Heliyon 10 (2024) e34220

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Molecular characteristics of patients with colorectal signet-ring cell carcinoma with different ABO blood groups

Wan-Ning Zhang [a,1], Wei-Jie Liang [b,1], Ying Zhang [a,1], Ming-Jian Liang [a,1], Ming-Juan Zhang [a], Qi Chen [a], Zhou-Pei Mo [a], Mei-Yi Wu [a], Xue-Zi Weng [a], Rui Han [a], Yong-Neng Liang [a], Miao-La Ke [a,2], Wen-Qian Lin [a,*,2]

[a] State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, 510060, China
[b] Department of Laboratory Medicine, Guangzhou First People's Hospital, South China University of Technology, Guangzhou, 510180, China

ARTICLE INFO

Keywords:
Colorectal neoplasms
Carcinoma
Signet ring cell
ABO blood-group system
Mutation
HLA antigens

ABSTRACT

Background: Colorectal signet-ring cell carcinoma (SRCC) is a rare subtype of malignant adenocarcinoma, accounting for approximately 1 % of colorectal cancer (CRC) cases. Its biomarkers and molecular characteristics remain controversial, and there are no specific therapeutic targets or strategies for its clinical treatment.

Methods: A retrospective study was conducted between January 2010 and December 2021. 1058 colorectal cancer cases from the Sun Yat-sen University Cancer Center and 489 cases from the Tumor Genome Atlas Project were included in the analysis, of which 64 were SRCC. Data extraction included patient demographics, blood types and risk factors, including clinical variables and genomics (either a 19-gene panel NGS or 1021-gene panel NGS). Univariate analyses were performed to identify factors significantly associated with overall survival.

Results: The blood groups of 27 (42.2 %), 18 (28.1 %), 12 (18.8 %), and seven (10.9 %) patients were classified as O, A, B, and AB, respectively. We found that O was a unique blood group characterized by a low frequency of KRAS mutations, a high frequency of heterozygosity at each HLA class I locus, and a high tumor mutational burden (TMB). Patients in blood group A with high-frequency KRAS mutations and those in blood group B with anemia and metabolic abnormalities required targeted treatment. Furthermore, genetic alterations in SRCC differed from those in adenocarcinoma and mucinous adenocarcinoma.

Conclusions: Our study revealed genomic changes in SRCC patients across different blood groups, which could advance the understanding and precise treatment of colorectal SRCC.

1. Introduction

Colorectal cancer (CRC) ranks as the third most common type of cancer worldwide [1], with adenocarcinoma (AC) [2] being its predominant histological subtype. Conversely, signet-ring cell carcinoma (SRCC) constitutes a rare subtype, comprising approximately

* Corresponding author. Department of Blood Transfusion, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in Southern China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, 510060, China.
E-mail address: linwq@sysucc.org.cn (W.-Q. Lin).
[1] These authors contributed equally to this work and share first authorship.
[2] Senior authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e34220
Received 8 May 2023; Received in revised form 4 July 2024; Accepted 5 July 2024
Available online 6 July 2024

1–3% [3] cases, distinguished by the presence of >50 % of tumor cells with prominent intracytoplasmic mucin, often displacing the nucleus [4,5]. Rare cancers can be defined as cancers with a prevalence of fewer than five cases per 100,000 people [6], and colorectal SRCC is undoubtedly a rare tumor because of its extremely low incidence [7]. Colorectal SRCC are associated with younger age and higher tumor grades [8,9]. It is more often diagnosed in patients with advanced-stage disease [10] and usually responds poorly to cytotoxic chemotherapy and radiotherapy compared to patients with conventional adenocarcinoma [11,12].

Many molecular studies have suggested different mechanisms of oncogenesis in SRCC and AC [13]. SRCC is characterized by a high rate of *MUC2* expression owing to the accumulation of mucin in the intracytoplasm [14]. SRCC is also associated with a high frequency of MSI-H [15] and mutations in the Ras/MAPK, PI3K/AKT, and EMT pathways [16–18]. Evidence has shown that colorectal SRCC remains a vague entity that resembles AC in some clinicopathological and molecular aspects [17] as well as mucinous adenocarcinoma (MAC). Exploring the differences in molecular characteristics is important for understanding the carcinogenic mechanism of SRCC.

The genomic landscape of colorectal SRCC has been debated. SRCC is still considered an unfavorable and unfamiliar subtype of the disease. Although colorectal SRCC is different from AC in terms of gene expression and histology, patients with SRCC currently receive treatments based on the same standard guidelines for classical AC because no clinical guidelines have been developed specifically for this entity [19]. Blood group antigens on erythrocyte membranes are inherited but are also found in the gastrointestinal mucosa [20]. The levels of gut microbiomes, such as *Faecalibacterium* and *Bacteroides,* are associated with ABO genes, and blood antigens are used as a preferred source of energy in the intestine [21]. However, a limited number of studies have investigated the potential association between ABO blood groups and the risk of developing bowel cancer, and have not distinguished between bowel cancer subtypes [22,23]. Thus, it is necessary to recognize blood groups based on molecular characteristics to guide individualized treatment of patients with SRCC.

In this study, we illustrate for the first time the differences in the molecular characteristics of ABO blood groups in SRCC. Additionally, we combined both the genomic and clinical variables of the ABO blood groups to generate a nomogram model with more accurate predictions than the clinical risk factors for prognosis.

Table 1
Summary of clinical characteristics of colorectal SRCC.

Variables	ABO blood group			
	A	B	O	AB
Age				
<30	6 (33.33)	2 (16.67)	4 (14.81)	1 (14.29)
30-60	9 (50.00)	7 (58.33)	17 (62.96)	5 (71.43)
≥60	3 (16.67)	3 (25.00)	6 (22.22)	1 (14.29)
Gender				
Male	13 (72.22)	4 (33.33)	22 (81.48)	5 (71.43)
Female	5 (27.78)	8 (66.67)	5 (18.52)	2 (28.57)
Smoking history				
Yes	6 (33.33)	3 (25.00)	15 (55.56)	0 (0.00)
No	12 (66.67)	9 (75.00)	12 (44.44)	7 (100.00)
Pathological stage				
I	0 (0.00)	0 (0.00)	1 (3.70)	1 (14.29)
II	2 (11.11)	1 (8.33)	2 (7.41)	2 (28.57)
III	4 (22.22)	1 (8.33)	13 (48.15)	4 (57.14)
IV	12 (66.67)	10 (83.33)	11 (40.74)	0 (0.00)
Family history				
Yes	5 (27.78)	2 (16.67)	7 (25.93)	0 (0.00)
No	13 (72.22)	10 (83.33)	20 (74.07)	7 (100.00)
Drinking history				
Yes	7 (38.89)	1 (8.33)	6 (22.22)	0 (0.00)
No	11 (61.11)	11 (91.67)	21 (77.78)	7 (100.00)
Treatment				
Surgery	14 (77.78)	8 (66.67)	24 (88.89)	5 (71.43)
Radiotherapy	3 (16.67)	2 (16.67)	7 (25.93)	3 (42.86)
Chemotherapy	16 (88.89)	11 (91.67)	26 (96.30)	6 (85.71)
Immunotherapy	0 (0.00)	1 (8.33)	2 (7.41)	1 (14.29)
Metastatic site				
Peritoneum	10 (55.56)	7 (58.33)	8 (29.63)	0 (0.00)
Lung	1 (5.56)	1 (8.33)	0 (0.00)	0 (0.00)
Bone	3 (16.67)	1 (8.33)	2 (7.41)	0 (0.00)
Liver	4 (22.22)	0 (0.00)	1 (3.70)	0 (0.00)
Urinary system	1 (5.56)	1 (8.33)	1 (3.70)	0 (0.00)
DL	12 (66.67)	5 (41.67)	4 (14.81)	0 (0.00)
No	6 (33.33)	2 (16.67)	16 (59.26)	7 (100.00)
Total	18	12	27	7

The number in the parentheses is the percentage of this category. DL: distant lymph node.

