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ORIGINAL SCIENTIFIC REPORT

Prognosticators of Long-Term Outcomes of TNM Stage II Colorectal Cancer: Molecular Patterns or Clinicopathological Features

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Abstract

Background Patients with stage II colorectal cancer (CRC) have a higher risk of recurrence when they have certain risk factors, including clinical and pathological patterns. However, as the prognostic role of molecular patterns for stage II disease is still unclear, this study aimed to investigate it.

Methods A total of 509 patients with stage II CRC were enrolled, and all clinical, pathological, and molecular data were collected. Molecular patterns included microsatellite instability (MSI); elevated microsatellite alterations at selected tetranucleotides (EMAST) status; and expression of *RAS*/*RAF* genes, genes of the APC pathway, and other gene mutations. The endpoints were oncological outcomes, including overall survival (OS), cancer-specific survival (CSS), disease-free survival (DFS), local recurrence (LR), and distant recurrence (DR). Cox regression analysis was used.

Results Numerous molecular patterns influenced the oncological outcomes on univariate analysis, but no variable reached significance in LR. On multivariate analysis, a mucinous component (MC) > 50% ($P < 0.01$) was significant for OS and CSS. Lymphovascular invasion (LVI; $P < 0.01$), MC > 50% ($P < 0.01$), and EMAST-H ($P = 0.02$) significantly influenced DFS, whereas LVI ($P < 0.01$), MC > 50% ($P < 0.01$), and *TP53* mutation ($P = 0.02$) were significant for DR.

Conclusions In this study, MSI, EMAST, and *RAS*/*RAF* alterations did not influence the oncological outcomes. Overall, LVI and MC were two significant prognostic factors for DFS and DR. Thus, the histopathology, rather than the genes, plays a major role in the prognosis of patients with stage II CRC.

Introduction

Colorectal cancer (CRC) is the most common cancer in Taiwan since 2013 [1]. Available treatments for CRC include resection, chemotherapy, radiation therapy, targeted therapy, and immunotherapy. In the era of precision medicine, molecular analysis for CRC is important for not only pathogenesis but also treatment [2]. The accumulation of genetic or chromosomal alterations results in the development of CRC. Loss of heterozygosity [3, 4] and gene mutations were predictive factors for CRC [5–7]. Moreover, deficiency in mismatch repair (MMR) proteins causes accumulation of DNA errors. This dysfunction

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mainly occurs over genomic areas with short repetitive nucleotide sequences and is known as microsatellite instability (MSI) [8, 9]. High level of MSI (MSI-H) is defined as ≥ 2 gene mutations for MMR proteins, accounting for approximately 15% of CRC cases. Elevated microsatellite alterations at selected tetranucleotides (EMAST) is another MSI variation, and it is also a prognostic factor for CRC [10].

Resection is the primary treatment for stage I–III CRC. Adjuvant chemotherapy improved 5-year survival and disease-free survival (DFS) of patients with stage III disease but not those with stage II disease [11]. Per the American Society of Clinical Oncology and the National Comprehensive Cancer Network recommendations, adjuvant chemotherapy may benefit patients with stage II CRC with poorly differentiated histology, lymphovascular invasion (LVI), perineural invasion (PNI), < 12 lymph node samples, inadequate margins, bowel obstruction, or localized perforation [12, 13]. However, pathological findings or clinical conditions rather than molecular patterns were used in the studies. While the predictive value of many mutant genes has been studied, most previous studies combined all stages of CRC, making the molecular effects unclear. Therefore, this study aimed to investigate the prognostic roles of mutant genes, MSI/EMAST status, and several histopathological features for pathological stage II CRC.

Materials and methods

Patients and clinical data

Between March 2000 and November 2010, 560 patients with pathological stage II CRC underwent curative surgery at the Division of Surgery of Colon and Rectum, Taipei General Hospital in Taiwan. Patients were excluded if they were followed up for < 30 days; received neoadjuvant radiotherapy for rectal cancer; had synchronous cancer, non-adenomatous tumor, familial adenomatous polyposis, or hereditary nonpolyposis CRC; or died due to surgical complications. Totally, 509 patients were enrolled in this study. Adjuvant chemotherapy, including oral tegafur, oral capecitabine, and intravenous 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX), was administered for patients with pathological stage II CRC with risk factors (T4 tumor; poorly differentiated histology; LVI; PNI; bowel obstruction; lesions with localized perforation; or close, indeterminate, or positive margins). Adjuvant chemotherapy was performed based on the combined decision of the surgeons, physicians, and patients. Patients were followed up every 3 months in the first 2 years and every 6 months in the following 3 years. Serum carcinoembryonic antigen (CEA) levels were checked at every visit. Imaging was performed according to the patient's condition, including computed tomography (CT), abdominal ultrasonography, chest radiographs, positron emission tomography, and colonoscopy. Abdominal CT performed every 12 months and if unexpected issues were observed, such as intestinal obstruction and intra-abdominal infection. Colonoscopy was performed 1 year after surgery; the interval was determined according to prior colonoscopy results. All clinical data were prospectively collected and retrospectively reviewed using medical records. Recurrence was defined as newly suspicious lesions on imaging with elevated serum CEA levels, as confirmed on multidiscipline team conference, or pathological evidence. The time between surgery to the date of death or last follow-up was overall survival (OS), and that between surgery to the date of death due to CRC was cancer-specific survival (CSS). DFS was the surgery date to the date of recurrence (including local recurrence [LR] and distant recurrence [DR]) or last follow-up.

