Gastric Carcinomas With Lymphoid Stroma

An Evaluation of the Histopathologic and Molecular Features

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Gastric Carcinomas

- 5年生存率<30%
- 遗传和环境因素

1.吸烟、饮食
2.感染性因素：幽门螺杆菌感染、EBV感染
3.遗传性息肉病
4.慢性萎缩性胃炎/肠上皮化生

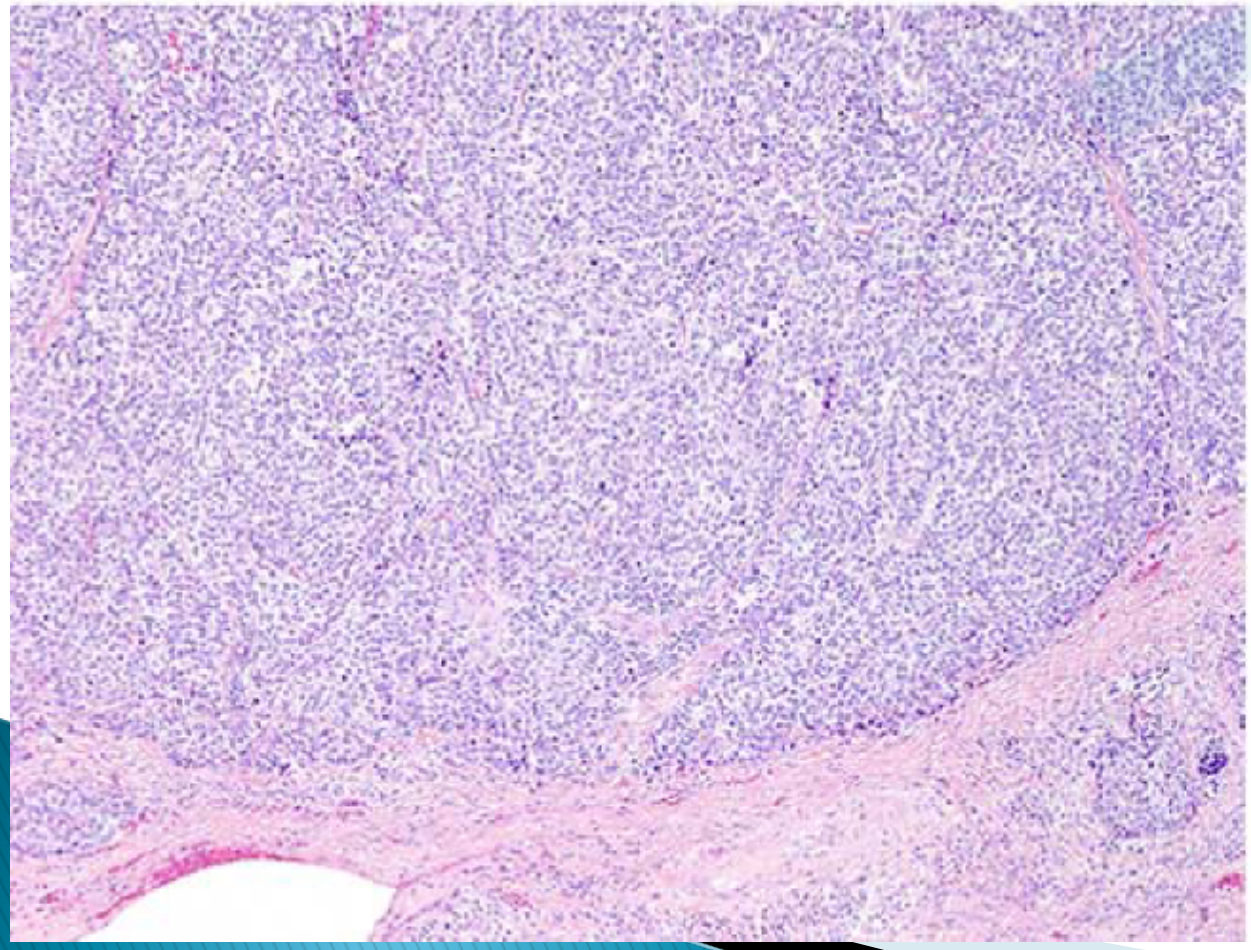
Gastric Carcinomas With Lymphoid Stroma(GCLS)

