	1	2	14	44	1.000
PDK1	0	1	15	45	1.000
PIK3C3	0	1	15	45	1.000
PIK3CA	1	4	14	42	1.000
PIK3R1	0	1	15	45	1.000
PKD1	1	3	14	43	1.000
PKHD1	3	8	12	38	1.000
PMS1	1	2	14	44	1.000
PMS2	0	1	15	45	1.000
POLD1	0	1	15	45	1.000
POLH	0	1	15	45	1.000
PRDM1	0	1	15	45	1.000
PRDM12	1	3	14	43	1.000
PRF1	0	1	15	45	1.000
PRKACA	0	1	15	45	1.000
PRSS3	1	2	14	44	1.000
PTPN11	0	2	15	44	1.000
PTPRD	0	1	15	45	1.000
QKI	0	1	15	45	1.000
RAC1	0	1	15	45	1.000
RAD51	1	2	14	44	1.000
RAD51C	0	1	15	45	1.000
RARA	0	1	15	45	1.000
RB1	0	2	15	44	1.000
RECQL4	0	1	15	45	1.000
RET	0	2	15	44	1.000
RHOA	1	2	14	44	1.000
SDHB	0	2	15	44	1.000
SGK1	0	1	15	45	1.000
SMAD4	0	1	15	45	1.000
SMARCA4	1	2	14	44	1.000
SMO	0	1	15	45	1.000
SOX21	0	1	15	45	1.000
SRY	0	1	15	45	1.000
STAT3	0	1	15	45	1.000
STK11	0	2	15	44	1.000
TET2	0	2	15	44	1.000
TGFBR2	1	2	14	44	1.000
THADA	0	1	15	45	1.000
TOP1	0	2	15	44	1.000
TP53	4	12	11	34	1.000
TSC1	0	1	15	45	1.000
TSC2	1	2	14	44	1.000
TSHR	0	1	15	45	1.000
TTF1	1	3	14	43	1.000
TUBB3	0	1	15	45	1.000
TUBB4A	0	2	15	44	1.000
UGT1A1	0	2	15	44	1.000
UGT1A10	0	1	15	45	1.000
UGT1A3	0	1	15	45	1.000
UGT1A4	0	1	15	45	1.000
UGT1A5	0	1	15	45	1.000
UGT1A6	0	1	15	45	1.000
UGT1A7	0	1	15	45	1.000
UGT1A8	0	1	15	45	1.000
UGT1A9	0	1	15	45	1.000
VHL	0	1	15	45	1.000
WAS	0	2	15	44	1.000
XPC	0	1	15	45	1.000
ZNF217	0	1	15	45	1.000

A, CLDN18.2 expressed in <40% of tumor cells; B, CLDN18.2 expressed in ≥40% of tumor cells.

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Table S6 Relation of CNVs and CLDN18.2 expression

All_genes_list	CNV_and_A	CNV_and_B	WT_and_A	WT_and_B	Fisher.test
CDK12	2	1	13	45	0.147
CCNE1	1	0	14	46	0.246
ERBB3	1	0	14	46	0.246
FGFR4	1	0	14	46	0.246
MED12	1	0	14	46	0.246
RECQL4	2	2	13	44	0.251
CASC3	1	1	14	45	0.434
CDC6	1	1	14	45	0.434
CSF3	1	1	14	45	0.434
ERBB2	1	1	14	45	0.434
ERCC2	1	1	14	45	0.434
GATA2	1	1	14	45	0.434
GRB7	1	1	14	45	0.434
GSDMA	1	1	14	45	0.434
GSDMB	1	1	14	45	0.434
LRRC3C	1	1	14	45	0.434
MED24	1	1	14	45	0.434
MSL1	1	1	14	45	0.434
NR1D1	1	1	14	45	0.434
ORMDL3	1	1	14	45	0.434
PSMD3	1	1	14	45	0.434
RAPGEFL1	1	1	14	45	0.434
RARA	1	1	14	45	0.434
WIPF2	1	1	14	45	0.434
ZPBP2	1	1	14	45	0.434
CCND1	0	4	15	42	0.564
RPTOR	0	3	15	43	0.569
FLT4	2	4	13	42	0.630
NOTCH1	2	9	13	37	0.716
BAP1	0	2	15	44	1.000
CDK6	0	2	15	44	1.000
CDKN2A	1	3	14	43	1.000
CDKN2B	1	3	14	43	1.000
CREBBP	0	1	15	45	1.000
EGFR	0	1	15	45	1.000
EXT2	0	2	15	44	1.000
FANCA	0	1	15	45	1.000
FGFR1	1	2	14	44	1.000
FGFR3	0	2	15	44	1.000
FLCN	0	1	15	45	1.000
FLT1	0	1	15	45	1.000
FLT3	0	1	15	45	1.000
HNF1A	1	3	14	43	1.000
IKBKE	1	2	14	44	1.000
IKZF3	0	1	15	45	1.000
KRAS	0	1	15	45	1.000
MDM2	0	1	15	45	1.000
MET	0	1	15	45	1.000
MIEN1	0	1	15	45	1.000
MITF	0	1	15	45	1.000
MUTYH	0	1	15	45	1.000
MYD88	0	1	15	45	1.000
NSD1	0	1	15	45	1.000
PAN3	0	1	15	45	1.000
PRDM1	0	1	15	45	1.000
SMO	0	2	15	44	1.000
TNFAIP3	0	1	15	45	1.000
TNFRSF14	1	2	14	44	1.000
TSC1	0	1	15	45	1.000
TSC2	0	1	15	45	1.000
WRN	0	1	15	45	1.000
WT1	1	2	14	44	1.000
XPC	1	2	14	44	1.000

A, CLDN18.2 expressed in <40% of tumor cells; B, CLDN18.2 expressed in ≥40% of tumor cells.

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