Pathological analysis

All specimens were sent to the Department of Pathology and Laboratory Medicine for further pathological analysis, including invasion depth, lymph node metastasis, LVI, PNI, mucinous component (MC), histological differentiation, and inflammation around the tumor. Tumor samples were collected and immediately frozen in liquid nitrogen in the Biobank of Taipei Veterans General Hospital. After review board approval (IRB 2017-06-004BC), we obtained tissue samples from the Biobank.

Gene mutation characterization with MassARRAY

The MassARRAY method was used for gene mutation analysis, including those in the RAS pathway (KRAS, HRAS, NRAS, and BRAF), PI3K pathway (PIK3CA, PTEN, and AKT1), and APC-TP53-FBXW7 pathway. These genes were identified based on the profiles in the COSMIC cancer database. Polymerase chain reaction (PCR) and extension primers were analyzed (MassARRAY Design 3.1 software, Sequenom, San Diego, CA, USA). Default single-base extension settings and parameters were used, but the maximum multiplex level input was adjusted to 15 [6]. Mutation alleles were manually designed to be lower in mass than the reference alleles by either forward or reverse extension. DNA amplification was performed with PCR primer pools, and unincorporated nucleotides were inactivated by shrimp alkaline phosphatase. A single-base extension reaction was conducted by using a mixture of dideoxynucleotides and extension primers, which were promptly hybridized adjacent to the mutations. PCR was performed in 384-well plates in volumes of 5

μL including 1 pmol of the corresponding primers, 10 ng tumor DNA, and HotStar Reaction Mix (Qiagen). The PCR procedure was 94 °C for 15 min followed by 40 cycles at 94 °C (20 s), 56 °C (30 s), and 72 °C (60 s), with the last extension at 72 °C (3 min). During the primer extension step, every sample was denatured at 94 °C followed by 40 cycles of 94 °C (5 s), 52 °C (5 s), and 72 °C (5 s). Cation exchange resin was added for salt removal. Multiplexed PCR was spotted onto SpectroCHIP II arrays, and DNA fragments were resolved by using MALDI-TOF on a MassARRAY Analyzer 4 system (Sequenom). Every spectrum was analyzed using Typer 4.0 software (Sequenom) for mutation. Putative mutations were filtered by manual review.

MSI and EMAST analysis

MSI detection was conducted as previously described [14]. Five reference microsatellite markers (D5S345, D2S123, BAT25, BAT26, and D17S250) were used according to the international criteria for MSI determination [15]. Primer sequences of the genes were acquired from GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). MSI-H was defined as instability in $\geq$ 2 loci of the five markers, whereas MSI-L and MSS were defined as one loci instability and no evidence of instability, respectively. EMAST detection was performed similarly. Five reference microsatellite markers (D20S82, D20S85, D8S321, D9S242, and MYCL1) were used. Primer sequences of genes were obtained from GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Specimens with at least one loci instability were EMAST positive. Analysis was performed twice if the results were equivocal.

Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 22 (IBM Corp., Chicago, IL, USA). Categorical variables were compared using the Chi-squared test and Fisher's test, whereas continuous variables were analyzed using the t-test. Kaplan–Meier method was used for survival analysis. All relatively significant prognostic factors in univariate analysis ($P < 0.2$) were applied to multivariate analysis using Cox regression with the forward-selection method. P values < 0.05 were considered statistically significant.

Results

Patient characteristics

The mean age was 71.7 ± 11 years, and the mean follow-up time was 66 months (range 1–163); the dropout numbers are shown in Fig. 1. Totally, 361 men (70.9%) and