2. Materials and methods

2.1. Clinical specimens and study design

This single-center, observational, retrospective study was conducted at the Sun Yat-sen University Cancer Center (SYSUCC). A total of 64 consecutive cases of colorectal SRCC between January 2010 and December 2021 were retrospectively examined (Table 1). Inclusion criteria were as follows (a) pathologically confirmed colorectal SRCC by two pathologists independently through H&E staining, disease staging was performed according to the AJCC, 8th edition; (b) complete medical records containing genomic and clinical variables; (c) absence of previous or concurrent malignancy; and (d) follow-up for more than 3 months after the definite diagnosis. The Institutional Review Board of Sun Yat-Sen University Cancer Center approved this study, and the requirement for informed consent was waived by the ethics review boards (B2022-353-01, Guangdong, China). Patients' medical records and the follow-up tracking system were used to record clinical characteristics, treatment response and follow-up outcomes. None of the patients had received antitumor therapy prior to biopsy sampling.

We compared the molecular features of SRCC with those of two different intestinal cancer subtypes in SYSUCC and TCGA cohorts to reveal the genetic profile of SRCC. We collected 949 colorectal AC and 45 MAC samples from the ChangKang project (https://changkang.hapyun.com/) and obtained 52 cases of colorectal MAC and 437 cases of AC from the TCGA database (https://portal.

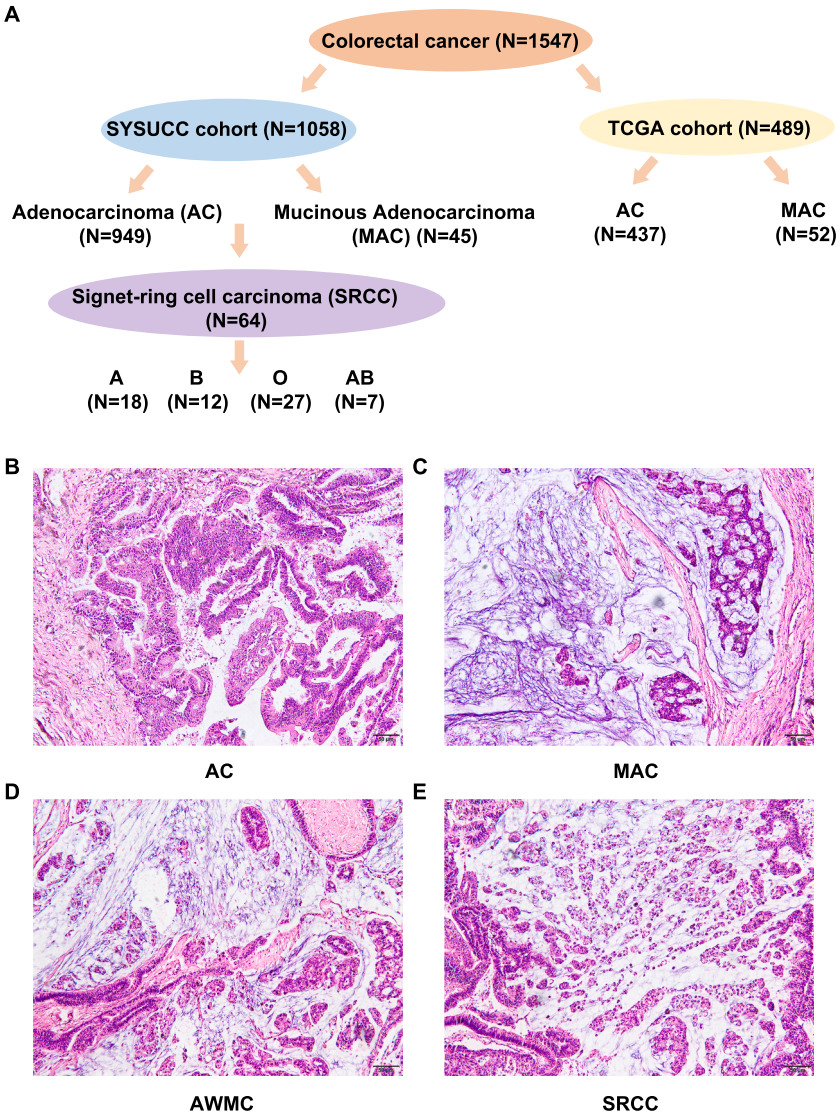

Fig. 1. HE staining of colorectal cancer (HEx100). A. Patient demographics. B. HE staining of Adenocarcinoma (AC) showing classical adenocarcinoma component. C. HE staining of Mucinous adenocarcinoma (MAC) showing mucin pool. D. HE staining of AWMC showing both classical adenocarcinoma component and mucinous component. E. HE staining of Signet ring cell carcinoma (SRCC) showing scattered signet ring cells.

gdc.cancer.gov/)(Fig. 1A). The genomic landscape of Chinese colorectal cancer from the Chang Kang project is currently the largest cancer genome study in China and the samples were also from our center. In addition, to demonstrate the differences in SRCC across ethnic groups, we compared genomic data from our center with another SRCC cohort (39 samples) in Northern Ireland [15]. Because the Northern Ireland study used a narrow panel sequencing approach, we chose to compare genes with mutation status determined in both datasets and compared microsatellite status and overall survival for samples from both datasets. Because only the samples from our center contain blood group information, the section on associated blood groups is only discussed for the 64 samples. As for the gene profiles, we compared the SRCC samples from our center with adenocarcinoma and mucinous carcinoma samples from our center and

Table 2
Univariate analyses of OS in patients with colorectal SRCC.

Variables	No. of patients	Overall Survival	
		HR (95 % CI)	*P* value
ABO blood group			
O	27	Reference	
A	18	2.649(0.838, 8.371)	0.097
B	12	3.023(0.919, 9.943)	0.069
AB	7	1.920(0.453, 8.131)	0.376
Gender			
Male	44	Reference	
Female	20	0.939(0.360, 2.450)	0.898
M stage			
M0	31	Reference	
M1	33	3.280(1.262, 8.522)	0.015
N stage			
N0	14	Reference	
N2	31	5.040(1.127, 22.537)	0.034
TNM stage			
Stage 2	9	Reference	
Stage 3	22	7.980(1.037, 61.423)	0.046
Peritoneal metastasis			
No	38	Reference	
Yes	26	3.330(1.372, 8.083)	0.008
Bone metastasis			
No	58	Reference	
Yes	6	2.748(0.591, 12.771)	0.197
Liver metastasis			
No	59	Reference	
Yes	5	2.713(0.784, 9.390)	0.115
Lung metastasis			
No	62	Reference	
Yes	2	15.365(2.794, 84.501)	0.002
DL metastasis			
No	43	Reference	
Yes	21	1.904(0.805, 4.504)	0.143
Surgery			
No	13	Reference	
Yes	51	0.630(0.242, 1.644)	0.345
Immunotherapy			
No	60	Reference	
Yes	4	0.373(0.049, 2.832)	0.340
Radiation			
No	49	Reference	
Yes	15	0.799(0.305, 2.093)	0.648
Chemotherapy			
No	5	Reference	
Yes	59	1.431(0.191, 10.731)	0.728
Transfusion			
No	54	Reference	
Yes	10	0.779(0.180, 3.370)	0.738
PT		1.542(1.161, 2.049)	0.003
INR		108.816(5.295, 2236.403)	0.002
TBA		1.036(1.014, 1.059)	0.001
HDL-C		5.148(1.477, 17.950)	0.010
ApoA1		6.943(1.368, 35.243)	0.019
CHO		1.572(1.084, 2.279)	0.017

HR: hazard ratio; CI: confidence interval.
M1: With distant metastasis; N2: 4 or more regional lymph node metastasis.
PT: Prothrombin time; INR: International normalized ratio; TBA: Total bile acid; HDL-C: High-density lipoprotein cholesterol; ApoA1: Apolipoprotein A1; CHO: cholesterol.

