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Original Article

Early Explosive Recurrence of Hepatocellular Carcinoma after Radical Resection: Risk Factors and Clinical Significance

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Abstract

Background and objectives: Although many patients with hepatocellular carcinoma (HCC) achieved significant prognostic improvement through surgical treatment, rapid and aggressive intrahepatic dissemination within months after radical resection was occasionally encountered. To date, there has been no dedicated literature addressing this phenomenon.

Methods: In this case-control study, we proposed the concept of early explosive recurrence (EER), which was defined as simultaneous development of no less than three recurrent lesions involving at least three different Couinaud's segments within 6 months following radical resection. A total of 325 patients (17 EER patients and 308 controls) were retrospectively reviewed.

Results: The incidence of EER of early HCC patients was 5.2%. EER was manifested as either multiple nodular (n = 11) or diffusely infiltrative type (n = 6). EER patients had significantly worse survival (hazards ratio, 48.7; 95% confidence interval: 19.9, 119.0). At multivariate analysis, increased tumor number, enlarged tumor size, positive microvascular invasion (MVI) and Glypican-3 (GPC3) were significant risk factors for EER. Tumor immune microenvironment (TIME) gene expression profile analysis showed that patients with EER presented higher transcriptional levels of CCL20, NT5E, and TDO2 as well as a lower transcriptional level of HLA-DQ1. Gene set enrichment analysis revealed that gene sets involving Th1 and Th2 differentiation (p = 0.016) and Th17 cell differentiation (p = 0.049) were enriched in control group.

Conclusions: Some surgically treated HCC patients developed EER with poor prognosis. The clinicopathological risk factors of EER included tumor number, tumor size MVI, and GPC3. Specific TIME profiles facilitate intrahepatic metastasis and progression of HCC.

Keywords: Hepatocellular carcinoma; Hepatectomy; Radical resection; Early explosive recurrence.

Abbreviations: AFP, alpha-fetoprotein; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; CCL20, C-C motif chemokine ligand 20; CD, cluster of differentiation; CI, confidence interval; CTL, cytotoxic T lymphocytes; CYT, cytolytic activity; DC, dendritic cell; DCP, des-gamma-carboxy prothrombin; DEG, differentially expressed genes; DI, diffusely infiltrative; EER, early explosive recurrence; GEP, gene expression profile; GO, gene ontology; GPC3, glypican-3; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; HCVAb, anti-hepatitis C virus antibodies; HLA, human leukocyte antigen; IFN-γ, interferon gamma; KEGG, Kyoto Encyclopedia of Genes and Genomes; MN, multiple nodular; mRECIST, modified response evaluation criteria in solid tumors; MVI, microvascular invasion; NK, natural killer; NLR, neutrophil-to-lymphocyte ratio; NR, not reached; NR (Table 1), not relevant; NT5E, ecto-5′-nucleotidase; OR, odds ratio; OS, overall survival; PLT, platelets; ROC, Receiver Operating Characteristic; PRF1, perforin; PT, prothrombin time; RFS, recurrence-free survival; SD, stable disease; TACE, transarterial chemoembolization; TB, serum total bilirubin; TDO2, tryptophan 2,3-dioxygenase; Teff, T-effector; Th1, T-helper 1; Th2, T-helper 2; Th17, T-helper 17; TIME, tumor immune microenvironment; TIS, T-cell inflamed signature; Treg, regulatory T cells; WBC, white blood cell.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies and is estimated to be responsible for nearly 830,180 deaths per year in the world.[1] Advances in diagnostic imaging and widespread application of screening programs in high-risk populations have improved the detection of HCC at early stages. In selected patients with preserved liver function and early-stage HCC, liver resection is associated with a 5-year survival rate of approximately 70%.[2] However, recurrence rates at 5 years following liver resection have been reported to exceed 70%.[3] The recurrence of HCC is often divided into early (less than 2 years) and late recurrence (more than 2 years) according to time to recurrence after surgery.[4] It was hypothesized that early recurrence is most likely the consequence of occult metastasis from the initial tumor, whereas late recurrence is mainly attributable to a second primary lesion.

In clinical practice, rapid (within a few months) and aggressive (involving several segments) recurrence of HCC after radical resection was occasionally encountered. It often manifests as multifocal lesions without clear boundaries and infiltrates the whole liver on magnetic resonance scan, resembling a "bloom of fireworks" and has a dismal prognosis. The impressive and distinctive nature suggests it belongs to a discrete subset of early recurrence and should be treated distinctly. However, previous studies concerning this topic are limited. In the current study, we recapitulated such a phenomenon and solemnly named it early explosive recurrence (EER). It is important to note that many EER patients were eligible surgical candidates upon preoperative evaluation, suggesting that there may be a deeper immunological mechanism behind such scenarios.

During tumor progression, both tumor and stromal cells orchestrate a strongly immunosuppressive tumor milieu, and ultimately shape the tumor immune microenvironment (TIME).[5] For instance, tumor-specific T cells and natural killer (NK) cells mediate tumor immunity, while macrophages are gradually hijacked and switched to promote survival and metastasis of tumor cells as the tumor progresses.[6] In the immunosuppressive microenvironment, persistent antigen stimulation induces T-cell exhaustion, which prevents the effective killing of tumor cells.[7] Based on multiple cell types involved in the HCC development, novel classifications of TIME have been proposed.[8,9] Notably, specific TIME subtypes including enrichment in effector T cells and, presence of tertiary lymphoid structures were associated with response to systemic therapy.[10,11] However, the correlation between TIME and postoperative recurrence is rarely reported. In this regard, a comprehensive evaluation of TIME in EER patients may promote the understanding of the tumor-immune interaction during tumor progression and intrahepatic metastasis in HCC.

In this study, we investigated the clinical and pathological risk factors which contribute to the development of EER in Chinese HCC patients. Furthermore, the specific TIME profile of EER was evaluated by performing gene expression profile (GEP) analysis on surgical specimens. Our results may help interpret the potential mechanism of EER phenomenon and improve treatment strategies for early HCC patients. We present the following article in accordance with the STROBE reporting checklist.

Methods

Definition and radiologic characteristics of EER

The EER phenomenon was occasionally encountered in clinic during routine follow-up of patients receiving hepatectomy as initial treatment for HCC. For the convenience of subsequent research, the precise definition of EER was refined as the following criteria (simplified as a "3-3-6" rule): (1) resection should be radical as confirmed by pathology in patients with resectable HCC. HCC with vascular invasion or extrahepatic metastases, which hamper the attainability of radical resection and patients underwent palliative or tumor-reductive surgery were excluded; (2) simultaneous development of no less than three intrahepatic recurrent lesions; (3) the involvement of at least three different Couinaud's segments (the involvement of the original surgical zone or extrahepatic metastasis is allowed but not required); (4) time from hepatectomy to recurrence which meets the above criteria should be less than 6 months. EER can only be diagnosed if all the above four criteria are met. Recurrence was diagnosed based on the radiological profile of newly onset low-density, T1 hypo-intense, T2, and diffusion hyperintense lesions with its typical enhancement manner on CT or magnetic resonance scan. EER can be subgrouped as multiple nodular (MN) or diffusely infiltrative (DI) subtype (Fig. 1). Specifically, the DI HCC was characterized by the lack of a well demarcated boundary on cross-sectional imaging, and was often associated with portal invasion.[12] Miliary enhancement was occasionally observed in the DI subset.