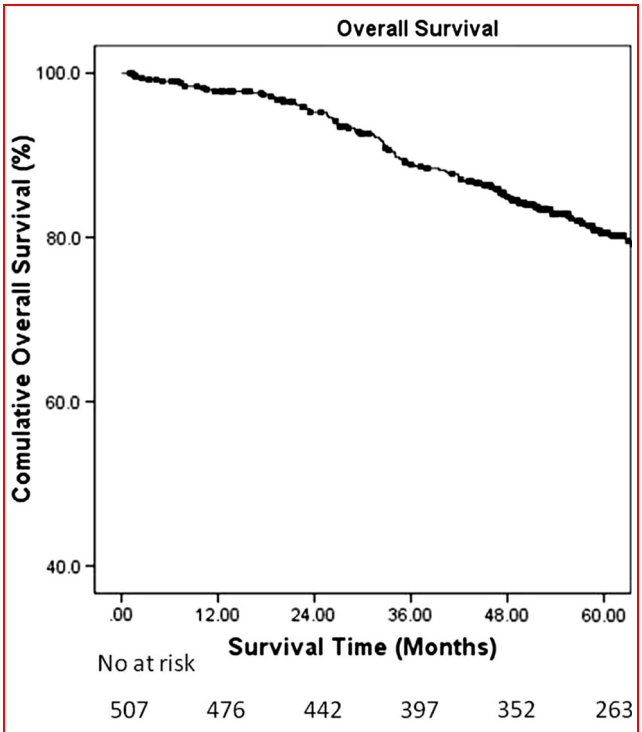

Fig. 1 Five-year overall survival and numbers at risk for all patients

148 women (29.1%) were enrolled. Patients characteristics are presented in Table 1. Most patients (477, 93.7%) had pT3 tumors, and 32 (6.3%) had pT4 tumors. Poorly differentiated histology was observed in 21 patients (4.1%), while 487 (95.7%) had mild or moderate differentiation. Totally, 486 cases (95.5%) had $\leq$ 50% MC while 21 (4.1%) had > 50%. In all, 39 (7.7%) patients had LVI and 11 (2.2%) had PNI. Eighty-two (16.1%) patients received adjuvant chemotherapy, including oral chemotherapy in 35 (6.9%) and FOLFOX treatment in 47 (9.2%).

MSI-H was diagnosed in 68 patients (13.4%), and 65 (12.8%) had EMAST high. Six patients (1.2%), 192 (37.7%), and 23 (4.52%) had AKT1, KRAS, and BRAF mutations, respectively. Totally, 156 patients (30.65%) had APC mutations, whereas 160 (31.43%) had TP53 mutation. Seventy-four patients (14.54%) had PIK3CA mutation. Nine patients (1.77%) and 59 patients (11.6%) developed LR and DR, respectively.

Univariate analysis

Many variables were used for univariate analysis of OS, CSS, DFS, LR, and DR (Table 2). For OS, univariate analysis demonstrated that older age ($P < 0.001$), female sex ($P = 0.039$), high postoperative CEA levels ($P = 0.029$), MC > 50% ($P = 0.001$), and AKT1 mutation ($P = 0.006$) were significant prognostic factors. For CSS,

Table 1 Patients clinicopathological features ($N = 509$)

Characteristic	No	%
Age	71.7 ± 11	(Year-old)
Gender		
Male	361	70.9
Female	148	29.1
Follow-up time	65.96 ± 36.18 (1–163)	(Months)
Tumor location		
Right-side colon (proximal to splenic flexure)	148	29.1
Left side colon (distal to splenic flexure)	230	45.2
Rectum	131	25.7
pT stage		
pT3	477	93.7
pT4	32	6.3
Preoperative CEA level		
< 5 mg/dl	337	66.2
≥ 5 mg/dl	160	31.4
NA	12	2.4
Postoperative CEA level		
< 5 mg/dl	252	49.51
≥ 5 mg/dl	18	3.54
NA	239	46.95
Adjuvant C/T		
Nil	427	83.9
Oral C/T (tegafur, capecitabine)	35	6.9
FOLFOX	47	9.2
pT stage versus adjuvant C/T		
pT3 without C/T	412	80.9
pT3 with oral C/T	32	6.3
pT3 with FOLFOX	33	6.5
pT4 without C/T	15	2.9
pT4 with oral C/T	3	0.6
pT4 with FOLFOX	14	2.8
Pathological features		
Lymphovascular invasion	39	7.7
Perineural invasion	11	2.2
Inflammation around the tumor	95	18.7
Tumor differentiation		
Mild + moderate	487	95.7
Poor	21	4.1
NA	1	0.2
Mucinous component		
≥ 50%	486	95.5
> 50%	21	4.1
NA	2	0.4
MSI-H	68	13.4
EMAST-H	65	12.8
MSI-H and EMAST-H	41	8.1
APC pathway	286	56.2
APC	156	30.65
TP53	160	31.43
FBXW7	44	8.64
SMAD4 pathway	41	8.06
SMAD4	19	3.73
TGFβR	23	4.52
PI3K pathway	83	16.31
PIK3CA	74	14.54
PTEN	4	0.79
AKT1	6	1.18
RAS-RAF pathway	229	44.99
$BRAF^{V600E}$	23	4.52
KRAS	192	37.72
HRAS	11	2.16
NRAS	19	3.73
Total recurrence	66	12.97
Local recurrence	9	1.77
Distant recurrence	59	11.6
Liver recurrence	22	4.32
Lung recurrence	30	5.89
Bone recurrence	1	0.20
Peritoneal recurrence	9	1.77
Other recurrence	6	1.18

CEA serum carcinoembryonic antigen, *C/T* chemotherapy, *FOLFOX* 5-fluorouracil/leucovorin/oxaliplatin, *MSI* microsatellite instability, *EMAST* elevated microsatellite alterations at selected tetranucleotides

female sex ($P = 0.03$), MC > 50% ($P < 0.001$), KRAS mutation ($P = 0.046$), HRAS mutation ($P = 0.045$), and AKT1 mutation ($P = 0.001$) were significant for prognosis. For DFS, preoperative CEA level ($P = 0.049$), LVI ($P = 0.001$), MC > 50% ($P = 0.003$), and EMAST-H ($P = 0.039$) were significant prognostic factors. There was no significant risk factor for LR (Table 3). However, significant risk factors for DR were LVI ($P = 0.002$), MC > 50% ($P = 0.01$), AKT1 mutation ($P = 0.037$), and TP53 mutation ($P = 0.036$).