- 淋巴上皮样癌和髓样癌
- 罕见，占胃腺癌1%~4%
- 通常累及近端胃或胃残端
- 常见于男性
- >80%病例与EB病毒感染有关

Gastric Carcinomas With Lymphoid Stroma

- 无或发育差的腺管结构和突出的间质淋巴细胞浸润
- 肿瘤细胞呈片分布，间质浸润的淋巴细胞使肿瘤细胞与间质界限不清，肿瘤周围亦可见大量淋巴细胞浸润

A



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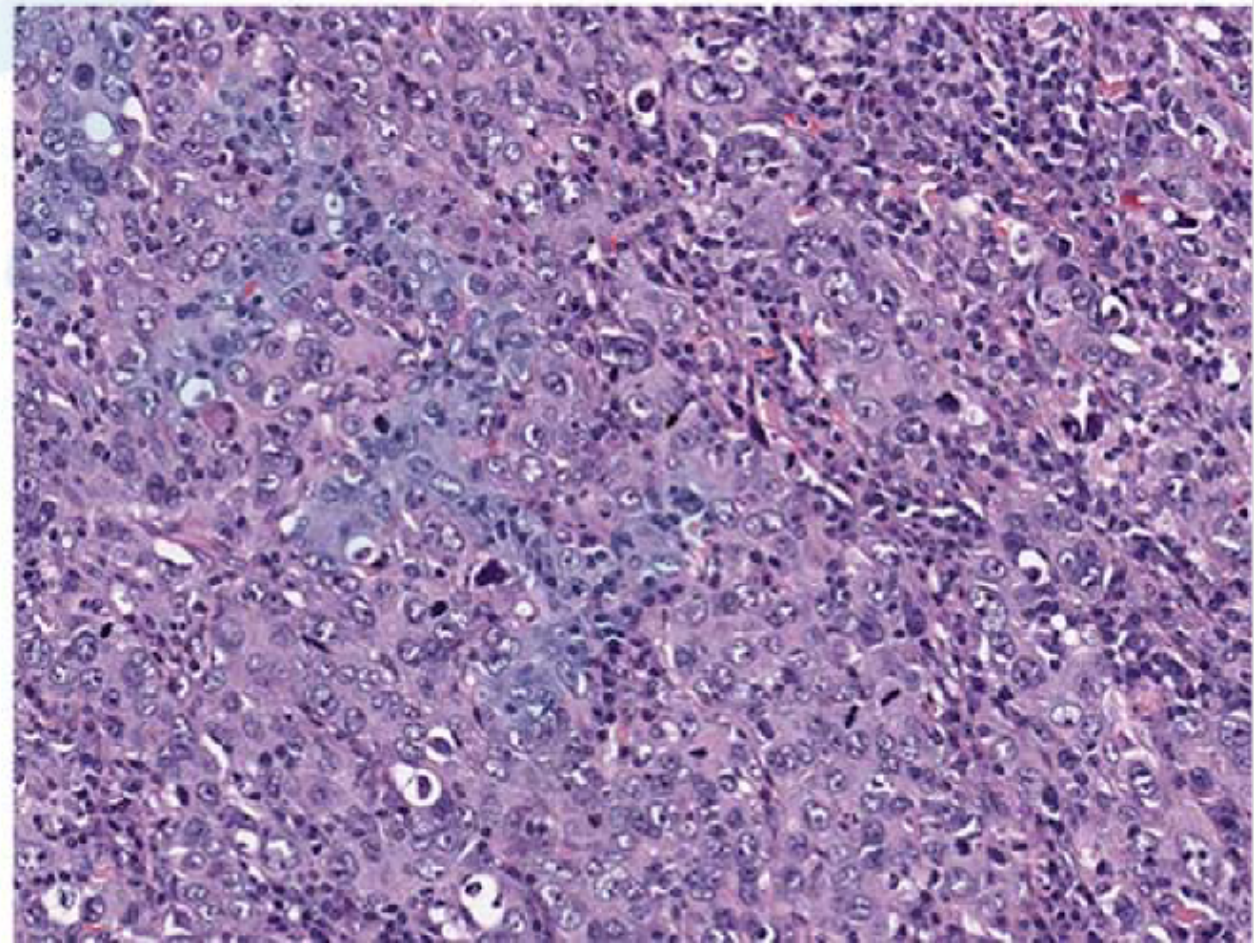
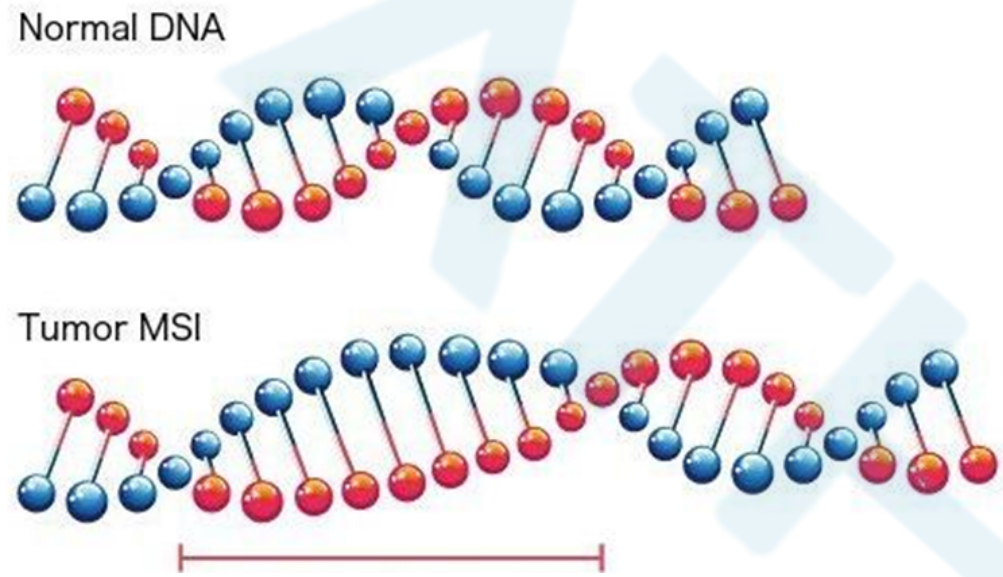


