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Molecular insights into the development of hepatic metastases in colorectal cancer: a metastasis prediction study

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Abstract. – OBJECTIVE: Colorectal cancer is presently the third most commonly diagnosed cancer in the United States. In this study, we identified molecular differences between hepatic and non-hepatic metastases in colorectal cancer and evaluated their prognostic significance.

MATERIALS AND METHODS: We downloaded primary data from the NCBI Gene Expression Omnibus (GSE6988, GSE62321, GSE50760, and GSE28722). To identify the molecular differences, we used the Significance Analysis of Microarray method. We selected nine prognostic genes (SYTL2, PTPLAD1, CDS1, RNF138, PIGR, WDR78, MYO7B, TSPAN3, and ATP5F1) with hepatic metastasis prediction score in colorectal cancer (hereafter referred to as LASSO Score). We confirmed the prognostic significance of the LASSO Score by using Kaplan-Meier survival analysis, multivariate analysis, the time-dependent area under the curve (AUC) of Uno's C-index, and the AUC of the receiver operating characteristic curve at 1-5 years.

RESULTS: Survival analysis revealed that a high LASSO Score is associated with a poor prognosis in colorectal cancer patients with hepatic metastases (p = 0). Analysis of C-indices and AUC values from the receiver operating characteristic curve further supported this prediction by the LASSO Score. Multivariate analysis confirmed the prognostic significance of the LASSO Score (p = 1.13e-06).

CONCLUSIONS: This study reveals the biological mechanisms underlying hepatic metastases in colorectal cancer and will help in developing targeted therapies for colorectal cancer.

Key Words:
Colorectal cancer, Hepatic metastases, LASSO, Prognosis.

Introduction

Colorectal cancer is the second most and third most common cancer among women and men, respectively[1]. It accounts for approximately 10% of cancer-related deaths[2]. In the year 2013, 771,000 people died due to colorectal cancer worldwide, making it the fourth most common global cause of cancer-related deaths after lung, liver, and stomach cancer[3]. Distant metastasis occurs mainly in the liver and is the major cause of death in colorectal cancer patients. Depending on the stage of primary tumors, the occurrence of liver metastasis ranges from 20% (Stage II) to 70% (Stage IV)[4].

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Aggressive liver resection in colorectal cancer patients with liver metastasis can presumably improve the five-year survival rate, but in more than 70% of the patients are not recommended a curative resection[5]. However, even when resection is combined with modern adjuvant systemic regimes, it is curative in only 20% of patients[6,7], with 70% developing recurrence, primarily in the liver[8]. Multidisciplinary treatments, evolving chemotherapy agents, and patient care have improved the overall five-year survival rates from <8% to 25%-40%[9,10]. Among patients without liver resection, the median survival rate is approximately 14-21 months[11,12]. The present chemotherapeutic regimens report a median survival rate of 20 months. Patients with unresectable metastases have a median survival rate of 4-12 months[11-17].

Although recent advances have led to an increase in the patients' overall survival with unresectable liver metastasis[18,19], a thorough understanding of the molecular biology of metastases is essential for detecting liver metastasis in its early stages and for optimizing the risk stratification to monitor liver metastasis. The present diagnostic imaging tools, such as contrast-enhanced computed tomography, positron emission tomography-CT, and magnetic resonance imaging can detect colorectal cancer liver metastasis[20]; however, these modalities are of limited value due to their inability to efficiently identify early metastatic lesions and the costs associated with advanced imaging. Considering these clinical challenges, the development of metastasis-specific molecular biomarkers that can help in predicting outcomes and developing more effective treatment therapies is warranted.

In the current era of genomic medicine and targeted therapies, biomarkers have emerged as important prognostic and predictive factors to guide systemic therapy and patient selection for surgery. We analyzed four publicly accessible data sets from the Gene Expression Omnibus (GEO) to develop a system that predicts hepatic metastasis and prognosis of colorectal cancer (LASSO Score) in colorectal cancer patients.

Materials and Methods

Patient Data and Study Design

Genomic and clinical data were accessed from the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). We used three independent cohorts (GSE6988, GSE62321, and GSE50760) to find common differentially expressed genes (DEGs), and further used GSE28722 to obtain metastasis prediction scores. We only included patients with colon adenocarcinoma in this study. The number of patients is described in Table I.