Multivariate analysis

Relatively significant ($P < 0.2$) prognostic factors on univariate analysis were used for multivariate analysis (Table 4). For OS, multivariate analysis confirmed that older age ($P < 0.001$) and MC > 50% ($P = 0.003$) were significant. For CSS, female sex ($P = 0.004$), MC > 50% ($P = 0.005$), and AKT1 mutation ($P = 0.003$) were significant. For DFS, preoperative CEA ≥ 5 mg/dL

Table 2 Univariate analysis of prognostic factors for overall survival, cancer-specific survival, and disease-free survival

Characteristics	Overall survival			Cancer-specific survival			Disease-free survival		
	HR	CI	*P* value	HR	CI	p value	HR	CI	*P* value
Age (>mean/ < mean)	3.985	2.587–6.138	< 0.001	1.513	0.867–2.641	0.145	0.888	0.547–1.439	0.629
Gender (male/female)	1.544	1.021–2.334	0.039	2.209	1.078–4.526	0.03	0.935	0.557–1.57	0.8
Preoperative CEA (≥5 mg/dl/ < 5 mg/dl)	1.234	0.862–1.765	0.25	1.672	0.958–2.918	0.07	1.661	1.002–2.754	0.049
Postoperative CEA (≥5 mg/dl/ < 5 mg/dl)	2.107	1.079–4.115	0.029	0.485	0.066–3.568	0.477	1.702	0.603–4.807	0.315
Adjuvant C/T									
Nil	1	–	0.063	1	–	0.67	1	–	0.354
Oral C/T (tegafur, capecitabine)	0.33	0.105–1.038	0.058	0.547	0.133–2.258	0.404	1.2	0.478–3.0	0.7
FOLFOX	0.638	0.35–1.16	0.141	1.11	0.5–2.47	0.81	1.64	0.83–3.23	0.15
Pathological features									
LVI (±)	0.929	0.472–1.831	0.832	0.982	0.354–2.724	0.973	2.781	1.487–5.201	0.001
PNI (±)	0.318	0.044–2.28	0.254	0.818	0.113–5.92	0.842	2.063	0.647–6.575	0.221
Inflammation around the tumor (±)	1.22	0.798–1.865	0.359	1.053	0.529–2.096	0.884	0.963	0.515–1.8	0.906
Mucinous component (>50%/ ≤ 50%)	2.611	1.468–4.644	0.001	4.481	2.108–9.529	< 0.001	3.274	1.494–7.176	0.003
Tumor differentiation (poor/ well + moderate)	0.466	0.148–1.468	0.192	0.417	0.058–3.021	0.387	0.744	0.182–3.041	0.681
MSI-H (±)	0.652	0.36–1.184	0.16	0.694	0.276–1.745	0.438	0.654	0.283–1.514	0.322
EMAST-H(±)	0.688	0.388–1.221	0.201	0.659	0.262–1.656	0.375	0.295	0.093–0.939	0.039
MSI-H and EMAST-H(±)	0.679	0.344–1.341	0.265	0.595	0.185–1.913	0.384	0.322	0.079–1.315	0.115
RAS-RAF pathway (involve/else)	1.164	0.825–1.643	0.387	1.684	0.978–2.9	0.06	1.379	0.851–2.235	0.193
KRAS (±)	1.238	0.873–1.757	0.231	1.731	1.01–2.967	0.046	1.395	0.858–2.269	0.18
$BRAF^{V600E}$ (±)	1.218	0.568–2.613	0.613	1.258	0.392–4.037	0.7	1.416	0.515–3.893	0.5
HRAS (±)	1.208	0.492–2.964	0.681	2.853	1.025–7.938	0.045	1.937	0.607–6.182	0.264
NRAS (±)	0.594	0.189–1.867	0.373	0.987	0.24–4.056	0.985	0.825	0.202–3.372	0.789
PI3K-AKT pathway (involve/else)	1.007	0.631–1.607	0.977	1.388	0.715–2.697	0.333	0.827	0.409–1.67	0.596
PIK3CA (±)	0.859	0.516–1.432	0.561	1.044	0.492–2.216	0.91	0.69	0.315–1.51	0.353
PTEN (±)	NA	NA	NA	NA	NA	NA	NA	NA	NA
AKT1 (±)	4.024	1.484–10.912	0.006	6.958	2.166–22.355	0.001	3.997	0.977–16.35	0.054
SMAD pathway (involve/else)	0.96	0.517–1.782	0.896	0.905	0.326–2.51	0.848	0.538	0.169–1.715	0.295
SMAD4 (±)	0.735	0.272–1.99	0.545	0.473	0.065–3.426	0.459	0.381	0.053–2.748	0.339
TGFβ (±)	1.013	0.472–2.177	0.973	1.201	0.373–3.86	0.759	1.518	0.929–2.48	0.096
APC pathway (involve/else)	1.119	0.79–1.584	0.527	1.415	0.811–2.47	0.222	1.096	0.672–1.786	0.714
APC (±)	0.967	0.658–1.421	0.863	1.39	0.792–2.44	0.252	0.949	0.556–1.617	0.846
TP53 (±)	0.982	0.676–1.426	0.924	1.034	0.581–1.842	0.909	1.518	0.929–2.48	0.096
FBXW7 (±)	1.501	0.889–2.533	0.128	1.38	0.59–3.227	0.458	0.854	0.343–2.127	0.735