MSI：微卫星不稳定，指与正常组织相比，在肿瘤中某一微卫星由于重复单位的插入或缺失而造成的微卫星长度的任何改变，出现新的微卫星等位基因现象。

MMR：DNA错配修复基因，它的表达缺失可引起DNA复制过程中错配的累积，导致微卫星不稳定（MSI）的发生，与肿瘤的发生密切相关。如 ：结直肠癌（15 %)、子宫内膜癌、胃癌、卵巢癌、肝胆管癌、泌尿系肿瘤等。

故无论从蛋白水平检测MLH1、MSH2、MSH6、PMS2等分子，还是在基因水平检测MSI状态均有助于判断该类病因。现已证明二者的符合性达到97.8%。



MATERIALS AND METHODS

1.483 surgically resected gastric carcinoma specimens

2.35 of which contained areas of carcinoma with lymphoid stroma

3.Clinicopathologic information：age, sex, race, tumor location, stage

Histopathologic Evaluation

- Displayed a sheet-like,syncytial growth of tumor cells, prominent lymphocytic infiltration, and a dense lymphoid infiltrate at the advancing edge of the tumor
- Evaluated foci of GCLS the number of tumor infiltrating lymphocytes (×400/HPF) ,cytologic features, mitotic activity, and peripheral lymphoid aggregates

Histopathologic Evaluation

- Lymphovascular and perineural invasion were Noted
- Other histologic patterns were noted and quantified when present in morphologically heterogenous cases.
- The background gastric mucosa : H. pylori infection, chronic gastritis, intestinal metaplasia.

Immunohistochemical Studies

- MLH1, MSH2, MSH6, PMS2 were performed to assess for MMR deficiency
- Tumors with complete loss of staining in tumor cell nuclei -MMRD (eg, loss of MLH1/PMS2, MSH2/MSH6, isolated loss of PMS2, or loss of MSH6)
- Tumors with retained nuclear staining for all MMR proteins were deemed MMR -proficient (MMR-P).

Immunohistochemical Studies

- β-catenin：strong nuclear staining of at least 10%
- HER2 ：incomplete basolateral staining, or complete membranous staining
- PD-L1 ：any amount of membranous PD-L1 staining,and the extent of staining was recorded.