TCGA, respectively, and compared them with SRCC samples from centers in Northern Ireland to depict the differences in the mutational features of SRCC more comprehensively.

2.2. Pathologic evaluation

The slides from patients with a documented pathological diagnosis of signet-ring cells were retrieved for review and confirmation of the percentage of cells present. Two experienced gastrointestinal pathologists independently evaluated the haematoxylin and eosin (H&E) stained slides from the tumors. (Fig. 1). Classical adenocarcinoma (AC), mucinous adenocarcinoma (MAC), adenocarcinomas

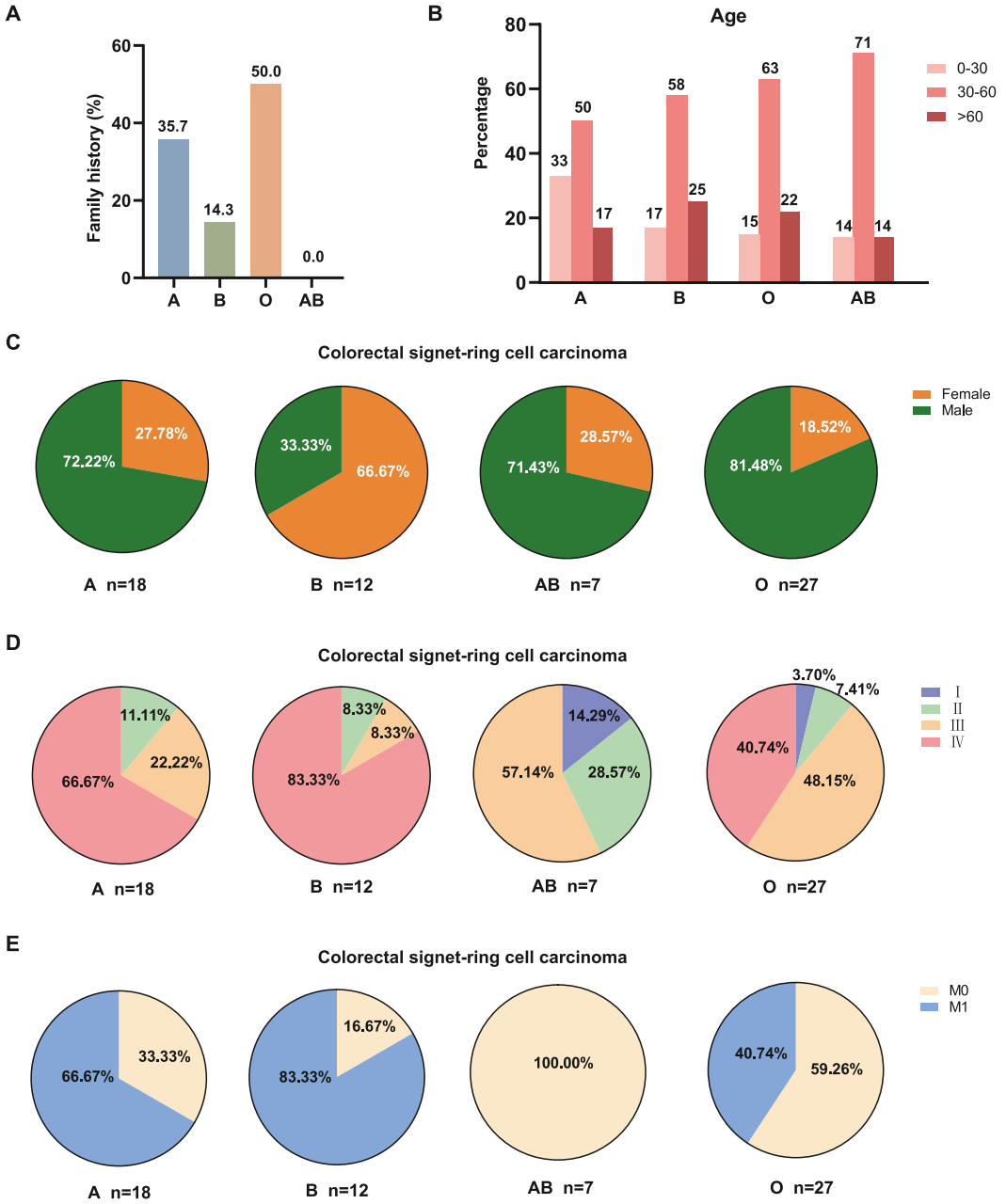

Fig. 2. Summarized characteristics of 64 colorectal SRCC patients with different ABO blood groups. A. Family history of SRCC patients with different ABO blood groups. B. Age distribution of SRCC patients with different ABO blood groups. C. Gender distribution of SRCC patients with different ABO blood groups. D. Pathology stage distribution of SRCC patients with different ABO blood groups. E. Metastases distribution of SRCC patients with different ABO blood groups.

with intra or extracellular mucin secretion (AWMC), and signet-ring cell carcinoma (SRCC) are shown in Fig. 1B–E. Tumors were categorised based on the proportion of signet ring cells, with a cut-off of ≥50 % defining SRCC. This designation typically indicates displacement and moulding of the nucleus (Fig. 1E).

2.3. Clinical characteristics

The following information was extracted from the medical records of patients: age, sex, ABO blood group, metastasis site, date of last follow-up and date of death (Table 1 and Table S1). The staging was performed according to the American Joint Committee on Cancer/Union for International Cancer Control TMN Staging System (version 8, 2017). The overall survival time (OS) was defined as the interval between the date at which the metastatic disease was diagnosed and the date of death from any cause. For those who were alive at the time of analysis, the last follow-up date was considered the point of censoring.

2.4. NGS and alteration identification

Primary SRCC samples from 10 patients were sequenced using a predefined set of validated assays. (v 1.0; Sequenom Inc., San Diego, CA, USA), The aforementioned methods were employed for the identification of 238 candidate mutations, which were found to be harbored by 19 cancer-associated genes: *ABL1, AKT1, AKT2, BRAF, CDK, EGFR, ERBB2, FGFR1, FGFR3, FLT3, HRAS, JAK2, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA*, and *RET* (Table S2). Mutations in each oncogene are listed in Supplemental Table S1. The methods have also been described in earlier papers [24]. High-performance liquid chromatography (HPLC) with purified water was used as a blank control and normal human leukocytes were used as negative controls. The assay protocols are presented in Table S3. The analysis of the matrix chips was conducted using MassARRAY Typer software (v4.0; Sequenom Inc.) with a predefined cutoff mutation frequency of 1 % [25].