HR hazard ratio, *CI* confident ratio, *LVI* lymphovascular invasion, *PNI* perineural invasion, *NA* not available, + mutant type, − wild type, *CEA* serum carcinoembryonic antigen, *C/T* chemotherapy, *FOLFOX* 5-fluorouracil/leucovorin/oxaliplatin, *MSI* microsatellite instability, *EMAST* elevated microsatellite alterations at selected tetranucleotides

($P = 0.043$), LVI ($P = 0.001$), MC > 50% ($P = 0.001$), and EMAST-H ($P = 0.017$) were significant. LVI ($P = 0.001$), MC > 50% ($P = 0.003$), and TP53 mutation ($P = 0.009$) were significant for DR.

Discussion

In this retrospective study, multivariate analysis revealed that MC > 50% was important for OS and CSS, and MC > 50% and LVI were significant poor prognostic factors for DFS and DR.

Table 3 Univariate analysis of risk factors for local recurrence and distant recurrence

Characteristics	Local recurrence			Distant recurrence		
	HR	CI	*P* value	HR	CI	*P* value
Age (≥mean/ < mean)	1.079	0.289–4.028	0.91	0.81	0.486–1.35	0.419
Gender (male/female)	0.555	0.149–2.068	0.38	0.995	0.571–1.731	0.985
Preoperative CEA (≥5 mg/dl/ < 5 mg/dl)	1.019	0.254–4.077	0.979	1.663	0.972–2.845	0.064
Postoperative CEA (≥5 mg/dl/ < 5 mg/dl)	NA	NA	NA	1.957	0.687–5.57	0.208
Adjuvant C/T						
Nil	1	–	0.115	1	–	0.71
Oral C/T (tegafur, capecitabine)	0.5	0.96–25.67	0.056	1.03	0.371–2.86	0.96
FOLFOX	3.0	0.58–15.73	0.188	1.38	0.65–2.92	0.403
Pathological features						
LVI (±)	1.493	0.187–11.94	0.706	2.869	1.489–5.528	0.002
PNI (±)	NA	NA	NA	2.329	0.728–7.451	0.154
Inflammation around tumor (±)	1.284	0.267–6.182	0.755	0.994	0.516–1.914	0.985
Mucinous (>50%/ ≤ 50%)	3.255	0.406–26.078	0.266	3.051	1.31–7.105	0.01
Tumor differentiation (poor/well + moderate)	NA	NA	NA	0.837	0.204–3.431	0.805
MSI-H (±)	0.85	0.106–6.805	0.878	0.604	0.242–1.511	0.281
EMAST-H (±)	NA	NA	NA	0.334	0.104–1.066	0.064
MSI-H and EMAST-H (±)	NA	NA	NA	0.362	0.088–1.484	0.158
RAS-RAF pathway (involve/else)	1.029	0.276–3.835	0.965	1.54	0.922–2.57	0.099
KRAS (±)	1.414	0.38–5.269	0.605	1.502	0.9–2.508	0.119
$BRAF^{V600E}$ (±)	NA	NA	NA	1.604	0.581–4.429	0.361
HRAS (±)	NA	NA	NA	2.172	0.678–6.957	0.192
NRAS (±)	NA	NA	NA	0.928	0.227–3.805	0.918
PI3K-AKT pathway (involve/else)	1.502	0.312–7.237	0.612	0.699	0.318–1.54	0.375
PIK3CA (±)	1.685	0.35–8.114	0.515	0.533	0.213–1.332	0.178
PTEN (±)	NA	NA	NA	NA	NA	NA
AKT1 (±)	NA	NA	NA	4.507	1.099–18.486	0.037
SMAD pathway (involve/else)	NA	NA	NA	0.606	0.19–1.937	0.398
SMAD4 (±)	NA	NA	NA	0.426	0.059–3.080	0.398
TGFβ (±)	NA	NA	NA	0.736	0.18–3.015	0.67
APC pathway (involve/else)	0.231	0.048–1.116	0.068	1.373	0.81–2.329	0.24
APC (±)	0.297	0.037–2.38	0.253	1.127	0.625–1.946	0.669
TP53 (±)	0.617	0.128–2.971	0.547	1.735	1.038–2.902	0.036
FBXW7 (±)	NA	NA	NA	0.97	0.388–2.426	0.949