Enrollment of study patients

This study was designed as a retrospective case-control study and approved by the ethical committee of Eastern Hepatobiliary Surgery Hospital (2018-1-001). Written informed consent was obtained from all participants. The inclusion criteria of the study population were: (1) Resectable HCC without extrahepatic spread and macrovascular invasion in well compensated patients before operation, which was confirmed by magnetic resonance imaging (MRI) [Barcelona Clinic Liver Cancer (BCLC) stage 0, A, and B]. (2) Resection should be radical as confirmed by pathology. (c) Complete follow-up data could be obtained. The exclusion criteria were: (1) Patients who underwent preoperative antitumoral treatment including systemic or locoregional therapies; (2) HCC with vascular invasion or extra-hepatic metastases; (3) Patients who underwent palliative or tumor-reductive surgery; (4) Resections for recurrent HCC; (5) Patients who died within 30 days after operation of surgical-related complications; (6) Patients lost to follow-up. In our clinical database, from 2015-07 to 2021-12, 438 patients underwent liver resection for HCC consecutively. According to the selection criteria, 325 patients were finally included in our study (Fig. S1). During the follow-up period, 25 patients showed recurrence within 6 months after hepatectomy, of whom 17 patients met the criteria of EER. The study patients were divided into two groups: those with EER (n = 17) and controls (those without EER, n = 308). The operative procedure was determined based on the anatomical location of tumors and liver function. Radical resection was defined as removal of all recognizable tumors with a clear margin. The clinical and pathological information of the 325 patients was extracted for further analysis. A gross lesion adjacent to the major focus (with a distance of <1 cm) and with a diameter of <1 cm was regarded as satellites. Otherwise, multiple foci were diagnosed.[13]

Follow-up protocol

After discharge, tumor markers alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) were monitored and MRI was conducted every 3 months for 2 years. Then, we screened patients by tumor marker measurement and ultrasound every 3 months, and MRI every 6 months. Recurrence was diagnosed based on the

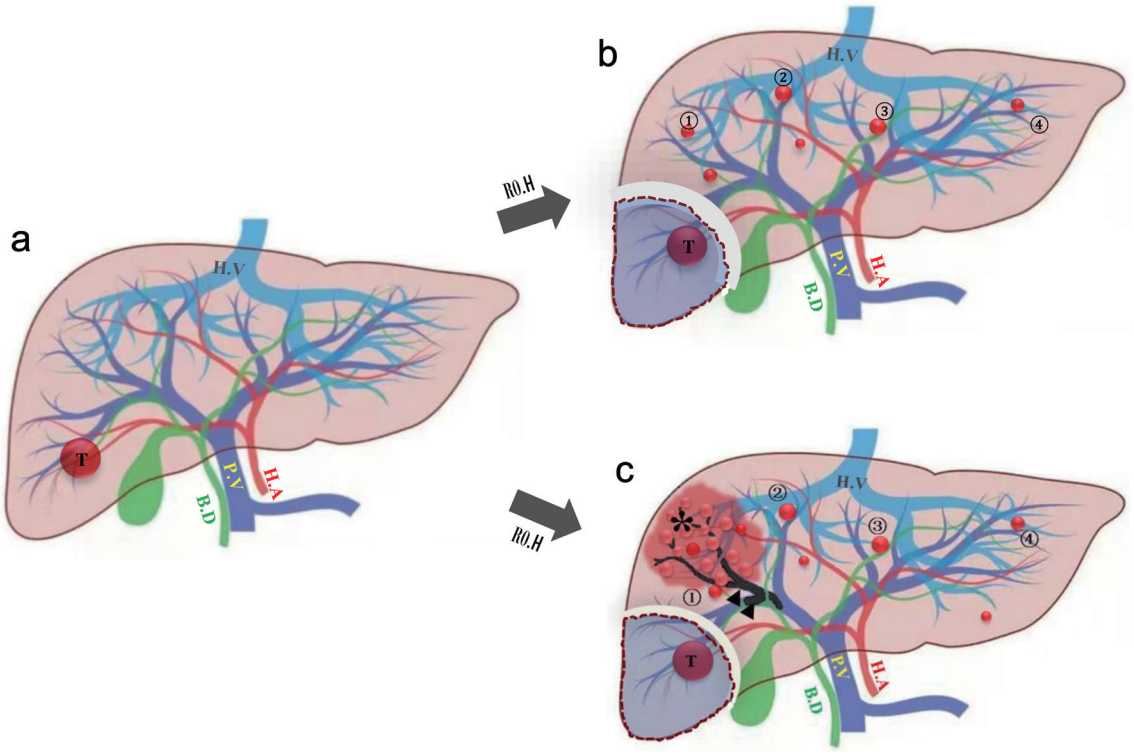

Fig. 1. Illustration of EER of HCC after R0 hepatectomy. EER is defined as the simultaneous development of at least three recurrent lesions spanning at least three distinct Couinaud's segments within 6 months following radical resection (the 3–36 rule). Based on the radiological features, EER can be classified into two subtypes, namely the MN subtype and the DI subtype. (a) Resectable HCC without extrahepatic spread and macrovascular invasion in well compensated patients before operation; (b) MN subtype of EER; (c) DI subtype of EER, characterized by the lack of well-demarcated boundary on cross-sectional imaging (asterisk) and the propensity to involve the adjacent portal vein (arrowheads). Arrowheads, portal vein invasion with the forming of tumor thrombus is a common but not necessary feature of DI HCC; Asterisk, recurrent lesions with ill-demarcated boundary, which is indicative of the permeative growth pattern; B.D, bile duct; DI, diffusely infiltrative; EER, early explosive recurrence; H.A, hepatic artery; H.V, hepatic vein; HCC, hepatocellular carcinoma; MN, multiple nodular; P.V, portal vein; R_0.H, R_0 hepatectomy; T, resectable HCC without gross vascular invasion at initial assessment; Red balls, ①②③④, recurrent lesions.

combined findings of these clinical examinations. Patients who developed recurrence were treated with repeat hepatic resection, transcatheter arterial embolization, or systemic agents. Treatment responses for these recurrent lesions were based on modified response evaluation criteria in solid tumors (mRECIST).[14] If extrahepatic recurrence was suspected (on the basis of clinical symptoms or unexplained elevation of a-fetoprotein level), chest CT, brain MRI, or whole-body bone scintigraphy were also performed. Models of recurrence were classified as EER of non-EER, according to the study definition. Recurrence-free survival (RFS) was defined as the time from date of resection to date of first recurrence or death by any cause, whichever happened first. Overall survival (OS) was defined as the time from date of resection to date of death regardless of the cause of death.