Detection of EBV in Tumor Cells

- Strong signal in tumor cell nuclei

DNA Extraction

Assessment for MSI by Polymerase Chain Reaction

- MSI- in at least 2 markers unstable
- MSS -at all markers stable

NGS Analysis

Statistical Methods

RESULTS

Clinicopathologic Features of Study Patients Compared With Those of TCGA Set

TABLE 2. Clinicopathologic Features of Gastric Carcinomas With Lymphoid Stroma and TCGA Cohort*

	Gastric Carcinomas With Lymphoid Stroma			TCGA Cohort		
	EBV⁺/MMR-P (N = 7)	EBV⁻/MMR-D (N = 12)	EBV⁻/MMR-P (N = 12)	EBV⁺/MMR-P (N = 26)	EBV⁻/MMR-D (N = 64)	EBV⁻/MMR-P (N = 205)
Male/female ratio	5/2	3/1	3/1	21/5	7/9	133/72
Mean age (y)	69	71	71	64	71	65
Location (%)						
Proximal stomach	43	42	83	77	48	60
Distal stomach	57	58	17	23	47	38
Not available	—	—	—	0	5	2
Overall pathologic stage (%)						
Localized (stages I and II)	71	83	75	42	59	49
Advanced (stages III and IV)	29	17	25	58	30	47
Tumor infiltrating lymphocytes/ HPF (mean)	85	86	65	—	—	—
Mitotic figures/10 HPF (mean)	18	14	24	—	—	—
Perineural invasion (%)	14	25	8			
Lymphovascular invasion (%)	71	67	83	—	—	—
Alternate component present (%)	43	58	83	—	—	—
Intestinal type	75	42	43	—	—	—
Mucinous type	25	25	0	—	—	—
Percentage of total volume (mean)	35	34	8	—	—	—
Background mucosal disease (%)						
Helicobacter pylori-associated chronic gastritis	43	33	33	—	—	—
Chronic gastritis, not otherwise specified	57	50	17	—	—	—
Intestinal metaplasia	57	58	50	—	—	—
Outcome (%)						
Alive with no evidence of disease	50	67	57			
Alive with disease	25	8	14			
Dead of disease	17	8	29			
Dead of other causes	8	8	—			
Not available	—	8	—			

*The results shown here are in part based upon data generated by TCGA Research Network: http://cancergenome.nih.gov/.

RESULTS

Clinicopathologic Features of Study Patients

- Most study patients were older adult men (male/female: 23/8)
- mean age 70 years (40 to 91 y)
- Nineteen (58%) patients were white, 6 (10%) were African American, and only 1 patient was of Asian descent.
- Eighteen (58%) in the proximal stomach (cardia and body/fundus), 13 (42%) in the distal stomach(antrum). 83% of EBV−/MMR-P in the proximal stomach ,only 42% of EBV−/MMR-D tumors
- 76% of gastric carcinomas with lymphoid stroma were localized (stage I or II)

RESULTS

Clinicopathologic Features of Study Patients

- Tumor-infiltrating lymphocytes (mean,78/HPF), EBV+/MMR-P(mean, 85/HPF) , EBV−/MMR-D(mean, 86/HPF),EBV−/MMR-P (mean, 65/HPF), but the differences were not significant
- Heterogenous growth Patterns

EBV+/MMR-P (43%-glandular,<10%)

EBV−/MMR-D (7/58% ,5-glandular ; and/or 3-mucinous ,30% to 50%)

EBV−/MMR-P(83%, 9-glandular ;and/or 3-mucinous ,70%),1 displayed focal signet ring cell differentiation

RESULTS

Clinicopathologic Features of Study Patients

- Overall recurrence rates

  EBV+/MSS, EBV−/MSI, EBV−/MSS (43%, 9%, 33%)
- Rates of death from disease

  EBV+/MSS, EBV−/MSI, EBV−/MSS(29%, 9%, 17%)

RESULTS

Immunohistochemical Features and In Situ Hybridization

TABLE 3. Immunohistochemical Features of Gastric Carcinomas With Lymphoid Stroma

	n (%)			
Immunohistochemical Stain	**EBV⁺/ MMR-P (N = 7)**	**EBV⁻/ MMR-D (N = 12)**	**EBV⁻/ MMR-P (N = 12)**	***P***
Nuclear β-catenin	1 (14)	0	2 (17)	0.42
Membranous HER2	0	0	0	1.0
PD-L1 staining in tumor cells				
<1%	2 (29)	3 (25)	5 (42)	
1%-4%	2 (29)	3 (25)	1 (8)	1.0
5%-25%	0	2 (17)	3 (25)	0.41
26%-50%	1 (13)	0	2 (17)	0.42
51%-75%	0	1 (8)	0	1.0
76%-100%	2 (29)	3 (25)	1 (8)	0.60
PD-L1 in peritumoral cells	5 (71)	10 (83)	8 (67)	0.68