HR hazard ratio, *CI* confident ratio, *LVI* lymphovascular invasion, *PNI* perineural invasion, *NA* not available, + mutant type, − wild type, *CEA* serum carcinoembryonic antigen, *C/T* chemotherapy, *FOLFOX* 5-fluorouracil/leucovorin/oxaliplatin, *MSI* microsatellite instability, *EMAST* elevated microsatellite alterations at selected tetranucleotides

In our study, 37.7% had KRAS mutation, similar to previous studies [16, 17]. KRAS mutation is a poor prognostic factor for CSS on univariate analysis ($P = 0.046$), but it does significantly influence OS or DFS. The predictive role of KRAS mutation has been diverse [6, 16, 18]. The conflicting results were owing to different patient populations. The prevalence rate of BRAF mutation in our study is 4.52%, but it was not a significant prognostic factor for oncological outcomes. BRAF mutation causes 2.25 times higher mortality than wild-type BRAF in CRC [19], and the effect of BRAF mutation has been reported more than that of KRAS mutation [17]. Hence, the influence of BRAF mutation is more apparent in stage III or IV CRC, but not in stage I and II CRC. Accordingly, KRAS and BRAF mutations were not significant high-risk factors for stage II CRC.

The PI3K/AKT/mTOR cascade is an alternative pathway for carcinogenesis, with increasing data implicating its importance in CRC [20]. AKT1 is a bridge between PI3Ks and mTORs, but only a few studies have mentioned its

Table 4 Multivariate analysis of risk factors for overall survival, cancer-specific survival, disease-free survival, and distant recurrence

Characteristics	Overall survival			Cancer-specific survival			Disease-free survival			Distant recurrence		
	HR	CI	*P* value	HR	CI	*P* value	HR	CI	*P* value	HR	CI	*P* value
Age (≥M/ < M)	3.049	1.812–5.131	< 0.001									
Gender (M/F)				3.855	1.545–9.617	0.004						
Preoperative CEA (≥5 mg/dl/ < 5 mg/dl)							1.688	1.016–2.804	0.043			
LVI (±)							3.219	1.661–6.24	0.001	3.166	1.589–6.312	0.001
Mucinous component (>50%/ ≤ 50%)	2.76	1.412–5.396	0.003	3.227	1.428–7.29	0.005	4.014	1.812–8.892	0.001	3.639	1.55–8.547	0.003
EMAST-H (±)							0.241	0.075–0.777	0.017			
AKT1 (±)				8.848	2.143–36.53	0.003						
TP53 (±)										2.048	1.196–3.507	0.009

HR hazard ratio, *CI* confident ratio, *LVI* lymphovascular invasion, + : mutant type, − wild type

predictive role in CRC owing to its low prevalence [21]. In our study, only six patients had AKT1 mutation, and four of them died of CRC. Although AKT1 mutation was a significantly poor prognostic factor, the number of cases was small. Nevertheless, more studies are needed to investigate the importance of AKT1 in CRC, although several clinical trials of AKT inhibitors for CRC are ongoing [22].

Characteristics of patients with MSI-H include right-side colon predominance, young age, poor differentiation, mucinous cell type, and better prognosis [14, 23]. Patients with MSI-H stage II CRC do not benefit from adjuvant 5-FU chemotherapy [24]. In our study, MSI-H did not influence any oncological outcomes in stage II CRC. As the prevalence rate of CRC (13.36%) was similar to that in other studies, the significance of MSI-H is probably regional (stage III) or systemic (stage IV). EMAST in our study was not a significant prognostic factor, even when combining analysis with MSI status. The result differs from previous results [25, 26], mainly considering the prevalence. In Western countries, EMAST-H was observed in 23–50% of CRC [27], whereas about 37% of patients with CRC presented EMAST-H in Japan [28]. However, only 13% of patients had EMAST-H in this study, probably owing to racial differences or different populations. In summary, the predictive role of EMAST in stage II disease is still controversial, and further investigation is needed.

MC > 50% is mucinous adenocarcinoma, a subtype of adenocarcinoma. MC > 50% is observed in advanced stage disease and associated with poor survival [29, 30]. However, a previous large cohort study with > 1000 patients revealed that MC was an insignificant prognostic factor for stage II and III CRC after curative surgery [31]. In our study, for localized CRC, MC was a poor prognostic factor for OS, CSS, and DFS and risk factor for DR, similar to another large cohort study [32]. Adjuvant chemotherapy or close follow-up should be performed for stage II CRC patients with MC > 50%. Additionally, LVI has known prognostic significance [33–35], and our study confirmed this finding. CRC with LVI is associated with a higher risk of recurrence, so adjuvant chemotherapy should be performed for stage II CRC [36]. In our hospital, this concept is acceptable for most doctors.