NanoString platform for gene expression analysis

Six patients from each group were randomly selected for GEP analysis. Histologic macro-dissection was performed on the 5 µm section tumor tissue slides. RNA was isolated from dissected tumor tissue using RNeasy FFPE kit (Qiagen, Hilden, Germany). A tissue surface area of approximately 50 mm^2 was used to harvest the necessary amount of RNA (~50 ng). RNA was input directly into the nCounter platform (NanoString Technologies, Seattle, WA, USA) for the hybridization reaction. Subsequent transcriptome analysis is based on the 289-immuno-gene panel. This panel allows simultaneous analysis of 289 genes involved in tumor-immune interaction. All transcriptomic data was quality controlled for imaging, binding density, positive control linearity, positive control limit of detection, according to the manufacturer's instructions. The raw data of each sample and gene were standardized against internal External RNA Controls Consortium controls to eliminate technical variability of the assay, and then counts were normalized to the geometric mean of endogenous housekeeping genes followed by $\log_2$ transformation.

Analysis of differentially expressed genes (DEGs)

The R package "limma" (version 4.1.0) was used for DEG. Limma powers differential expression analyses for RNA sequencing and microarray studies.[15] Limma returned empirical Bayes moderated-t *p*-values and adjusted *p*-values (Q-value) to correct for multiple comparisons testing using the Benjamini-Hochberg method. The significance of determining DEGs was a *p*-value of < 0.05 and expression fold change ≥ 2 or $\leq 1/2$. Kyoto Encyclopedia of Genes and Genomes (KEGG) canonical pathway enrichment analysis was performed using WebGestaltR v 0.1.1.

Gene set enrichment analysis (GSEA)

GSEA is a computational method that determines whether a predefined set of genes [those belonging to a specific gene ontology (GO) term or KEGG pathway] shows statistically significant, con-

cordant differences between two biological states.[16] We performed GSEA analysis using the clusterProfiler package gseKEGG and gseGO functions in R, with parameters of 1,000 permutations and a *p*-value cutoff of 0.05, are ranked according to the fold change in expression of 289 immune-related genes in the two groups. The enrichment statistic was set to classic for this analysis.

Immune infiltration deconvolution analysis

For immune cell infiltration assay, marker genes of 14 immune cell types, including B cells, dendritic cells, macrophages, exhausted $CD8^+$ T cells, $CD8^+$ T cells, neutrophils, mast cells, cytotoxic cells, Tregs, NK $CD56^{dim}$ cells, NK cells, $CD45^+$ leukocytes, and Th1 cells were retrieved as previously reported.[17] We further divided macrophages into macrophages M1 and macrophages M2 as described previously.[18,19] M1 (classical) macrophages are capable of inhibiting tumor growth by direct cytotoxicity and antibody-dependent cell-mediated cytotoxicity, while M2 macrophages support tumor progression by promoting angiogenesis, tumor cell invasion and metastasis.[20] All TIME cell infiltration scores were calculated as arithmetic mean of the constituent genes.

Study of TIME signatures

For study of TIME signatures, several gene sets containing genes associated with immune-associated biological processes were constructed according to previous reports.[21–24] Those signature reporting systems include cytotoxic T lymphocytes levels, cytolytic activity score, T-effector (Teff) gene signature and an 18-gene T-cell inflamed signature. Specifically, the cytolytic activity score was calculated from the geometric mean of the normalized read counts for perforin (referred to as PRF1) and granzyme A,[24] the T-cell inflamed signature score contained interferon-gamma (IFN-γ)–responsive genes related to antigen presentation, chemokine expression, cytotoxic activity, and adaptive immune resistance which are necessary for clinical benefit on immunotherapy,[21] the Teff gene signature was defined by CD8A, EOMES, PRF1, IFN-γ, and CD274.[25]

Statistical analysis

Quantitative variables were reported as median and interquartile range. Univariable and multivariable logistic regression models were used to identify factors associated with EER. Variables with *p*-values < 0.20 in univariate analysis were included in the multivariate regression analysis. For GEP analysis, the Wilcoxon rank sum test was used to compare the estimated immune cell types and immune signature scores between two groups. Boxplots were generated in R with the ggplot2 package, indicating the median and interquartile range. Survival functions were estimated using the Kaplan–Meier method and differences in survival rates between the study groups were compared using a Cox proportional hazards regression model. $p < 0.05$ was considered statistically significant.

Results

Patients

Based on the principle of case-control studies, we retrospectively included 17 patients who met the criteria of EER as cases and 308 patients as controls. Of the 325 patients, 315 had early-stage HCC (BCLC stage 0 or A), and the remaining 10 patients had intermediate-stage HCC (BCLC stage B) before resection. The median follow-up time was 35.7 months. During the entire follow-up period, recurrence was seen in 104 patients. Twenty-five patients had recurrence within 6 months, of whom 17 were diagnosed with EER. The incidence of EER in early HCC patients was 5.2%. Patients were followed up for 22.5 [95% confidence interval (CI): 8.8, not reached (NR)] and 36.2 (95% CI: 33.4, 39.7) months in the case and control groups, respectively. The clinical and pathological characteristics of these patients are shown in Table 1. The demographic and clinical characteristics of the study patients are shown in Table S1.

EER phenomenon after radical resection of HCC

The median time to EER was 3.1 (95% CI: 2.2, 3.7) months. According to the radiological profiles, 11 patients were subclassified as an MN subtype, the other six patients were subclassified with a DI subtype. A representative case of MN and DI subtypes was shown in Figure 2. In the MN subgroup, the median number of EER lesions was 6 (3–7), the median number of the involved segments was 4 (3–4.25). In the DI subgroup, the median number of the involved segments was 3 (3–8). Lung metastasis was found in three patients in the MN subgroup, and portal vein invasion was found in one patient in the DI subgroup. The median OS of EER patients was 16.9 (95% CI: 9.3, 20.4) months. EER was significantly associated with worse prognosis (hazards ratio, 48.7; 95% CI: 19.9, 119.0, $p < 0.001$, compared with an NR median OS in the control group; Fig. 3a). We also tested whether EER was associated with poor survival in recurred HCC. Patients in control group were divided into recurred (non-EER) and recurrence-NR groups. The median OS of non-EER patients was also NR. Using Cox proportional hazards regression model, the hazards ratio for EER was 20.1 (95% CI: 7.9, 51.3), compared with non-EER patients (Fig. 3b).

Treatment of EER of HCC

In the MN subgroup, two patients underwent reoperation, and nine received transarterial chemoembolization (TACE). Three patients treated with TACE also received a TKI (sorafenib or lenvatinib) and PD-1 monoclonal antibody combination therapy. Three patients treated with TACE also received TKI monotherapy as systemic treatment. In the DI subgroup, all six patients were treated with TACE. Four patients also received targeted therapy, three of whom also received PD-1 mAb. Based on the mRECIST 1.1 criteria, partial response was achieved in six of the 17 patients (35.3%), and a stable disease was achieved in two patients (11.8%). However, four patients experienced progressive disease after initial partial remission or stable disease. The median time to progression was 5.9 (95% CI: 2.1–9.1) months. Therapeutic results are summarized in Table S2 and Figure 3c.