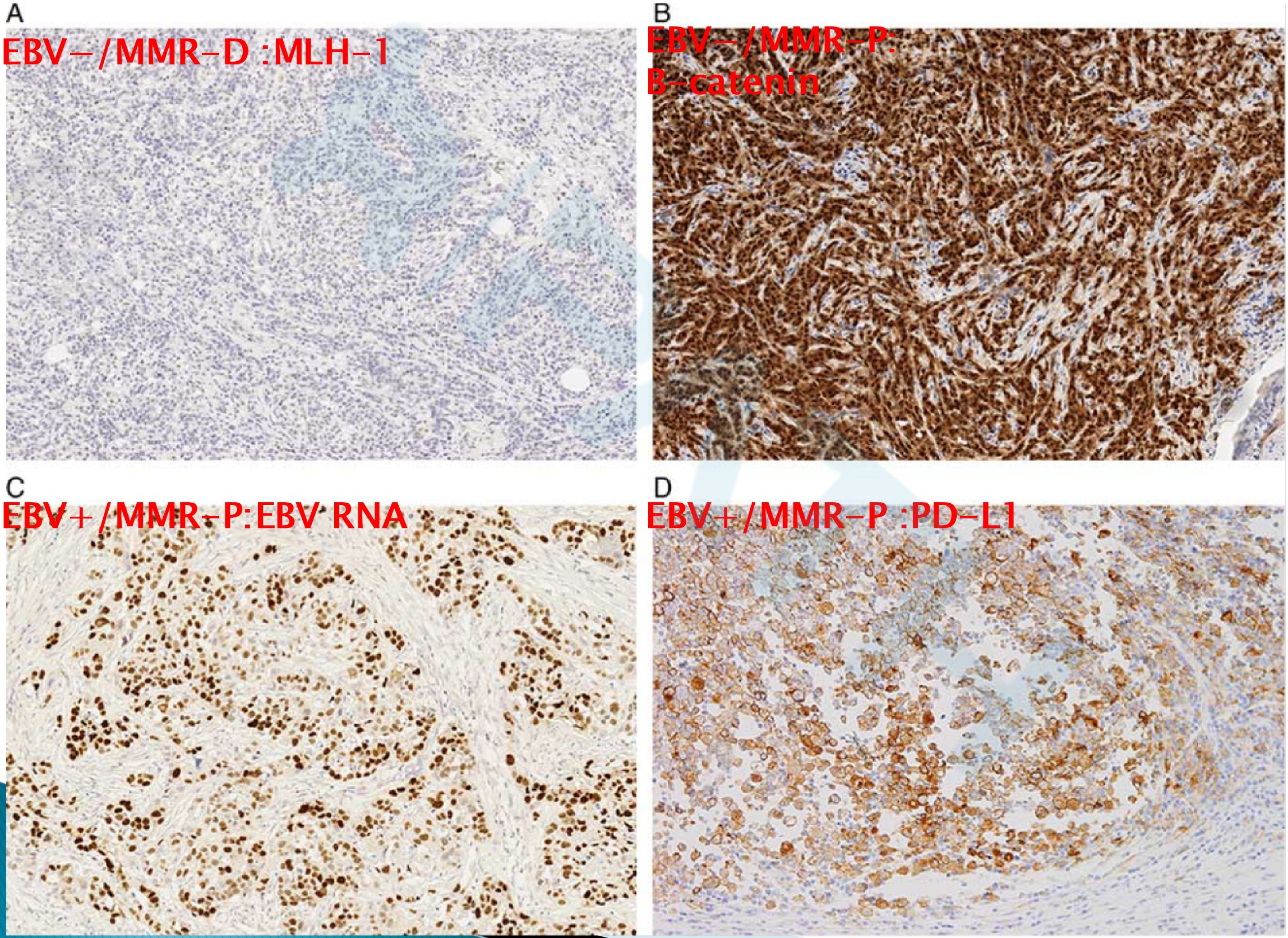
RESULTS

Immunohistochemical Features and In Situ Hybridization

- 3 β-catenin (1 EBV+/MMR-P ,2 EBV−/MMR-P)
- Lacked membranous staining for HER2
- 15(48%) PD-L1 >5%; 7 (23%) PD-L1 >50% (Diffuse) ;
- 75% cases PD-L1 staining of surrounding and infiltrating dendritic-type inflammatory cells.
- PD-L1 staining among subgroups of GCLS were no differences (extent or intensity)
- EBV−/MMR-D (2 loss of MSH2/MSH6, 10 loss of MLH1/PMS2) Nuclear