Statistical Analysis

We performed the Significance Analysis of Microarray (SAM) to find DEGs using the R package siggenes, which has been used in previously published studies for similar analyses[21]. We decided a Delta value with False Discovery Rate value as 0.05. Overrepresentation analysis was performed to identify enriched pathways of common DEGs using ConsensusPathDB (http://cpdb.molgen.mpg.de/). To develop a metastasis prediction score, we performed variable selection *via* the least absolute shrinkage and selection operator (LASSO) regression with leave-one-out cross-validation using the R package coxnet[22]. We performed survival analyses to predict metastasis-free survival for patients with colon cancer. Moreover, we evaluated the discriminatory power of the score *via* several methods [Kaplan-Meier curve, Uno's C index, area under the curve (AUC) value at a specific time, and Cox proportional hazard regression] using the R package survival and survAUC, as previously mentioned[23]. All statistical analyses were performed using the R studio software (ver. 1.1.453, Boston, MA, USA).

Table I. The number of colorectal cancer patients in each cohort.

Variables	GSE6988	GSE62321	GSE50760	GSE28722
Patients (n)	68	15	18	125
Normal colorectal mucosa	22	15	18	0
Colorectal primary tumor (adenocarcinoma)	44	15	18	125
Colorectal liver metastasis tumor (adenocarcinoma)	24	0	0	N/A

Table II. Common upregulated or downregulated genes in colorectal cancer with liver metastasis..

Gene symbol		
Upregulated genes		
GPT2	PTPLAD1	
Downregulated genes		
ABI3BP	AFG3L2	AIFM3
ATP2A3	ATP5F1	ATP8B1
ATPIF1	B3GALT5	C10orf99
CAPN5	CDS1	DHRS9
GAB1	LIMA1	MARVELD
MGLL	MLXIP	MYO5B
MYO7B	NDUFC1	PDE5A
PIGR	RIPK1	RNF138
S100A10	SCAMP2	SH3KBP1
SH3RF1	SIAE	SLC26A2
SLC44A2	SMAD2	SPINT1
SSPN	ST6GALNAC6	SYTL2
TAGAP	TCF7L2	TFCP2L1
TLR3	TMC4	TMEM54
TSPAN3	VSIG2	VTI1B
WDR78		

Results

Differentially Expressed Genes Associated With Hepatic Metastases in Colon Cancer

To evaluate the candidate genes involved in the progression of hepatic metastases in colon cancer patients, we analyzed differentially expressed genes (DEGs) in the normal individuals and three cohorts of colon cancer patients (GSE6988, GSE62321, and GSE50760) using the SAM method (Figure 1). We identified common DEGs within each cohort. A total of 85 common-upregulated and 260 common-downregulated genes were also identified from the three cohorts (Figure 1A). In these three cohorts, 1124 upregulated and 3855 downregulated genes were identified from GSE6988, 470 upregulated and 1910 downregulated genes were identified from GSE62321, and 5013 upregulated and 3319 downregulated genes were identified from GSE50760. The GSE6988 cohort was divided into two groups: hepatic and non-hepatic metastases. Of the 345 common DEGs, we identified 48 DEGs that promoted hepatic metastases in colon cancer patients (Figure 1B). The DEGs are listed in Table II. Moreover, pathway analysis of the 46 downregulated DEGs from colorectal cancer patients with hepatic metastases was performed (Table III). The 11 pathways with the most significant *p*-value are listed in Table III. Of these 48 genes, the GSE28722 cohort was subjected to LASSO analysis to determine the metastasis prediction score from the metastasis groups (Figure 1C). A total of nine prognostic genes (*SYTL2, PTPLAD1, CDS1, RNF138, PIGR, WDR78, MYO7B, TSPAN3,* and *ATP5F1*) for metastasis prediction score were selected (hereafter referred to as the LASSO Score) (Table IV). The regression coefficients for the risk score are described in Table IV.