Risk factors of EER of HCC

Univariate analysis revealed that AFP ($p = 0.034$), tumor number ($p = 0.002$), tumor size ($p < 0.001$), the sum of tumor size ($p < 0.001$), the presence of microscopic satellites ($p = 0.017$) and microvascular invasion (MVI) ($p = 0.002$) were significant risk factors for the occurrence of EER (Table 1). Multivariate analysis found that tumor number [$p = 0.001$; odds ratio (OR), 7.387; 95% CI: 2.369, 23.040], tumor size ($p < 0.001$; OR, 1.461; 95% CI: 1.218, 1.752), the presence of MVI ($p = 0.005$; OR, 7.912; 95% CI: 1.888, 33.152), and positive immunohistochemical staining of glypican-3 (GPC3, $p = 0.023$; OR, 6.542; 95% CI: 1.297, 33.009) were significant (Table 1). In the regression model, the log of odds of EER was equal to logit = 2 × number + 0.379 × size + 2.068 × MVI + 1.878 × GPC3 – 9.845. Consecutively, the risk of EER can be calculated by $r = [1 + \exp(-\text{logit})]^{-1}$. A nomogram was

Table 1. Comparison of clinical and pathological characteristics of the EER group and the control group

Parameter	Patients with EER, *n* = 17	Controls, *n* = 308	Univariate analysis		Multivariate analysis	
			Odds ratio	*p*-value	Odds ratio	*p*-value
Age in years	53 (45–59)	57.5 (50–65)	0.967 (0.925, 1.011)	0.149	NR	NR
Male sex, %)	16 (94.1)	264 (85.7)	2.667 (0.344, 20.618)	0.347	NR	NR
WBC as 10^9/L	4.8 (3.6–5.6)	5.1 (4.1–6.1)	0.741 (0.509. 1.078)	0.117	NR	NR
NLR	2.0 (1.8–2.4)	1.8 (1.3–2.4)	1.060 (0.798, 1.407)	0.687	NR	NR
PLT as 10^9/L	162 (110–196)	152 (119–193)	1.001 (0.992, 1.010)	0.721	NR	NR
TB in mmol/L	13.7 (10.2–15.9)	13.8 (10.8–18.5)	0.989 (0.936, 1.044)	0.695	NR	NR
Alb in g/dL	42.5 (41.1–46)	42.5 (40.3–44.7)	1.071 (0.941, 1.219)	0.296	NR	NR
ALT in IU/L	37 (23–48)	25 (18–37)	1.003 (0.992, 1.014)	0.562	NR	NR
AST in IU/L	32 (26–36)	23 (19–31)	1.004 (0.990, 1.019)	0.500	NR	NR
PT in s	11.6 (11.1–12.4)	11.4 (10.9–11.9)	0.994 (0.913, 1.082)	0.987	NR	NR
HBsAg-positive, %	15 (88.2)	249 (80.8)	1.777 (0.396, 7.984)	0.453	NR	NR
HCVAb-positive, %	1 (5.9)	8 (2.6)	2.328 (0.274, 19.764)	0.439	NR	NR
Log HBVDNA in IU/mL	2 (0–4)	0 (0–3)	1.175 (0.952, 1.451)	0.131	NR	NR
AFP in ng/mL	351.5 (19.8–1,210)	15.1 (3.8–166.4)	1.000 (1.000, 1.000)	0.034	NR	NR
DCP in mAu/mL	1,185 (344–13,993)	144 (40–759)	1.000 (0.999, 1.000)	0.362	NR	NR
Tumor number	1 (1–1)	1 (1–1)	5.206 (1.844, 14.692)	0.002	7.387 (2.369, 23.040)	0.001
Maximum tumor size in cm	5.5 (3–9.5)	3.5 (2.4–4.5)	1.347 (1.157, 1.569)	<0.001	1.461 (1.218, 1.752)	<0.001
Total tumor size in cm	6.5 (4–9.5)	3.5 (2.4–4.8)	1.376 (1.179, 1.608)	<0.001	NR	NR
BCLC stage B, %	2 (11.7)	8 (2.6)	5 (0.975, 25.621)	0.054	NR	NR
Resection margin in cm	0.8 (0–1.6)	0.6 (0–1.3)	1.372 (0.858, 2.193)	0.187	NR	NR
Poorly differentiated, %	15 (88.2)	295 (95.8)	0.331 (0.068, 1.599)	0.169	NR	NR
Gross satellite-positive, %	2 (11.7)	14 (4.5)	2.8 (0.582, 13.456)	0.199	NR	NR
Microscopic satellite-positive, %	12 (70.6)	122 (39.6)	3.659 (1.258, 10.645)	0.017	NR	NR
Satellite-positive, %	14 (82.4)	124 (40.3)	6.925 (1.949, 24.598)	0.003	NR	NR
MVI-positive, %	14 (82.4)	122 (39.6)	7.114 (2.003, 25.275)	0.002	7.912 (1.888, 33.152)	0.005
Glypican-3-positive, %	14 (82.4)	196 (63.6)	2.667 (0.750, 9.480)	0.130	6.542 (1.297, 33.009)	0.023
Cirrhosis, %	4 (23.5)	115 (37.3)	0.516 (0.164, 1.621)	0.258	NR	NR

AFP, alpha-fetoprotein; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BCLC, Barcelona Clinic Liver Cancer; DCP, des-γ-carboxy prothrombin; EER, early explosive recurrence, HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCVAb, anti-hepatitis C virus antibodies; MVI, microvascular invasion; NLR, neutrophil-to-lymphocyte ratio; NR, not relevant; PLT, platelets; PT, prothrombin time; TB, serum total bilirubin; WBC, white blood cell.