In addition to molecular patterns, there are other possible biomarkers for predicting oncological outcomes of stage II CRC. Circulating tumor DNA is used for screening and diagnosis [37], treatment, and post-treatment monitoring [38]. However, the extraction procedure and cutoff value are not standardized. Besides, high Ki-67 expression was also associated with low pT, pN stage of CRC [39] but the good prognostic effect of Ki-67 was more significant in stage IV rather than in stage I, and II CRC [40]. MicroRNAs are biomarkers for CRC, but their clinical application is still not conclusive [41]. Blood miR141 and tissue miR21, mRi181a, miR224, and miR226 were significant prognostic factors for CRC [42]. Moreover, tumor-infiltrating lymphocytes were good prognostic factors for CRC and predictor of the immunotherapy effect [43, 44]. However, we did not examine the above biomarkers, and these biomarkers have possible interactions with genes in the current study. Hence, further investigations are needed.

It was histopathological finding rather than most mutant genes that influence the prognosis of localized CRC in our study. With increased rates of screening colonoscopy, early CRC is more frequently found. After radical resection, histopathological results remain important references for treatment, even in the era of molecular oncology.

This study has some strengths. We evaluated many genes, the MSI/EMAST status, histopathology, and clinical data in a relatively large number of patients. Despite being a retrospective study, real-world data are presented. However, some limitations should be considered. We could not avoid retrospective bias. Only observational data were provided, but the effects of adjuvant chemotherapies were

not excluded. We also did not conduct multivariate analysis for LR, because no variables reached significance on univariate analysis. Furthermore, some data were lack, including CpG island methylator phenotype, comorbidities, performance status, leakage, and postoperative infection.

In conclusion, MSI, EMAST, and KRAS and BRAF mutations did not influence OS, CSS, and DFS of patients with stage II CRC. LVI and MC were significant prognostic factors for DFS and DR, while MC was significant for OS and CSS. Thus, histopathology, rather than genes, plays a major role in the prognosis of patients with stage II CRC.

Authors' contributions Tai-Chuan Kuan and Shih-Ching Chang drafted/revised this article and analyzed the data. Shih-Ching Chang was involved in conception and design. Jen-Kou Lin, Tzu-Chen Lin, Shung-Haur Yang, Jeng-Kae Jiang, Wei-Shone Chen, Huann-Sheng Wang, Yuan-Tzu Lan, Chun-Chi Lin, Hung-Hsin Lin, and Sheng-Chieh Huang contributed to acquisition of data.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

References

1. The Department of Health t E Y (2013) The Department of Health. Healthy statistics: Cancer Registry Annual Report in Taiwan Area, Taiwan, R.O.C
2. Dienstmann R, Vermeulen L, Guinney J et al (2017) Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat Rev Cancer 17(2):79–92
3. Lin JK, Chang SC, Yang YC et al (2003) Loss of heterozygosity and DNA aneuploidy in colorectal adenocarcinoma. Ann Surg Oncol 10(9):1086–1094
4. Chang SC, Lin JK, Lin TC et al (2005) Genetic alteration of p53, but not overexpression of intratumoral p53 protein, or serum p53 antibody is a prognostic factor in sporadic colorectal adenocarcinoma. Int J Oncol 26(1):65–75
5. Chang CC, Lin HH, Lin JK et al (2015) FBXW7 mutation analysis and its correlation with clinicopathological features and prognosis in colorectal cancer patients. Int J Biol Markers 30(1):e88–95
6. Lin CC, Lin JK, Lin TC et al (2014) The prognostic role of microsatellite instability, codon-specific KRAS, and BRAF mutations in colon cancer. J Surg Oncol 110(4):451–457
7. Chang YY, Lin PC, Lin HH et al (2016) Mutation spectra of RAS gene family in colorectal cancer. Am J Surg 212(3):537–544.e533
8. Sinicrope FA, Sargent DJ (2012) Molecular pathways: microsatellite instability in colorectal cancer: prognostic, predictive, and therapeutic implications. Clin Cancer Res 18(6):1506–1512
9. Markowitz SD, Bertagnolli MM (2009) Molecular origins of cancer: Molecular basis of colorectal cancer. N Engl J Med 361(25):2449–2460
10. Carethers JM (2017) Microsatellite instability pathway and EMAST in colorectal cancer. Curr Colorectal Cancer Rep 13(1):73–80
11. Gill S, Loprinzi CL, Sargent DJ et al (2004) Pooled analysis of fluorouracil-based adjuvant therapy for stage II and III colon cancer: who benefits and by how much? J Clin Oncol 22(10):1797–1806
12. Benson AB, Schrag D, Somerfield MR et al (2004) American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 22(16):3408–3419
13. NCCN Clinical Practice Guideline in Oncology Colon Cancer, (2017).
14. Lin CC, Lai YL, Lin TC et al (2012) Clinicopathologic features and prognostic analysis of MSI-high colon cancer. Int J Colorectal Dis 27(3):277–286
15. Perucho M (1999) Correspondence re: C.R. Boland et al., A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res., 58: 5248–5257, 1998. Cancer Res 59(1):249–256
16. Farina-Sarasqueta A, van Lijnschoten G, Moerland E et al (2010) The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. Ann Oncol 21(12):2396–2402
17. Roth AD, Tejpar S, Delorenzi M et al (2010) Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60–00 trial. J Clin Oncol 28(3):466–474
18. Phipps AI, Buchanan DD, Makar KW et al (2013) KRAS-mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers. Br J Cancer 108(8):1757–1764
19. Safaee Ardekani G, Jafarnejad SM, Tan L et al (2012) The prognostic value of BRAF mutation in colorectal cancer and melanoma: a systematic review and meta-analysis. PLoS ONE 7(10):e47054
20. Johnson SM, Gulhati P, Rampy BA, et al (2010) Novel expression patterns of PI3K/Akt/mTOR signaling pathway components in colorectal cancer. J Am Coll Surg 210(5): 767–776, 776–768.
21. Danielsen SA, Eide PW, Nesbakken A et al (2015) Portrait of the PI3K/AKT pathway in colorectal cancer. Biochim Biophys Acta 1855(1):104–121
22. Bahrami A, Khazaei M, Hasanzadeh M et al (2018) Therapeutic potential of targeting PI3K/AKT pathway in treatment of colorectal cancer: rational and progress. J Cell Biochem 119(3):2460–2469
23. Vilar E, Gruber SB (2010) Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol 7(3):153–162
24. Carethers JM, Smith EJ, Behling CA et al (2004) Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. Gastroenterology 126(2):394–401
25. Devaraj B, Lee A, Cabrera BL et al (2010) Relationship of EMAST and microsatellite instability among patients with rectal cancer. J Gastrointest Surg 14(10):1521–1528
26. Garcia M, Choi C, Kim HR et al (2012) Association between recurrent metastasis from stage II and III primary colorectal tumors and moderate microsatellite instability. Gastroenterology 143(1):48–50.e41
27. Watson MM, Berg M, Soreide K (2014) Prevalence and implications of elevated microsatellite alterations at selected tetranucleotides in cancer. Br J Cancer 111(5):823–827
28. Yamada K, Kanazawa S, Koike J et al (2010) Microsatellite instability at tetranucleotide repeats in sporadic colorectal cancer in Japan. Oncol Rep 23(2):551–561