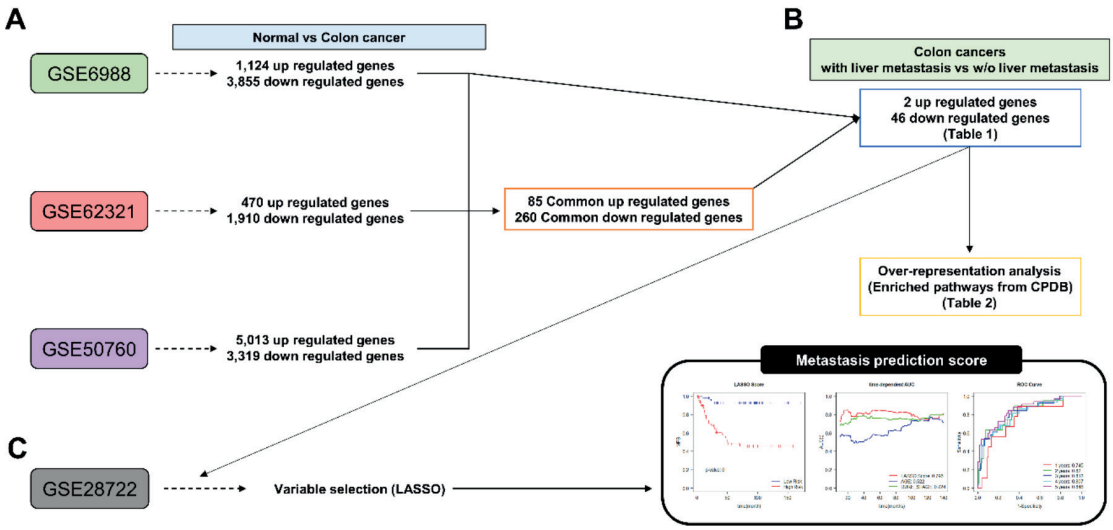

Figure 1. The running procedure of the LASSO Score.

Table III. Enriched pathways of downregulated genes in colorectal cancer with liver metastasis.

Pathways	Source	No. of genes in set	*p*-value	*q*-value	No. of overlapped genes	Overlapped genes
TLR3-mediated TICAM1-dependent programmed cell death	Reactome	6	8.76e-05	0.00517	2	TLR3, RIPK1
Signaling by MET	Reactome	61	0.000443	0.0131	3	GAB1, SH3KBP1, SPINT1
TICAM1, RIP1-mediated IKK complex recruitment	Reactome	21	0.0012	0.0212	2	TLR3, RIPK1
RIP-mediated NFkB activation via ZBP1	Reactome	23	0.00144	0.0212	2	TLR3, RIPK1
ZBP1(DAI) mediated induction of type I IFNs	Reactome	27	0.00198	0.0234	2	TLR3, RIPK1
Glycerophospholipid biosynthesis	Reactome	133	0.00419	0.0412	3	CDS1, MGLL, SLC442
Signaling by EGFR	Reactome	43	0.00498	0.042	2	GAB1, SH3KBP1
Gastric cancer–Homo sapiens (human)	KEGG	149	0.00575	0.0424	3	GAB1, TCF7L2, SMAD2
Cytosolic sensors of pathogen-associated DNA	Reactome	51	0.00695	0.0431	2	TLR3, RIPK1
Hepatocellular carcinoma-Homo sapiens (human)	KEGG	168	0.008	0.0431	3	GAB1, TCF7L2, SMAD2
Ion transport by P-type ATPases	Reactome	55	0.00804	0.0431	2	ATP2A3, ATP8B1

Table IV. Selection of genes and regression coefficients for risk score.

Gene symbol	Hazard ratio	Regression coefficients
SYTL2	2.508545	0.919703
PTPLAD1	0.399470	−0.91762
CDS1	0.438212	−0.82505
RNF138	0.567074	−0.56727
PIGR	0.625969	−0.46845
WDR78	0.969584	−0.03089
MYO7B	0.782271	−0.24555
TSPAN3	0.627621	−0.46582
ATP5F1	0.891459	−0.11490

Prognostic Value of the LASSO Score for Metastasis Prediction

To evaluate the prognostic value of the LASSO Score for hepatic metastases in colorectal cancer, we analyzed the Kaplan-Meier curves of the LASSO Score with a cutoff value (Figure 2A and Table V). The group with a high LASSO Score had a significantly shorter survival duration than that of the group with a low LASSO Score (Figure 2A). The prognostic value of the LASSO Score for hepatic metastases in colorectal cancer was further confirmed using multivariate analysis (Table V).

To assess the efficiency of the LASSO Score as a biomarker for hepatic metastases in colorectal cancer, we examined Uno's C-index for time-dependent AUC analysis (Figure 2B) and AUC values from one to five years for the ROCs (Figure 2C). The LASSO Score yielded high C-index values compared with the age and Dukes stage (LASSO Score: 0.796, AGE: 0.522, DUKE_STAGE: 0.724; Figure 2B). The ROC graphs revealed high AUC values for 1-5 years from the LASSO Score (1 year: 0.745, 2 years: 0.82, 3 years: 0.812, 4 years: 0.807, and 5 years: 0.846; Figure 2C). The five-year ROC graphs revealed high AUC values, compared with those of the ROC graphs for 1-4 years.

Discussion

Although multiple genetic factors contributing to hepatic metastases in colorectal cancer and its pathobiology have been studied extensively, the mechanisms underlying the progression of hepatic metastases in colorectal cancer remain unclear.

Table V. Univariate and multivariate analysis of metastasis-free survival.