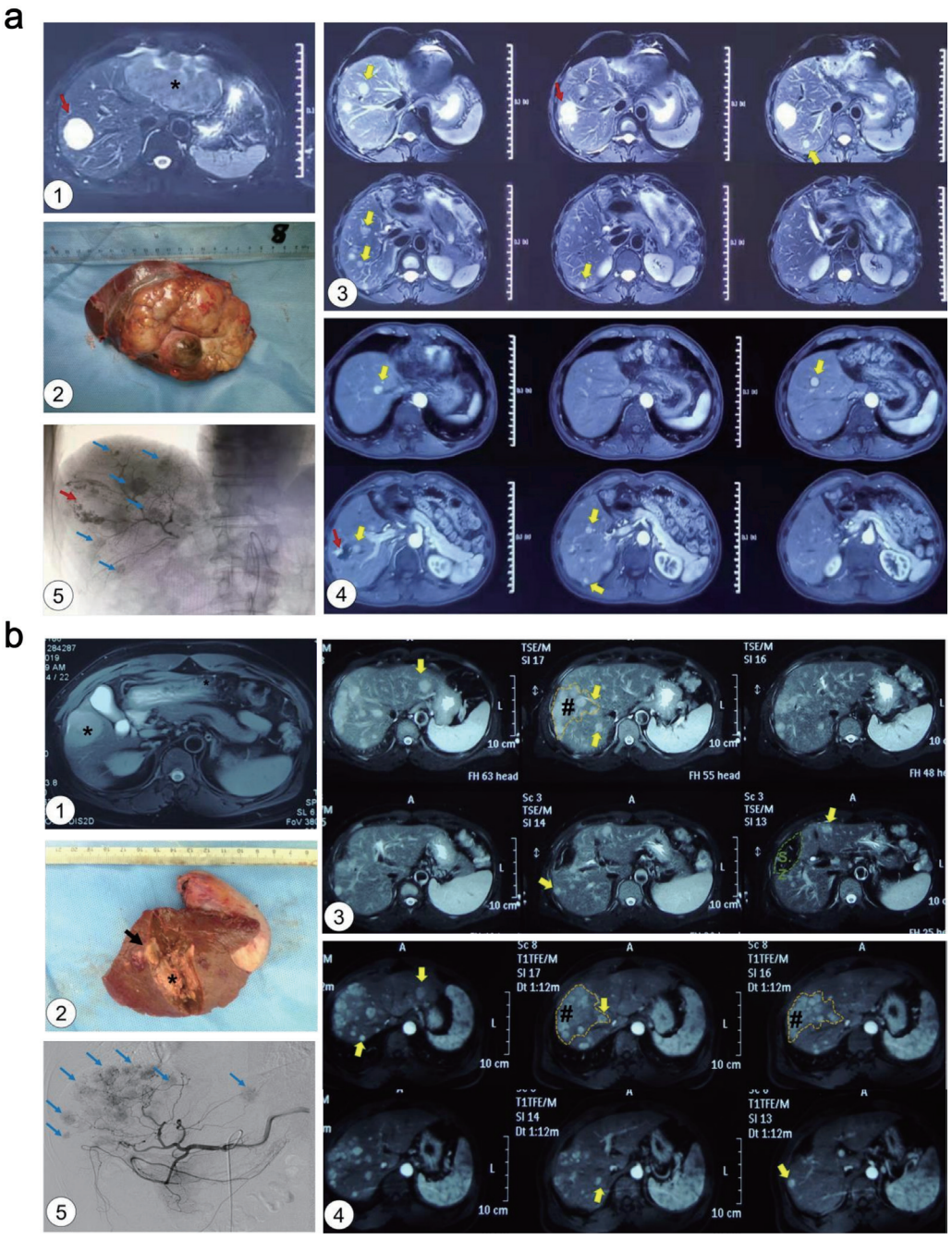

Fig. 2. Representative cases of EER of HCC after liver radical resection. (a) MN subtype of EER (Patient No. 15 in Table S3). (1) T2-weighted MRI before operation showed solitary HCC (asterisk, 11.5 cm×8.0 cm in size) involving segments II/III/IV without macrovascular invasion (red arrow denotes a hemangioma); (2) Gross specimen of left hemi-hepatectomy. HCC with confluent multinodular type and positive for MVI was confirmed by pathology. (3) and (4) Contrast-enhanced MRI image obtained 3.7 months after operation (T2-weighted and axial arterial phase T1-weighted) showed multiple recurrent nodules (yellow arrows) enhanced at arterial phase with washout at equilibrium phase (not shown), involving segments V/VI/VII/VIII without tumor thrombus or involvement of the surgical margin. (5) Upon recurrence, trans-arterial chemoembolization was performed and multiple lipiodol-staining nodules (blue arrows, red arrow denotes the hemangioma) resembling "bloom of fireworks" were seen by angiography. (b) The DI subtype of EER (Patient No. 8 in Table S2). (1) T2-weighted MRI before operation showed solitary HCC (asterisk, 4.3 cm × 4.0 cm in size) involving part of segment V/VI with no evidence of tumor thrombus. (2) Gross specimen of liver segmentectomy. HCC with positive for MVI was confirmed pathologically. A 1 cm satellite (black arrow) was found adjacent to the main tumor (asterisk). (3) and (4) Contrast-enhanced MRI image obtained 2.6 months after operation (T2-weighted and axial arterial phase T1-weighted) showed patchy areas of heterogeneous enhancement diffusely involving segments II, III, V, VII and VIII (number sign and yellow arrows) with ill-demarcated boundary. The SZ was free from local tumor recurrence and tumor thrombus was not obviously visible. (5) Multiple lipiodol-staining nodules (blue arrows) resembling "bloom of fireworks" were seen by angiography. DI, diffusely infiltrative; EER, early explosive recurrence; HCC, hepatocellular carcinoma; MN, multiple nodular; MRI, magnetic resonance image; MVI, microvascular invasion; SZ, surgical zone.