RESULTS

Immunohistochemical Features and In Situ Hybridization



RESULTS

Variant Rates of Commonly Altered Genes in Subgroups of Gastric Carcinoma With Lymphoid Stroma and TCGA Cohort

TABLE 4. Variant Rates of Commonly Altered Genes in Subgroups of Gastric Carcinoma With Lymphoid Stroma and TCGA Cohort*

	Gastric Carcinomas With Lymphoid Stroma (n [%])					TCGA Cohort (n [%])				
Affected Gene	EBV+/ MMR-P (N = 4)	EBV−/ MMR-D (N = 9)	EBV/ MMR-P (N = 11)	Total (N = 24)	*P*	EBV+/ MMR-P (N = 26)	EBV−/ MMR-D (N = 64)	EBV−/ MMR-P (N = 205)	Total (N = 295)	*P*
KRAS	1 (25)	5 (56)	1 (9)	7 (29)	0.07	1 (4)	15 (23)	12 (6)	28 (9)	<0.001
TP53	0	1 (11)	9 (82)	10 (42)	0.001	1 (4)	25(39)	112 (56)	138 (47)	<0.001
ARID1A	3 (75)	7 (78)	5 (45)	15 (63)	0.39	14 (56)	54 (84)	22 (11)	90 (31)	<0.001
PIK3CA	1 (25)	3 (33)	2 (18)	6 (25)	0.82	20 (80)	27 (42)	10 (5)	57 (19)	<0.001
PRKDC	3 (75)	6 (67)	5 (45)	14 (58)	0.55	1 (4)	25 (39)	5 (3)	31 (11)	<0.001
FGFR2	2 (50)	2 (22)	0	4 (17)	0.04	0	8 (13)	4 (2)	12 (4)	0.003
FGFR3	1 (25)	4 (44)	3 (27)	8 (33)	0.85	0	4 (6)	2 (1)	6 (2)	0.03
ERBB2	0	2 (22)	1 (9)	3 (13)	0.76	1 (4)	7 (11)	6 (3)	14 (5)	0.03
ERBB3	0	1 (11)	0	1 (4)	0.54	2 (8)	21 (33)	8 (4)	31 (11)	<0.001
MLL2	1 (25)	6 (67)	2 (18)	9 (38)	0.08	5 (20)	50 (78)	5 (3)	60 (20)	<0.001
MLL3	0	7 (78)	3 (27)	10 (42)	0.02	3 (12)	33 (52)	9 (5)	45 (15)	<0.001
EGFR	0	2 (22)	1 (9)	3 (13)	0.76	0	12 (19)	3 (2)	15 (5)	<0.001
SYNE1	2 (50)	4 (44)	5 (45)	11 (46)	1	1 (4)	47 (73)	33 (17)	81 (27)	<0.001
CDH1	0	3 (33)	2 (18)	5 (21)	0.52	0	5 (8)	24 (12)	29 (10)	0.13
CTNNB1	0	2 (22)	0	2 (8)	0.28	3 (12)	7 (11)	9 (5)	19 (6)	0.07
Mutation count (mean)	37	83	48	59	0.13	154	134	1311	396	<0.001

*The results shown here are in part based upon data generated by TCGA Research Network: http://cancergenome.nih.gov/.

RESULTS

Variant Rates of Commonly Altered Genes in Subgroups of Gastric Carcinoma With Lymphoid Stroma

- KRAS :EBV−/MMR-D (56%) *vs* EBV+/MMR-P (25%) and EBV−/MMR-P (9%)
- TP53 :EBV−/MMR-P (82%)*vs* EBV+/MMR-P (0%) and EBV−/MMR-D(11%)
- PIK3CA :EBV+/MMR-P (kinase domain /E542K), EBV−/MMRD(helical domain mutations /H1047R and R899C)
- High alterative rates :ARID1A, FGFR2/3, MLL2/3, PRKDC, and SYNE1 regardless of molecular subtype