29. Du W, Mah JT, Lee J et al (2004) Incidence and survival of mucinous adenocarcinoma of the colorectum: a population-based study from an Asian country. Dis Colon Rectum 47(1):78–85
30. Kang H, O'Connell JB, Maggard MA et al (2005) A 10-year outcomes evaluation of mucinous and signet-ring cell carcinoma of the colon and rectum. Dis Colon Rectum 48(6):1161–1168
31. Catalano V, Loupakis F, Graziano F et al (2012) Prognosis of mucinous histology for patients with radically resected stage II and III colon cancer. Ann Oncol 23(1):135–141
32. Park JS, Huh JW, Park YA et al (2015) Prognostic comparison between mucinous and nonmucinous adenocarcinoma in colorectal cancer. Medicine (Baltimore) 94(15):e658
33. Lim SB, Yu CS, Jang SJ et al (2010) Prognostic significance of lymphovascular invasion in sporadic colorectal cancer. Dis Colon Rectum 53(4):377–384
34. Huh JW, Lee JH, Kim HR et al (2013) Prognostic significance of lymphovascular or perineural invasion in patients with locally advanced colorectal cancer. Am J Surg 206(5):758–763
35. Chang S-CL, Wang H-S, Yang S-H, Jiang J-K, Lan Y-T, Lin T-C, Li F-Y, Chen W-S, Lin J-K (2012) Lymphovascular invasion determines the outcome of stage I colorectal cancer patients. Formosan Journal of Surgery 45:141–145
36. Nikberg M, Chabok A, Letocha H et al (2016) Lymphovascular and perineural invasion in stage II rectal cancer: a report from the Swedish colorectal cancer registry. Acta Oncol 55(12):1418–1424
37. Church TR, Wandell M, Lofton-Day C et al (2014) Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. Gut 63(2):317–325
38. Vidal J, Muinelo L, Dalmases A et al (2017) Plasma ctDNA RAS mutation analysis for the diagnosis and treatment monitoring of metastatic colorectal cancer patients. Ann Oncol 28(6):1325–1332
39. Melling N, Kowitz CM, Simon R et al (2016) High Ki67 expression is an independent good prognostic marker in colorectal cancer. J Clin Pathol 69(3):209–214
40. Li P, Xiao ZT, Braciak TA et al (2016) Association between Ki67 Index and Clinicopathological Features in Colorectal Cancer. Oncol Res Treat 39(11):696–702
41. Masuda T, Hayashi N, Kuroda Y, et al (2017) MicroRNAs as Biomarkers in Colorectal Cancer. Cancers (Basel) 9(9).
42. Gao S, Zhao ZY, Wu R et al (2018) Prognostic value of microRNAs in colorectal cancer: a meta-analysis. Cancer Manag Res 10:907–929
43. Rozek LS, Schmit SL, Greenson JK, et al (2016) Tumor-infiltrating lymphocytes, crohn's-like lymphoid reaction, and survival from colorectal cancer. J Natl Cancer Inst 108(8).
44. Ko YS, Pyo JS (2019) Clinicopathological significance and prognostic role of tumor-infiltrating lymphocytes in colorectal cancer. Int J Biol Markers: 1724600818817320.