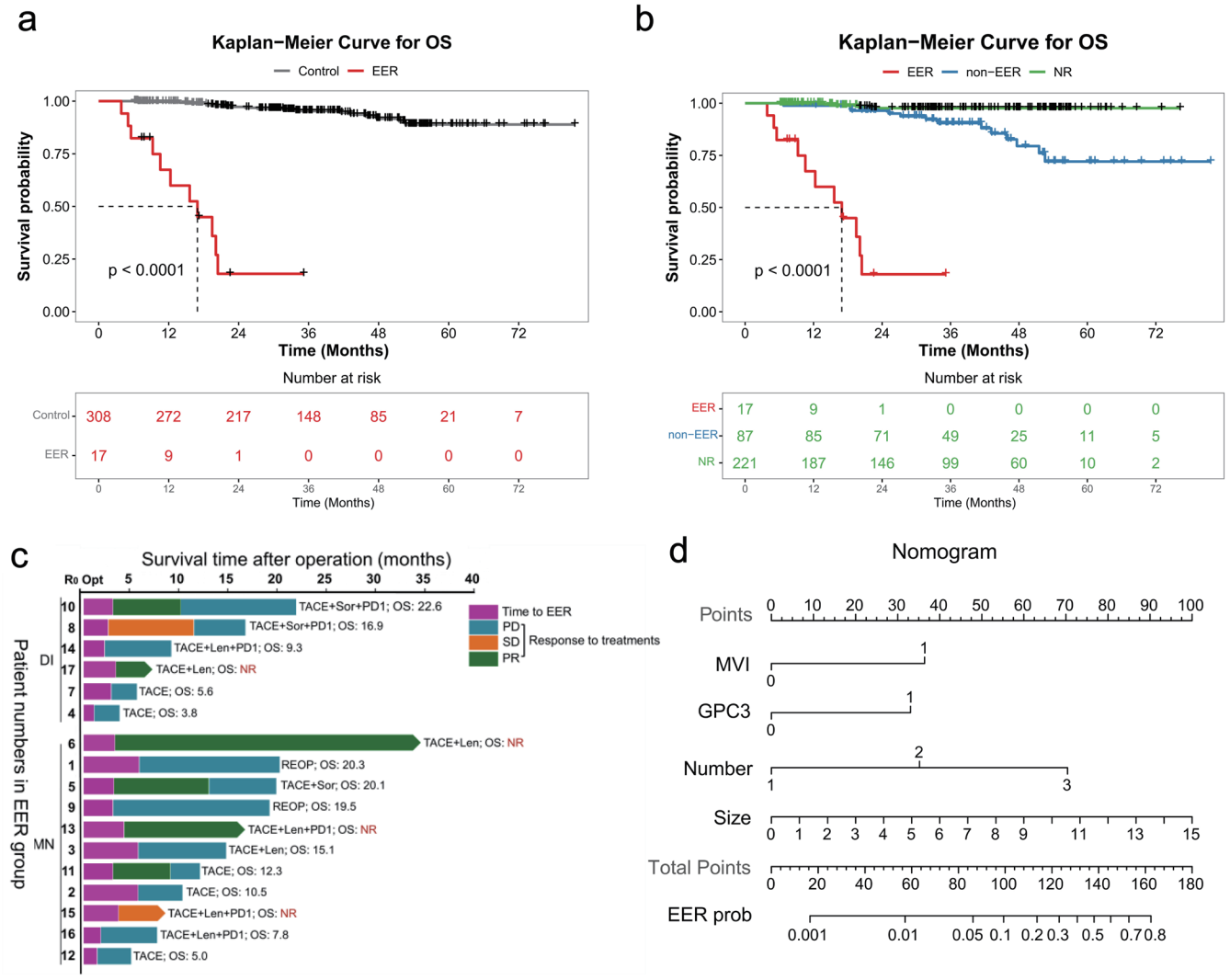

Fig. 3. Kaplan–Meier survival estimates of overall survival, treatment course, and risk factors of EER. (a) Median overall survival of EER patients was 16.9 (95% CI: 9.3, 20.4) months, compared with NR in the control group (hazard ratio, 48.7, 95% CI: 19.9, 119.0, $p < 0.001$). The censor date is indicated with plus symbols. (b) Patients in control group were divided into recurred (non-EER) and recurrence-NR groups. The hazards ratio for EER was 20.1 (95% CI: 7.9, 51.3, $p < 0.001$), compared with non-EER patients. The censor date is indicated with plus symbols. (c) Schematic diagram of the disease course and response patterns of EER patients, grouped by subtypes of EER. (d) Nomogram constructed based on logistic regression to predict postoperative EER. CI, confidence interval; DI, diffusely infiltrative subtype; EER, early explosive recurrence; Len, lenvatinib; MN, multiple nodular subtype; NR, not reached; OS, overall survival; PD, progressive disease; PD1, Programmed death-1 monoclonal antibody treatment; PR, partial remission; SD, stable disease; Sor, sorafenib; TACE, transarterial chemoembolization.

constructed based on logistic regression to predict postoperative EER (Fig. 3d). The area under the curve of ROC for the regression model was 0.897 (Fig. S2). This model can help clinicians in predicting the probability of EER after radical surgery.

TIME GEP analysis

To explore the possible immune biomarkers associated with EER, the TIME-associated GEP of the 12 patients randomly selected from the two groups (six patients in each group) were compared using a 289 gene panel (Fig. 4a). DEG analysis showed that EER patients had higher transcriptional levels of CCL20, NT5E, and TDO2 as well as a lower transcriptional level of HLA-DQ1, compared with the control group (Fig. 4b and Table S3). KEGG pathway enrichment analysis revealed no significant differences between the two groups. However, GSEA analysis revealed that gene sets involving Th1 and Th2 differentiation ($p = 0.016$; Fig. 4c) and Th17 cell differentiation ($p = 0.049$; Fig. 4d) were both enriched in the control group.

We further investigated the differences in cell type scores and TIME signatures between the two groups. No significant differences in the 16 infiltrating immune cell scores were detected between the two groups (all $p > 0.40$; Fig. S3). Among all measured TIME signatures (Fig. 5), there was a nonsignificant trend toward lower Teff scores in EER patients compared with the control group ($p = 0.065$; Fig. 5a). The Teff score of each patient and expression level of component genes was presented in Figure 5b and c. Significantly prolonged RFS was verified in patients with higher Teff scores by log-rank tests ($p = 0.022$; Fig. 5d).