A total of 11 patient samples were subjected to sequencing using a bespoke panel comprising 1021 genes related to cancer. The sequencing was performed at the Department of Molecular Diagnostics. The 1021 sequenced genes are listed in Table S4. Somatic variations were detected, including single nucleotide variations, small insertions and deletions (InDels), copy number variations, and gene fusions. The detailed protocols employed for each stage of the process, including sample preparation, genomic DNA extraction, library construction, target enrichment, next-generation sequencing (NGS), and data analysis, have been uploaded to the Research Data Deposit Public Platform (RDDA2022938685). Tumor mutational burden (TMB) was defined as the number of somatic mutations and insertion-deletion mutations per megabyte base within the coding regions of the tumor tissues (Tables S5–S6).

2.5. Statistical analysis

GraphPad Prism software, version 8, was used for data analysis. The Kaplan-Meier method was used to estimate survival distributions, the differences in OS were analyzed with the log-rank statistic and hazard ratios (HRs) were calculated using a univariate Cox regression analysis (Table 2, Tables S7–S8). All statistical tests were two-sided, and *P* values of less than 0.05 were deemed significant. Clinical follow-up was completed by March 2022.

2.6. Data sharing

The key raw data have been uploaded onto the Research Data Deposit public platform (RDD), with the approval RDD number of RDDA2022938685.

3. Results

3.1. Patient characteristics and metastatic patterns

We included 64, colorectal SRCC specimens obtained from Sun Yat-sen University Cancer Center. The clinicopathological characteristics of this cohort are summarized in Table 1 and Fig. 2. Of the 64 included patients, 44 were males (68.75 %) and 20 were females (31.25 %); the male to female ratio was 2.2. The age at which the diagnosis was made was, on average, 44.5 years (range, 20–91 years), and forty-five percent of patients(n = 29) were younger than 40 years at the time of diagnosis. Family history of cancer was reported in 21.88 % of the patients, whereas smoking history was reported in 34.38 % of the patients. Overall, 27 (42.2 %), 18 (28.1 %), 12 (18.8 %), and seven (10.9 %) patients had blood groups O, A, B, and AB, respectively.

A significant correlation between blood group and the prevalence of family history of cancer was identified. Specifically, individuals with blood group O exhibited a higher prevalence of family history of cancer, with 50 % reporting such history, compared to 35.7 % and 14.3 % of those with blood groups A and B, respectively (Fig. 2A). Thirty-three percent of the patients (6/18) in blood group A and 15 percent of the patients (4/27) in blood group O were younger than 30 years at the time of diagnosis (Fig. 2B). SRCC was significantly more prevalent in females in blood group B than in males in the other three blood groups (Fig. 2C). The proportions of patients with blood group O classified as having American Joint Committee on Cancer (AJCC) stages I, II, III, and IV disease were 3.70 %, 7.41 %, 48.15 %, and 40.74 %. Patients in A and B blood groups were more likely to belong to stage IV (Fig. 2D). Patients with blood group B had the highest rate of distant metastases, and those in blood group AB had the lowest rate (Fig. 2E).

Patients in blood group B had significantly worse OS than patients with blood group O (Fig. 3A–B), the prognosis of patients with

blood groups A and AB was not significantly different from that of blood group O (Figs. S1A–B). Six metastatic sites (the peritoneum, bone, liver, lungs, urinary system, and distant lymph nodes) were identified in the cohort. The lung was the least common metastatic site among those observed in patients diagnosed with metastatic colorectal SRCC, accounting for 6.1 % (2/33) of metastatic sites, respectively. The peritoneum was the most common metastatic site (72.7 %; 8/11) in patients with blood group O, and the distant lymph nodes were the most common metastatic sites (100 %; 12/12) in patients with blood group A (Fig. 3C). In colorectal SRCC, The prognosis for patients with peritoneal metastasis was found to be significantly worse than that of patients without metastasis ($P < 0.01$)

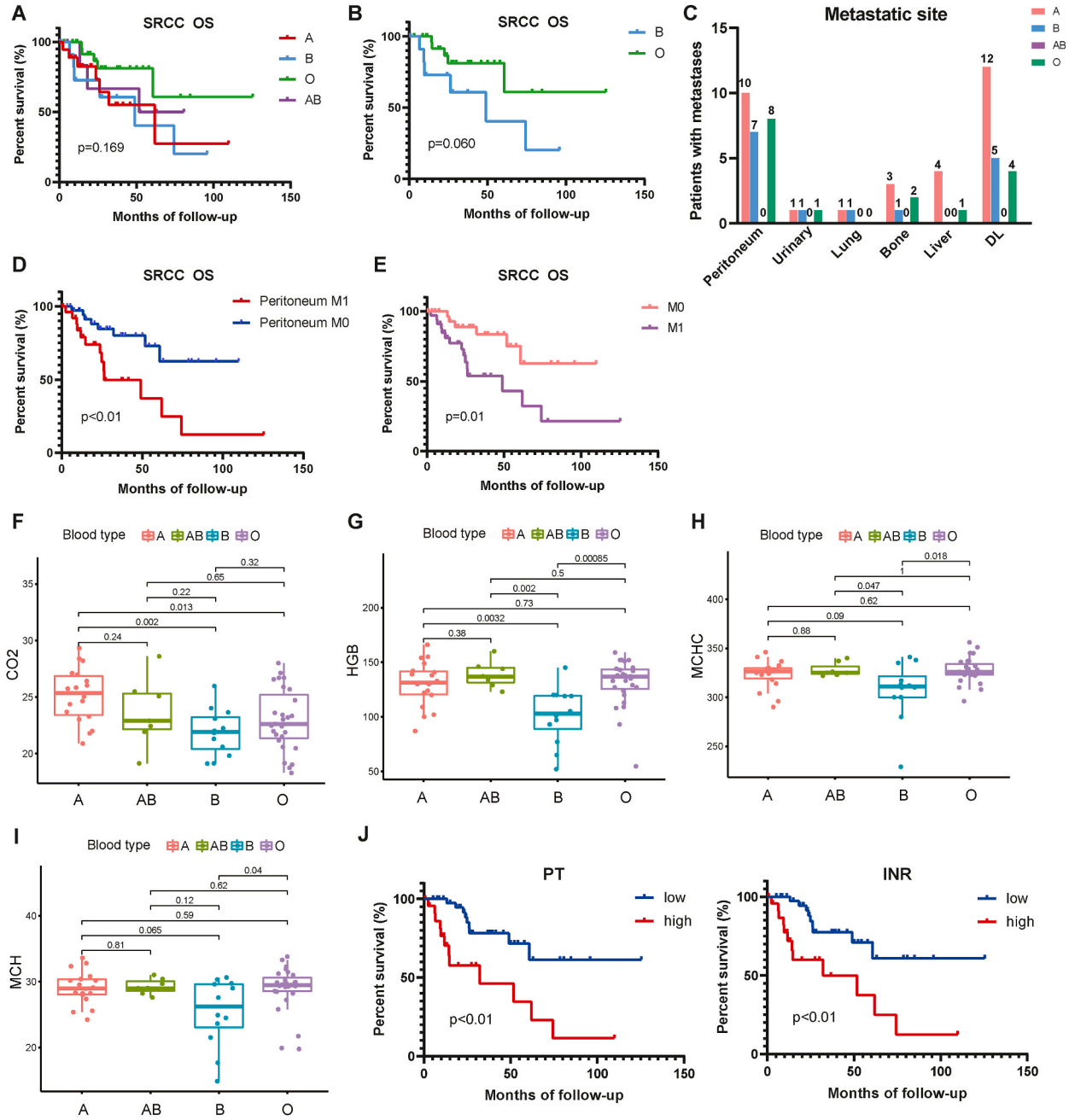

Fig. 3. Kaplan–Meier analysis of OS in colorectal SRCC patients and the differences in clinical variables between the four groups. A. Kaplan–Meier analysis of OS in patients with different blood groups (log-rank $P = 0.169$). B. Kaplan–Meier analysis of OS in patients with blood group B and O (log-rank $P = 0.060$). C. Distribution of distant metastatic sites in colorectal SRCC. D. Kaplan–Meier analysis of OS in patients with and without peritoneum metastasis (log-rank $P < 0.01$). E. Kaplan–Meier analysis of OS in patients with and without metastasis (log-rank $P = 0.01$). F. Serum CO2 levels of four blood groups. G. HGB levels of four blood groups. H. MCHC levels of four blood groups. I. MCH levels of four blood groups. J. Kaplan–Meier analysis of OS in patients with different groups (Coagulation-related clinical variables, log-rank P < 0.01).