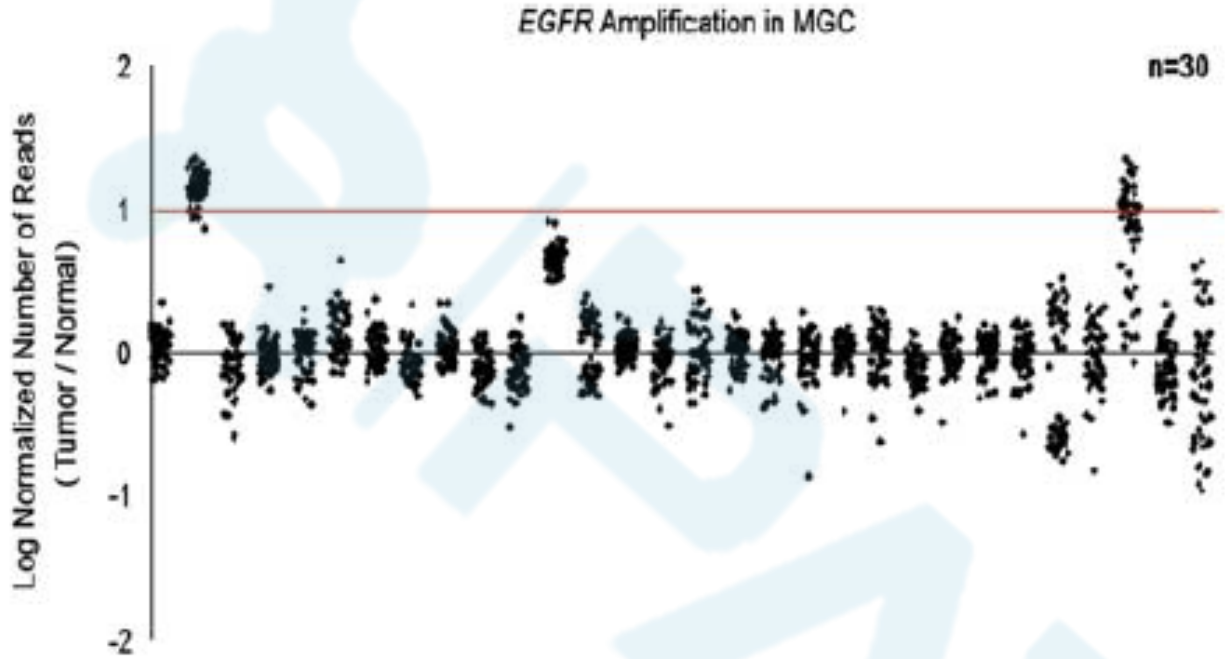
RESULTS

Variant Rates of Commonly Altered Genes in Subgroups of Gastric Carcinoma With Lymphoid Stroma

- The mean number of alterations
  EBV−/MMR-D (83) *vs* EBV−/MMR-P (44) ,
  EBV+/MMR-P (37)
- Mutations per megabase of sequence
  EBV−/MMR-D (46.5) *vs* EBV+/MMR-P (14.7), EBV−/MMR-P (15)

RESULTS

Variant Rates of Commonly Altered Genes in Subgroups of Gastric Carcinoma With Lymphoid Stroma



unequivocal amplification of EGFR in 2 EBV−/MMR-P and borderline EGFR amplification in 1 EBV−/MMR-D

Other amplified genes included MYC and CCNE1 in EBV−/MMR-P

RESULTS

Variant Rates of Commonly Altered Genes in Subgroups of Gastric Carcinoma With Lymphoid Stroma and TCGA Cohort

- KRAS variants:GCLS(29% ,most occurred in EBV−/MMR-D)*vs* TCGA(10%)
- PIK3CA variants:GCLS (25%)vs TCGA (20%)
- PIK3CA:GCLS and EBV+/MMR-P (25%) *vs* EBV+ tumors in TCGA (80%)
- FGFR3 mutations :GCLS (33%) *vs* TCGA (2%)

DISCUSSION

- Histologic subtype is enriched, EBV-encoded RNAs (22%) and mutually exclusive MMRD(39%)
- Unassociated with regional lymph node or distant metastases,despite their high-grade cytologic features and frequent lymphovascular invasion (74%)
- Most (65%) GCLS(EBV−/MMR-P) showed morphologic heterogeneity with discrete areas of glandular differentiation
- 83% EBV−/MMR-P in the proximal stomach, no associations between pathologic features and EBV status or MMR abnormalities