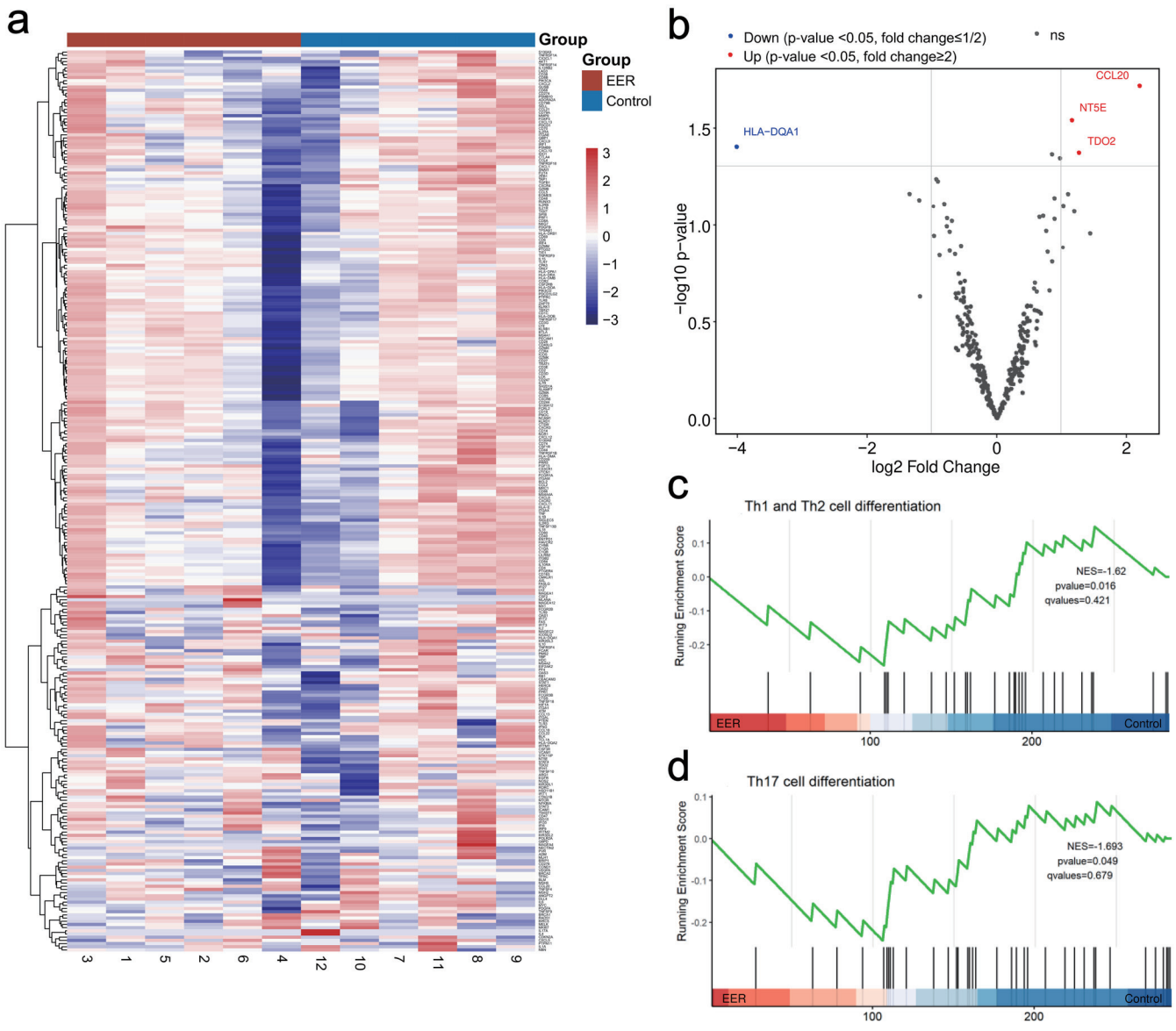

Fig. 4. Immune gene expression of patients in EER and control groups. (a) Heatmap of expression level of 289 genes in EER and control groups (rows denote genes and columns represent samples (relatively upregulated, red; down-regulated, blue). (b) DEGs in the EER and control groups. To compare the TIME profile of EER and control patients, we randomly selected six samples from each group. The R package "limma" (version 4.1.0) was used to identify DEGs. The significance criteria for determining DEGs (EER compared with control) was $p < 0.05$ and an expression fold change ≥2 or ≤1/2. Patients with EER had higher transcriptional levels of CCL20, NT5E and TDO2 and lower transcriptional levels of HLA-DQ1. (c, d) Enrichment of Th1 and Th2 (C) and Th17 (D) differentiation in control groups by gene set enrichment analysis. $n = 6$ in each group. CCL20, C-C motif chemokine ligand 20; DEG, Differentially expressed gene; EER, early explosive recurrence; HLA, human leukocyte antigen; NT5E, ecto-5′-nucleotidase; TDO2, tryptophan 2,3-dioxygenase; Th1, T-helper 1; Th2, T-helper 2; Th17, T-helper 17; TIME, tumor immune microenvironment.

Discussion

Rapid acceleration of HCC has been reported during a variety of treatment modalities, including immunotherapy, trans-arterial chemoembolization, and radiofrequency ablation.[26–28] Although postoperative recurrence of HCC is common, the special nature of EER warrants special treatment. To our knowledge, this is the first study to report the frequency, risk factors, and clinical significance of EER in a large cohort.

In this study, several risk factors, including tumor number, tumor size, the presence of MVI, and positive GPC3 staining on IHC appeared to promote EER of HCC after radical resection. Currently, most consensus opinions and guidelines recommend liver resection in cases without macrovascular invasion and extrahepatic metastases, multinodular HCC was not an absolute contraindication for surgery.[29–31] It should be noted that all patients included in our study were resectable by both anatomical and oncological criteria. On one hand, our results support a more rigorous patient selection strategy regarding tumor burden assessment before surgery. On the other hand, the EER risk model may help clinicians in planning adjuvant therapies for resected HCC.

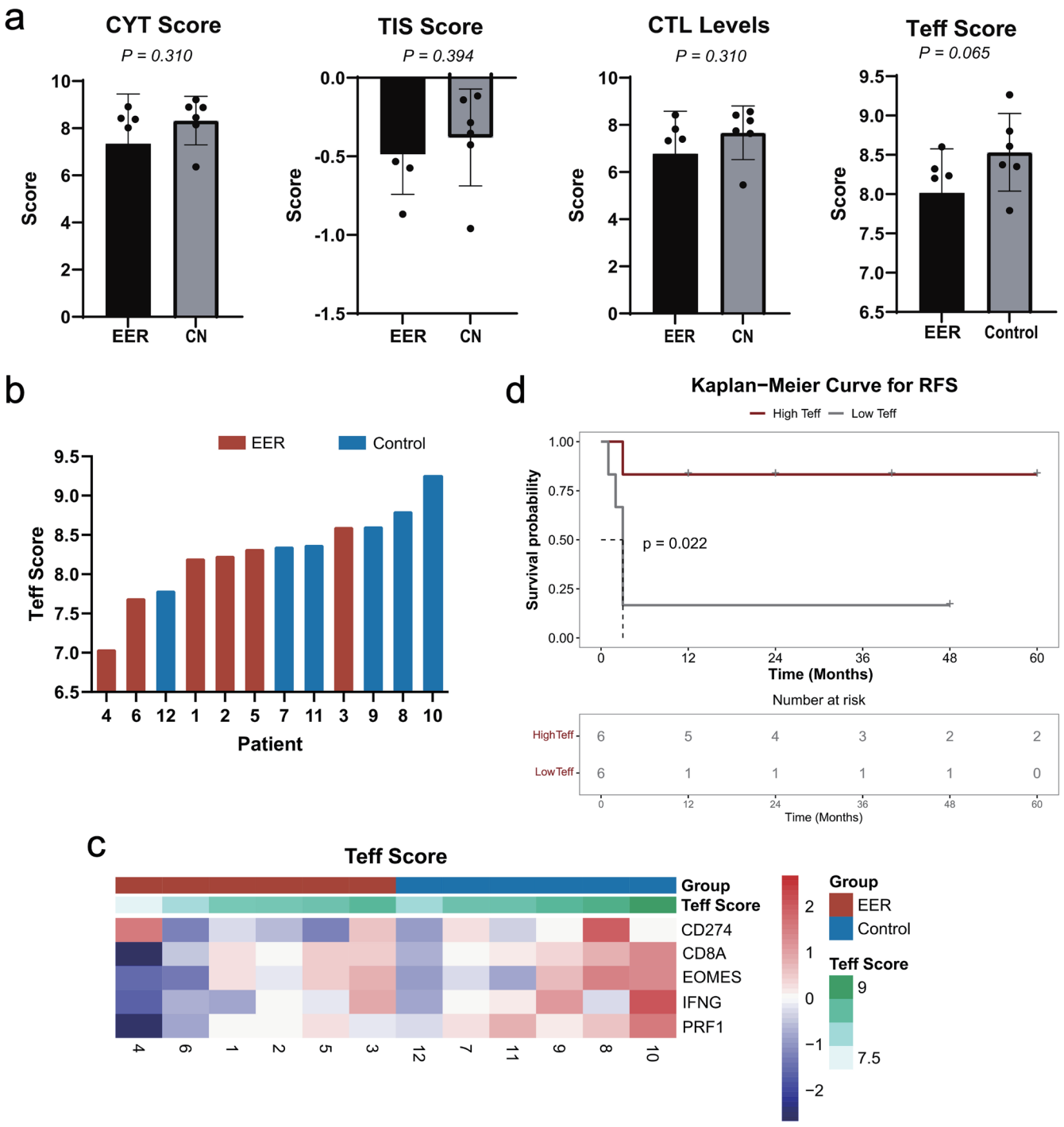

Fig. 5. Comparison of immune signatures between EER and control groups. (a) Comparison of four immune signatures (CYT score, TIS, CTL levels and Teff score) between EER and control groups (Wilcoxon rank sum test). EER patients showed a nonsignificant trend toward reduced Teff scores (p = 0.065). (b) Waterfall plot of Teff scores in individual patients in EER and control groups. (c) Heatmap of expression levels of genes constructing Teff score. (d) Association between Teff score and recurrence-free survival. CTL, cytotoxic T lymphocytes; CYT, cytolytic activity; EER, early explosive recurrence; RFS, recurrence-free survival; Teff, T-effector; TIS, T-cell inflamed signature.

MVI is a histological feature of HCC related to aggressive biological behavior and its prognosis prediction value has been validated.[32,33] Interestingly, a recent study reported that neoadjuvant trans-arterial infusion chemotherapy with FOLFOX could improve outcomes of HCC patients beyond the Milan criteria, with a significant reduction of MVI rate (39.0% vs. 11.4%).[34] Taken together, these results suggested that patients at risk of EER may be more suitable for first-line nonsurgical therapy.