(Fig. 3D). There was no significant difference in the prognostic impact of metastasis at other sites (Fig. S1C).

3.2. Clinical variables

The level of arterial PCO2 (PaCO2) in patients in blood group B was lower than that in patients in blood groups A and O ($P < 0.05$), suggesting that these patients had abnormal metabolic states (Fig. 3F). The RBC-related factors HGB, MCHC, and MCH were at their lowest levels in blood group B (Fig. 3G–I). Kaplan–Meier survival analysis and log-rank tests were conducted to evaluate the influence of several factors, including age, sex, histological grade, family history and clinical variables. Factors found to be significantly associated with worse OS were PT (>11.9 s), INR (>1.04), HDL-C (>1.23 mmol/L), serum TBA (>9.8 μmol/L), CHO (>4.64 mmol/L) and peritoneal metastasis (Fig. 3J, Fig. S1D, Table 2).

3.3. Molecular characteristics

A total of 11 cases were subjected to testing using the 1021-gene panel, while 10 cases were tested using the 19-gene panel. Genetic

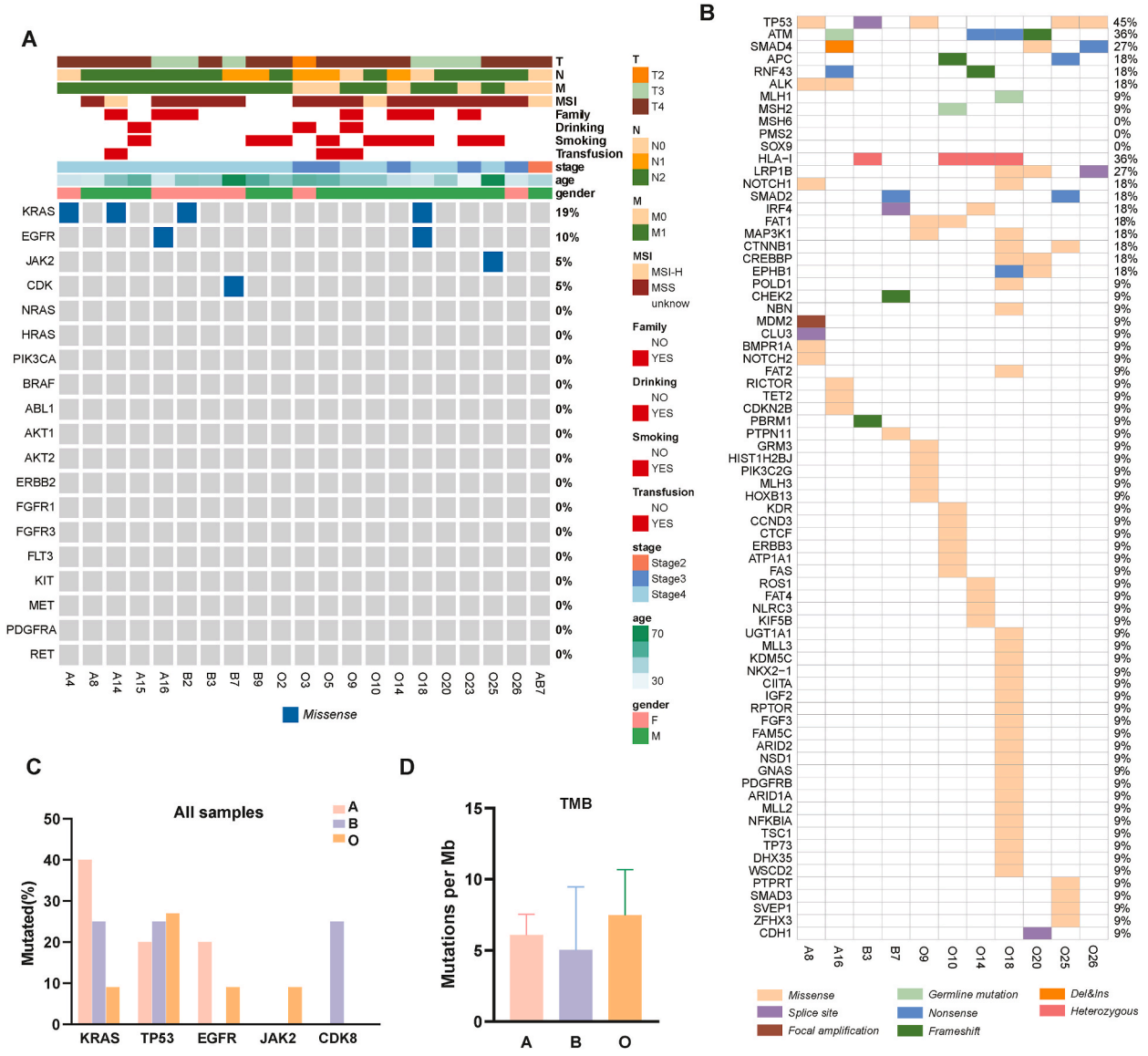

Fig. 4. The molecular features of colorectal SRCC patients with different ABO blood groups. A. Gene mapping of four blood groups in colorectal SRCC (19-gene panel). B. Gene mapping of four blood groups in colorectal SRCC (1021-gene panel). C. Comparison of mutation rates of cancer drivers of three blood groups in colorectal SRCC. D. Tumor Mutation Burden of three blood groups in colorectal SRCC.

mutations were detected in 66.7 % of SRCC tumors (14/21); details relating to gene mutation frequencies in the four blood groups are shown in Fig. 4. Compared with blood groups A and B, blood group O was commonly found in *KRAS* wild-type (WT) cells. Three cases (14.3 %, with high microsatellite instability) were considered hypermutated (Fig. 4A–C). The most common alteration in SRCC was a point mutation in *TP53* (four missense mutations and one splice site mutation, 5/11, 45 %), followed by HLA class I heterozygosity. (3/11, 36 %) and multiple mutations in *ATM* (3/11, 36 %). No BRAF mutations were detected in the cohort. By comparing mutated genes in different cancer signaling pathways, we found that the frequency of mutations in p53 (e.g., *TP53*) and TGF-β (e.g., *SMAD4*) pathways was higher, however the mutation burden in WNT, MAPK, and PI3K pathways were dramatically lower in colorectal SRCC (Fig. 4A–B). *TP53*, *ATM* mutations and HLA-I heterozygosity in patients were more prevalent in patients with blood group O than in