DISCUSSION

- GCLS:well circumscribed ，consist of diffuse, sheet-like growth of syncytial cells ，dense lymphoid infiltrates that obscure the interface between tumor cells and stroma
- Glandular differentiation is absent and cells contain large nuclei with open chromatin and 1 or several nucleoli
- GCLS harbor EBV-encoded RNAs（Asia ，80% EBV+ ）
- In western, EBV positivity rates are lower; (7%to 39%) -MMR-D or EBV-/ MMR -D

DISCUSSION

- The prognosis of GCLS is better --infiltrating lymphocytes,EBV +, or MMR -D(independently)
- Grogg et al found that higher numbers of tumor infiltrating lymphocytes were associated with improved survival, regardless of EBV and microsatellite status
- Lim et al found the 10-year disease-specific survival rates of patients with EBV+ and EBV− tumors to be 89.1% and 66.9% in 274 GCLS

DISCUSSION

Features of Gastric Carcinoma With Lymphoid Stroma Associated With Epstein-Barr Virus

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- EBV+ in GCLS patients is associated with a favorable prognosis, more obvious in advanced-stage GCLS
- EBV-negative GCLS is similar to conventional adenocarcinoma, and similar survival times.
- EBV status may be more important than the proportion of undifferentiated tumor cells in the diagnosis of GCLS and management of patients.

DISCUSSION

- High rate of PD-L1 staining (48%) among GCLS with similar rates of staining regardless of EBV and MMR status.
- PD-L1:EBV+/MMR-P and EBV−/MMR-D showed higher rates of positivity(>75%)*vs* EBV−/MMR-P, not statistical significance
- Elevated TMB at the molecular level and enhanced PD-L1 staining at the protein level; this subtype effectively respond to PD-L1inhibitors and similar agents

DISCUSSION

- Promoter methylation -tumor suppressor gene silencing among gastric carcinomas(EBV+ , MMR-D)
- The molecular features in GCLS are similar to gastric carcinomas in general. Not detect ERBB2 amplification in any of our cases, although this feature is present in conventional adenocarcinomas(up to 34%)
- (4%)ERBB3 alterations, others gastric carcinomas study(up to 12%)
- ARID1A (63%) and KRAS (29%) alterations among GCLS vs conventional gastric carcinomas,reflecting higher numbers of EBV+ and MMRD tumors

DISCUSSION

- Pattern of promoter hypermethylation distinct from that of MMR-D , results from EBV latent membrane protein 2A expression.Frequent alterations in PIK3CA and ARID1A , very low number of TP53 mutations
- High frequency of ARID1A alterations (75%) , no TP53 mutations (EBV+/MMR-P GCLS)
- 1(25%) PIK3CA mutation in our study*vs*(80%)EBV+ gastric cancers in TCGA
- PRKDC :75% of our EBV+/MMR-P *vs* 4% in TCGA

DISCUSSION

- 9p gains in EBV+ --increased expression of JAK2, CD274,PDCD1LG2; the latter 2 genes encode PD-L1 and PDL2.not find JAK2 alterations in EBV+/MMR-P
- (43% , EBV+)PD-L1 staining (>50% of the tumor cells)

DISCUSSION

- Sporadic tumors : MLH-1 promoter methylation, affecting important genes(CDKN2A, RUNX3, and CDH1)
- EBV−/MMR-D GCLS :ARID1A (78%) , PIK3CA (33%)mutations,FGFR2 (22%),FGFR3 (44%), and ERBB2 (22%)

DISCUSSION

- Chromosomal instability :chromosomal copy number alterations, but not mutation rates
- Reported:PIK3CA alterations (3%), but frequent TP53 mutations and amplifications (ERBB2, EGFR, and FGFR2
- In our study EBV-/MMR-P:PIK3-CA(18%) ,TP53 (82%), often in combination with amplifications of EGFR, MYC, and CCNE1

Summary

- Our results suggest GCLS show similar molecular features to those described in TCGA study
- GCLS are histologically indistinguishable regardless of molecular alterations,
- EBV−/MMR-P:proximal stomach,glandular differentiatio *vs* EBV+ or MMR-D
- GCLS : similar patterns of alteration with respect to cancer-related genes *VS* tubular and diffuse tumor types.
- HER2- ,not ERBB2 amplification, KRAS mutations, higher TMB, extensive PD-L1+ : less responsive to targeted therapy, but susceptibility to immune checkpoint inhibitors

Thanks for your attention