GPC3, a membrane-associated proteoglycan, is an important component of the extracellular matrix and is specifically upregulated in HCC.[35] It promotes tumor growth and metastasis by interacting with a variety of extracellular signal molecules, activating the downstream WNT and Hedgehog pathways and inducing epithelial-mesenchymal transition. Elevated GPC3 levels have been widely shown to be associated with poor prognosis in HCC.[36] In our previous study, low GPC3 levels were associated with better survival and RFS in patients with HCC who were treated with sorafenib.[37] In this study, GPC3 was shown to be an independent risk factor for developing EER.

Recently, TACE has been globally adopted as standard treatment for patients with intermediate-stage HCC. Median survival ranged from 19.4 months in uncontrolled investigations to 37 months in RCTs.[1] Combining TACE with targeted drugs and PD-1 monoclonal antibody further improved their efficacy.[38,39] However, in this study, although most EER patients received TACE on recurrence, the median OS was only 16.9 (95% CI: 9.3, 20.4) months, indicating the aggressive biological nature of EER. The dismal prognosis of EER further underscores the rationale of recurrence subclassification refinement and the demand for novel treatment strategies. The significance of proposing EER is that like hyperprogression caused by immunotherapy, EER may be triggered by surgery itself. It was reported that the expression of IL11 increased after hepatectomy, triggering the outgrowth of HCC cells.[40] Importantly, akin to the severe acute respiratory syndrome caused by coronavirus, primarily mediated by the phenomenon known as cytokine storm, EER may reflect a mechanism of tumor hypergrowth mediated by certain inflammatory mediators. Owing to the limitations of our research conditions, we could not capture the fluctuations of the levels of inflammatory mediators in the serum and liver of EER patients in the early postoperative period. However, we retrospectively analyzed surgical samples, that were currently available.

Patients with HCC who undergo surgical resection face a high risk of recurrence. Studies have reported that the level of $CD161^{+}CD8^{+}$ T cells with an innate-like low cytotoxic state increases in early relapse tumors,[41] and the abundance of regulatory T cells and follicular helper T cells can predict a poor prognosis after surgery.[42] This underscores the strong association between HCC recurrence and the underlying immune background. However, little is known about the immune-related factors influencing EER. In this study the GEP analysis showed that several genes associated with immune suppression, including *NT5E* (CD73) and *TDO2* were significantly upregulated in EER patients. Indeed, these two genes have been demonstrated to promote tumor progression in HCC and are currently under investigation as novel therapeutic targets.[43–45] NT5E (Nt5e, 5′ nucleotidase, ecto) is an extracellular nucleotide enzyme expressed on the surface of various immune cells. It catalyzes the hydrolysis of AMP into adenosine, which binds to adenosine receptors and inhibits the function of various immune cells.[46] TDO2 expression in the tumor microenvironment accounted for the release of kynurenine (Kyn), a potent immunomodulatory molecule.[47] Additionally, CCL20 exhibited a higher transcriptional level in the EER group. It is well-established that the CCL20-CCR6 axis promotes cancer progression directly by enhancing the migration and proliferation capacity of cancer cells and indirectly by remodeling the tumor microenvironment.[48] Previously, CCL20 has been reported to be involved in the progression of various malignant tumors,[49,50] and its expression level was associated with advanced stage and high recurrence rate of HCC.[51] The CCL20-CCR6 signaling axis is essential for the trafficking of Th17 cells.[52] However, GSEA analysis showed that Th17 cell enrichment was reduced in EER patients. Th17 cells have a dual role in tumor immunity. On one hand, Th17 cells promote tumor growth by inducing angiogenesis. On the other hand, Th17 cells drive antitumor immune responses by recruiting immune cells into tumors, activating effector CD8+ T cells, or even directly by converting toward Th1 phenotype and producing IFN-γ.[53] According to the existing results, the mechanism of CCL20 promoting EER may not be accomplished by recruiting Th17 cells.

The Teff signature represents antitumor effectiveness of T cells. A lower Teff signature has been linked to an inferior survival benefit in HCC patients undergoing immunotherapy.[11] In this study, we observed that EER patients had a lower Teff signature, indicating that weakened antitumor immunity may contribute to rapid and aggressive post-operative intrahepatic metastasis.

Limitations

This study has some limitations that should be mentioned. It was a single-center retrospective analysis with a relatively modest sample size. The TIME GEP analysis was performed in a limited subset of patients because of funding constraints, which may impact the generalizability of our conclusions. Additionally, the influence of humoral factors, such as cytokine fluctuations in serum and liver, on EER was not fully examined. Unilateral analysis of immune factors in the tumor microenvironment also presents methodological limitations. Therefore, the clinical and molecular biomarkers identified in this study require further validation in prospective controlled clinical trials. Future studies should aim for a more comprehensive understanding of EER and a more precise tailoring of treatment strategies for HCC, accounting for both the tumor's biological behavior and the immune profiles of the tumor microenvironment.

Conclusions

In conclusion, EER represents a unique form of early recurrence in HCC, characterized by distinct immune profiles, prognosis, and treatment responses. This study describes the terminology and classification for this specific subgroup of HCC patients for the first time. Given its notably poor prognosis, preventing EER is of paramount importance in the long-term management of HCC. Through an in-depth exploration of the clinicopathological and TIME profiles of patients experiencing EER after radical resection, we have gained a more comprehensive understanding of this aggressive recurrence pattern in HCC.

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/CSP.2023.00037.

Fig. S1. Flow diagram of the study. EER, early explosive recurrence; HCC, hepatocellular carcinoma.

Fig. S2. Area under the curve of receiver operating risk for the EER risk prediction model. AUC, Area under curve; EER, early explosive recurrence; ROC, Receiver Operating Characteristic.

Fig. S3. Comparison of 16 immune cell scores in the EER and control groups (Wilcoxon rank sum test). CD, cluster of differentiation; CN, control normal; DC, dendritic cells; EER, early explosive recurrence; NK, natural killer; Th1, T-helper 1; Th2, T-helper 2; Th17, T-helper 17; Treg, regulatory T cells.

Table S1. The demographic and clinical information of study patients.

Table S2. Treatment outcomes in 17 patients with EER.

Table S3. Transcriptional levels of 289-immuno-gene.

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Conflict of interest

SL was employed by the Jiangsu Simcere Diagnostics Co., Ltd. Dr. Guoyi Wu has been an editorial board member of *Cancer Screening and Prevention* since November 2021. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

YF, HZ, KY and GW Designed the study, KY, XL, WD and HZ acquired and analyzed the clinical, radiological and pathological data, SL analyzed and summarized the RNA sequencing data, KY and SL wrote the manuscript, YF and GW critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Ethical statement

This study was conducted in strict accord with the guidelines of the Helsinki Declaration as revised in 2013 and received full approval from the ethical committee of Eastern Hepatobiliary Surgery Hospital. Informed consent for this retrospective analysis was waived.

Data sharing statement

The raw dataset can be obtained by reaching out to the corresponding author by E-mail at fuyg1982@shu.edu.cn.

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