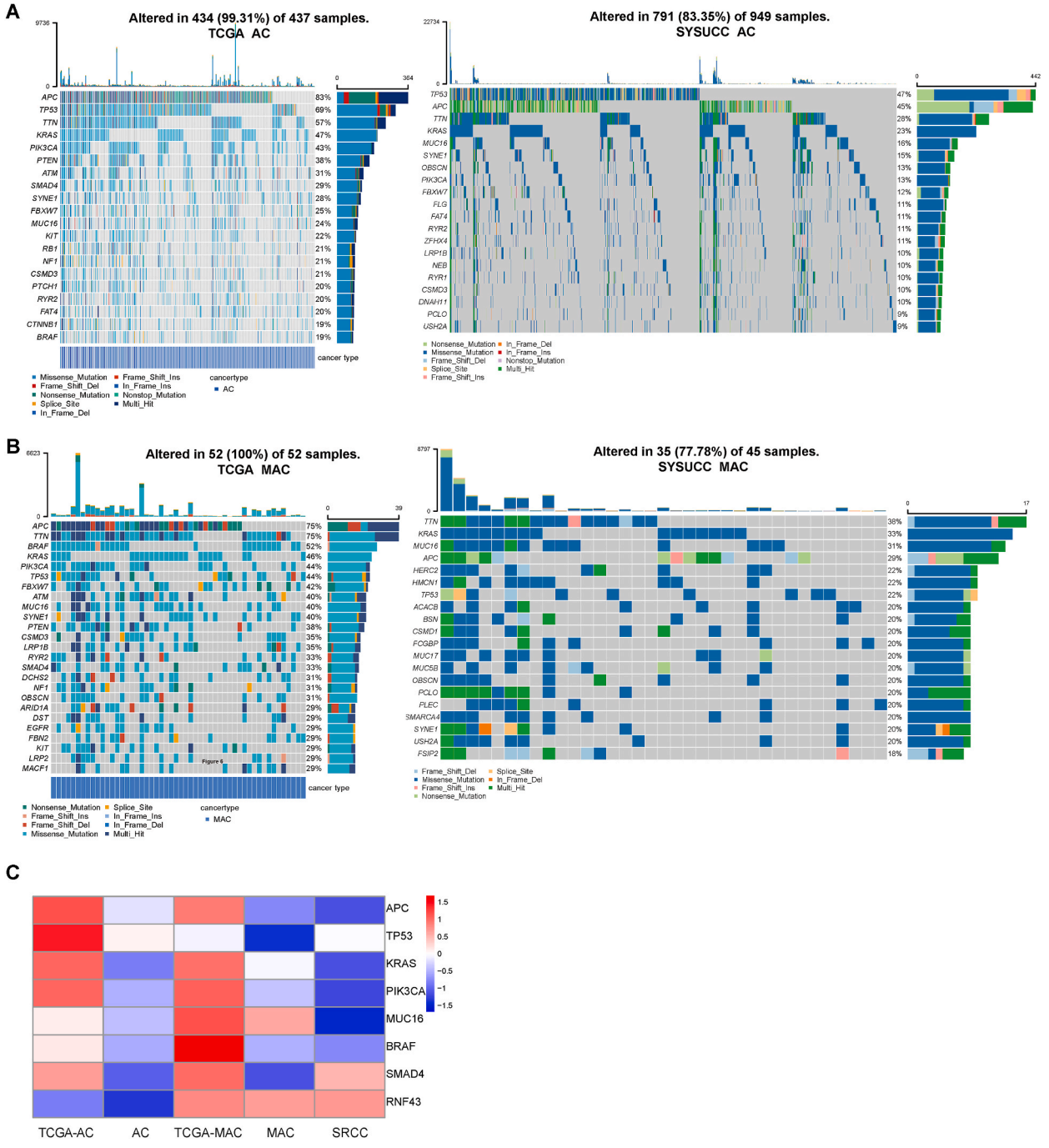

Fig. 5. Gene mapping of colorectal AC, MAC and SRCC. A. The molecular characteristics of AC. B. The molecular characteristics of MAC. C. High frequency mutations of AC, MAC and SRCC.

those with other blood groups. Patients in blood group O had higher TMB than those in the other groups (Fig. 4D).

We examined the genetic alterations among MAC, AC and SRCC using WES and used a database to compare the gene mutation frequencies between AC, MAC, and SRCC. The results showed that AC and MAC have different high-frequency driver genes, e.g., APC and TP53 are mutated more frequently in the AC group than in the MAC group, whereas MUC16 is more likely to be mutated in the MAC group (Fig. 5A and B). SRCC had a lower relative frequency of *APC*, *KRAS* and *PIK3CA* mutations than AC and MAC (Fig. 5). Cluster analyses of gene mutation frequency showed a significant difference between SRCC and other colorectal subtypes (Fig. 5C), SRCC had a higher relative frequency of *RNF43* mutations than AC, similar to MAC. These results indicate that the genetic alterations in SRCC are different from those in AC and MAC.

We also used the Northern Ireland' database to compare gene mutation frequencies, and the results were consistent with our data (Fig. 6A); the mutation rates of *KRAS* and *PIK3CA* were lower than those of intestinal adenocarcinoma in both groups. The validation cohort had 32 % *BRAF* mutations, whereas our sample of 21 patients had no mutations in this essential gene. The MSI sample accounted for 15.56 % of the microsatellite status in our cohort and 47.62 % in the other cohort (Fig. 6B). Patients in the SYSUCC cohort (n = 64) had a median overall survival of 61.9 months, while the median OS of the other cohort (n = 39) was 31.4 months (Fig. 6C).

4. Discussion

Colorectal SRCC is a distinct histological subtype based on its clinicopathological features, genetic features and clinical outcomes. We found that the mutation frequencies of classical bowel cancer driver genes such as *APC*, *PIK3CA*, and *KRAS* were significantly lower in SRCC than in the other two subtypes. This also corroborates the findings of a Fudan University study that the lack of activation of the MAPK and PI3K pathways may be responsible for the low proliferation phenotype of SRCC [4]. In contrast, the mutation rate of *RNF43* was significantly higher than that of adenocarcinoma (both TCGA and SYSUCC cohorts). *RNF43* is a key regulator of the WNT pathway, and its inactivation stabilizes *β-catenin* and leads to nuclear translocation of cells. This suggests that SRCC and AC/MAC activate the

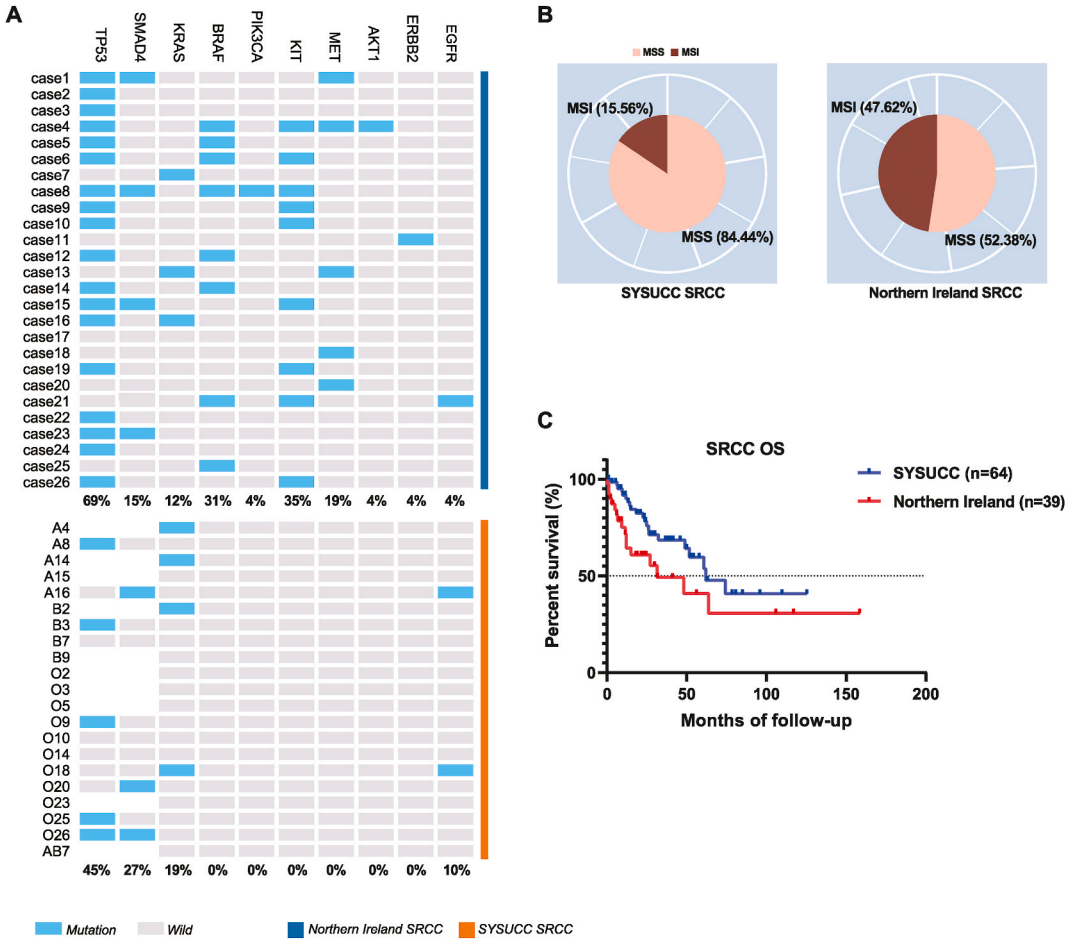

Fig. 6. Genomic differences between the SYSUCC cohort and the Northern Ireland cohort. A. Gene mapping of two centers. B. Microsatellite status of two centers. C. Kaplan–Meier analysis of OS.

Wnt-β-catenin pathway in different ways.

Second, ABO blood group antigens can influence tumorigenesis by altering intercellular adhesions, membrane signaling and immune surveillance [26]. Khalili et al. found no significant association between blood group B levels and the overall risk of colon cancer [27]. However, Urun et al. reported that ABO/Rh blood groups were significantly associated with CRC, and that there was no relationship between *KRAS* mutations and ABO blood groups [28]. None of the previous studies have analyzed the relationship between different subtypes of CRC and ABO blood groups. We found that patients with SRCC and blood group B were often diagnosed at an advanced stage and exhibited rapid disease progression. In addition, because of abnormal metabolism, PaCO2 levels were generally low in patients in blood group B, and the degree of anemia was more severe in patients in blood group B than in those in the other groups.

Lastly, colorectal SRCC is significantly associated with more peritoneal metastasis, lower *KRAS* or *BRAF* mutation rates [29], lower E-cadherin expression [30,31], and increased *MUC2* expression [32] than AC; however, some studies have shown that *BRAF* mutations must be closely associated with the presence of malignant signet ring cells, regardless of their percentages [33]. No BRAF mutations were detected in our cohort. The rate of BRAF mutations in SRCC was also extremely low in the Fudan cohort [4], which differs from the findings of previous studies, perhaps because of ethnic differences, which were largely based on the sequencing of European and American populations. Our results showed that the high-frequency mutations in blood group O were *TP53, ATM*, and HLA-I, while those in blood group A were *KRAS* and *ALK*. *KRAS* mutations influence multiple pathways, including the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR pathways, which regulate the cell cycle, promote cell growth, and suppress apoptosis, and play a crucial role in carcinogenic processes by inducing a plethora of inflammatory cytokines and chemokines, thereby promoting tumourigenesis and invasiveness [34]. The impact of *KRAS* mutations might explain the prognosis of patients in blood group A. Patients in blood group O had a higher TMB than the others did. HLA-I has been extensively used as a biomarker in immunotherapy, and its heterozygosity has been associated with disease progression [35]. Previous reports have shown that The presence of HLA-I homozygosity and a low mutation burden is significantly correlated with a reduction in survival compared to patients who exhibit heterozygosity for each class I locus. Furthermore, the combined effect of HLA class I heterozygosity and TMB on improved survival was found to be more pronounced than that of TMB alone [35,36]. Our results also showed that SRCC patients who were heterozygous at each HLA class I locus had better prognosis and belonged to blood group O. Moreover, patient O10 with an HLA-I heterozygous mutation survived for more than 10 years after receiving left hemicolectomy, appropriate chemotherapy and 12 doses of immunotherapy (Sintilimab Injection). In comparison with the Northern Ireland cohort, we found that the median survival of our patients was significantly longer, despite the lower proportion of MSI in our center sample. This may be due to the fact that four patients in our cohort received immunotherapy in addition to conventional surgery and radiation, and the overall survival of that patient who received immunotherapy was the best among all patients (both MSI-H and MSS groups). This study revealed, for the first time, the differences in the clinical features and molecular profiles of SRCC in different ABO blood groups. We performed exon sequencing in 21 cases of SRCC and found that patients with blood group O had the potential to benefit from immunotherapy and that people with blood groups A and B were at high risk of poor SRCC prognosis.

4.1. Implications and recommendations

SRCC is a rare subtype of colorectal cancer, previous SRCC studies have focused only on a few cancer gene mutations and expression or case reports. In our study, for the first time, we collected three subtypes of colorectal cancer from the SYSUCC and TCGA databases for molecular characterization and found that SRCC has a completely different mutation profile than AC and MAC. Precision therapeutic options should be sought based on the gene mapping features of SRCCs. Previous studies on the relationship between ABO blood groups and CRC have not been sufficiently illustrated. Our study revealed, for the first time, significant differences in laboratory parameters and molecular characteristics of SRCC patients with different ABO blood groups. Patients in blood group A with high-frequency KRAS mutations and those in blood group B with anemia and metabolic abnormalities required targeted treatment. Blood group O was heterozygous for each HLA class I locus and may benefit from immunotherapy.

4.2. Limitations

One of the limitations of this study was the small sample size due to the rarity of the tumor. The results of this study can be further confirmed if data from more research centers are available. However, our study provides data on the molecular characteristics of patients with different ABO blood groups that can be used for future high-quality and adequately powered studies on colorectal SRCC.

5. Conclusion

Colorectal SRCC represents a distinct subtype from common adenocarcinoma and mucinous adenocarcinoma, exhibiting markedly different molecular features. For instance, SRCC is characterized by an elevated frequency of mutations in *RNF43,* whereas driver genes, such as *APC* and *PIK3CA* exhibit low-frequency mutations. Our study revealed varied clinical features and molecular profiles of SRCC across different ABO blood groups. Furthermore, some clinical variables, such as PT and INR, have been proposed to predict the prognosis of SRCC. Finally, we identified blood group O as a potentially novel target for the immunotherapy of colorectal SRCC.

Funding

This research was supported by Health Commission of Guangdong Province Scientific Research Project (Grant No. A2023098).

Consent for publication

All authors reached an agreement to publish the study in this journal.

Ethics declarations

This study was reviewed and approved by The Institutional Review Board of Sun Yat-Sen University Cancer Center, with the approval number: B2022-353-01.

Informed consent was not required for this study because it was a retrospective study, clinical characteristics and follow-up results were retrieved from patient medical records and follow-up tracking system.

Data availability statement

All data to support the conclusions have been either provided or are otherwise publicly available.

The key raw data have been uploaded onto the Research Data Deposit public platform (RDD), with the approval RDD number of RDDA2022938685.

CRediT authorship contribution statement

Wan-Ning Zhang: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wei-Jie Liang:** Formal analysis, Data curation. **Ying Zhang:** Project administration, Formal analysis. **Ming-Jian Liang:** Visualization, Investigation. **Ming-Juan Zhang:** Investigation. **Qi Chen:** Supervision, Software. **Zhou-Pei Mo:** Validation, Software. **Mei-Yi Wu:** Investigation. **Xue-Zi Weng:** Project administration, Methodology. **Rui Han:** Investigation, Data curation. **Yong-Neng Liang:** Supervision, Investigation. **Miao-La Ke:** Writing – review & editing, Investigation, Data curation. **Wen-Qian Lin:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Professor Wen-Qian Lin for comments on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34220.

References

[1] E. Morgan, M. Arnold, Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from GLOBOCAN 72 (2) (2023) 338–344.
[2] L. Biller, D. Schrag, Diagnosis and treatment of metastatic colorectal cancer: a review, JAMA 325 (7) (2021) 669–685.
[3] A. Puccini, et al., Molecular profiling of signet-ring-cell carcinoma (SRCC) from the stomach and colon reveals potential new therapeutic targets, Oncogene 41 (26) (2022) 3455–3460.
[4] Y. Li, et al., Frequent RNF43 mutation contributes to moderate activation of Wnt signaling in colorectal signet-ring cell carcinoma, Protein & cell 11 (4) (2020) 292–298.
[5] Y. An, et al., Clinicopathological and molecular characteristics of colorectal signet ring cell carcinoma: a review, Pathol. Oncol. Res. 27 (2021) 1609859.
[6] S. Wang, et al., Comprehensive genomic profiling of rare tumors in China: routes to immunotherapy, Front. Immunol. 12 (2021) 631483.
[7] Global, regional, and national burden of colorectal cancer and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019, The lancet. Gastroenterology & hepatology 7 (7) (2022) 627–647.
[8] S. Belli, et al., Outcomes of surgical treatment of primary signet ring cell carcinoma of the colon and rectum: 22 cases reviewed with literature, Int. Surg. 99 (6) (2014) 691–698.
[9] P. Tawadros, et al., Adenocarcinoma of the rectum in patients under age 40 is increasing: impact of signet-ring cell histology, Dis. Colon Rectum 58 (5) (2015) 474–478.
[10] M. Weng, et al., Characteristics of primary signet ring cell carcinoma of colon and rectum: a case control study, BMC Gastroenterol. 22 (1) (2022) 173.
[11] X. Kong, et al., Characteristics and prognostic factors of colorectal mucinous adenocarcinoma with signet ring cells, Cancer Manag. Res. 9 (2017) 573–580.
[12] L. Pozos-Ochoa, et al., Prognosis of signet ring cell carcinoma of the colon and rectum and their distinction of mucinous adenocarcinoma with signet ring cells. A comparative study, Pathol. Oncol. Res. 24 (3) (2018) 609–616.

[13] A. Bellan, et al., Early signet ring cell carcinoma arising from colonic adenoma: the molecular profiling supports the adenoma-carcinoma sequence, Hum. Pathol. 50 (2016) 183–186.
[14] J. Nam, et al., Molecular characterization of colorectal signet-ring cell carcinoma using whole-exome and RNA sequencing, Translational oncology 11 (4) (2018) 836–844.
[15] M. Alvi, et al., Molecular profiling of signet ring cell colorectal cancer provides a strong rationale for genomic targeted and immune checkpoint inhibitor therapies, British journal of cancer 117 (2) (2017) 203–209.
[16] J.-Y. Nam, et al., Molecular characterization of colorectal signet-ring cell carcinoma using whole-exome and RNA sequencing, Translational Oncology 11 (4) (2018) 836–844.
[17] K. Korphaisarn, et al., Signet ring cell colorectal cancer: genomic insights into a rare subpopulation of colorectal adenocarcinoma, British journal of cancer 121 (6) (2019) 505–510.
[18] K. Tajiri, et al., Investigation of clinicopathological characters and gene expression features in colorectal signet-ring cell carcinoma utilizing CMS classification, Mol Clin Oncol 14 (5) (2021) 98.
[19] N. Hugen, et al., Colorectal signet-ring cell carcinoma: benefit from adjuvant chemotherapy but a poor prognostic factor, Int. J. Cancer 136 (2) (2015) 333–339.
[20] A. Gampa, et al., Relationships between gastrointestinal microbiota and blood group antigens, Physiol. Genom. 49 (9) (2017) 473–483.
[21] E. Lopera-Maya, et al., Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project, Nat. Genet. 54 (2) (2022) 143–151.
[22] J. Huang, et al., ABO blood type and the risk of cancer - findings from the Shanghai Cohort Study, PLoS One 12 (9) (2017) e0184295.
[23] A. Al-Sawat, et al., Relationship between ABO blood group and the risk of colorectal cancer: a retrospective multicenter study, Journal of clinical medicine research 14 (3) (2022) 119–125.
[24] K. Korphaisarn, et al., Signet ring cell colorectal cancer: genomic insights into a rare subpopulation of colorectal adenocarcinoma, Br. J. Cancer 121 (6) (2019) 505–510.
[25] J. Peng, et al., Oncogene mutation profile predicts tumor regression and survival in locally advanced rectal cancer patients treated with preoperative chemoradiotherapy and radical surgery, Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 39 (7) (2017) 1010428317709638.
[26] A. Al-Sawat, et al., Relationship between ABO blood group and the risk of colorectal cancer: a retrospective multicenter study, J. Clin. Med. Res. 14 (3) (2022) 119–125.
[27] H. Khalili, et al., ABO blood group and risk of colorectal cancer, Cancer Epidemiol. Biomarkers Prev. 20 (5) (2011) 1017–1020.
[28] Y. Urun, et al., ABO and Rh blood groups and risk of colorectal adenocarcinoma, Asian Pac J Cancer Prev 13 (12) (2012) 6097–6100.
[29] H. Kim, et al., Genomic alterations in signet ring and mucinous patterned colorectal carcinoma, Pathol. Res. Pract. 215 (10) (2019) 152566.
[30] B.R. Song, C.C. Xiao, Z.K. Wu, Predictors of lymph node metastasis and prognosis in pT1 colorectal cancer patients with signet-ring cell and mucinous adenocarcinomas, Cell. Physiol. Biochem. 41 (5) (2017) 1753–1765.
[31] M.E. Borger, et al., Signet ring cell differentiation in mucinous colorectal carcinoma, J. Pathol. 212 (3) (2007) 278–286.
[32] Y. Li, et al., A novel human colon signet-ring cell carcinoma organoid line: establishment, characterization and application, Carcinogenesis 41 (7) (2020) 993–1004.
[33] S. Yalcin, O. Onguru, BRAF mutation in colorectal carcinomas with signet ring cell component, Cancer biology & medicine 14 (3) (2017) 287–292.
[34] S. Rahman, et al., Therapeutic targets of KRAS in colorectal cancer, Cancers 13 (24) (2021) 6233.
[35] D. Chowell, et al., Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy, Science 359 (6375) (2018) 582–587. New York, N.Y.
[36] B. Li, et al., Landscape of tumor-infiltrating T cell repertoire of human cancers, Nat. Genet. 48 (7) (2016) 725–732.