	Univariate analysis				Multivariate analysis			
Parameters	*p*	HR	95 CI		*p*	HR	95 CI	
Age	0.582	1.00805	0.9797	1.037	0.6841	1.006	0.9775	1.035
DUKE stage (A & B vs. C & D)	6.6e-07	6.175	3.013	12.66	0.00151	3.460	1.6074	7.447
LASSO-score	4.92e-11	5.1542	3.161	8.404	1.13e-06	3.739	2.1985	6.360

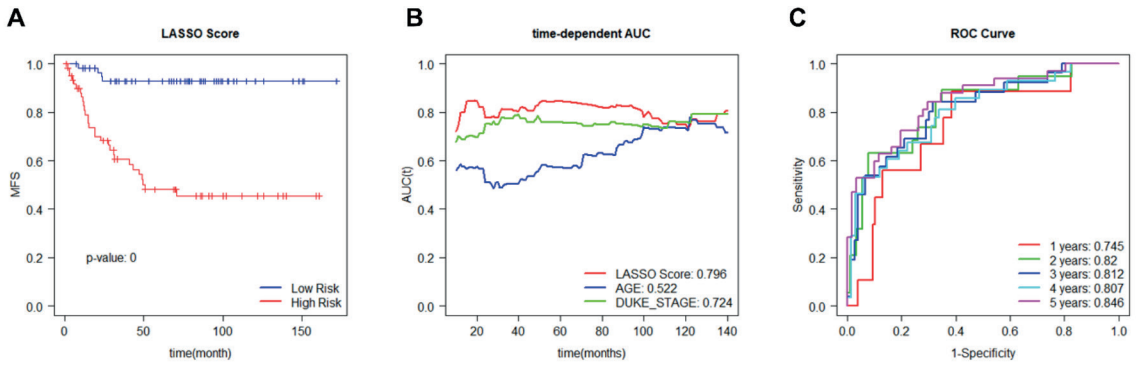

Figure 2. Analysis of the LASSO Score. **A,** Expression levels of genes are classified as low or high (blue or red lines, respectively) based on the comparison of their median cut-off values from the LASSO Score. **B,** Time-dependent area under the curve (AUC) of the groups for LASSO Score, age, and Dukes stage. **C,** Receiver operating characteristics (ROC) curves for selected years in terms of the LASSO Score.

The development of modern chemotherapeutic agents has led to an increase in long-term survival in nonresectable metastatic colorectal cancer patients. The median survival in patients can be extended by 4-6 months using systemic chemotherapy. Moreover, adjuvant chemotherapy may also improve the quality of life[24,25]. Systemic treatment with 5-fluorouracil-leucovorin, irinotecan, and oxaliplatin has been introduced[26]. Furthermore, the addition of monoclonal antibodies against vascular endothelial growth factor and bevacizumab to the combination chemotherapy significantly improved the survival rate[19].

Here, we systematically selected nine metastasis-predicting genes based on the LASSO Score.

Synaptotagmin-like 2 (*SYTL2*) interacts with Rab27a GTPase in cytotoxic T cells and participates in cytotoxic granule secretion[27]. Moreover, it regulates the morphology of melanocytes and controls melanosome distribution in the cell periphery[28]. In ovarian cancer, *SYTL2* is associated with the metastasis of ovarian cancer[29].

Protein tyrosine phosphatase-like A domain containing PTPLAD1 localized in endosomes is associated with the insulin receptor and affects insulin signaling[30]. CDP-diacylglycerol synthase 1 (CDS1) catalyzes the formation of CDP-diacylglycerol from phosphatidic acid[31]. Ring Finger Protein 138 (RNF138) is an E3 ubiquitin protein ligase that promotes DNA repair[32-34]. Polymeric immunoglobulin receptor (PIGR) is a transporter of polymeric IgA and IgM and is a prognostic biomarker for hepatocellular carcinoma. PIGR levels increase in response to viral or bacterial infections[35]. WD Repeat Domain 78 (WDR78) is essential for ciliary beating and for axonemal dyneins[36]. Myosin-7B (MYO7B) is an actin-based motor protein that promotes the accumulation of intermicrovillar adhesion complex components at the microvillar tips[37]. Tetraspanin-3 (TSPAN3) is an integral membrane protein and acts as a target of the RNA-binding protein Musashi-2, which plays a key role in AML[38]. ATP5F1 is a mitochondrial ATP synthase that utilizes an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation[39].

Conclusions

The development of biomarkers that can effectively predict patients' prognosis with hepatic metastases in colorectal cancer is warranted. Through SAM analysis, we tried to limit the possible candidates with accurate hepatic metastasis prediction scores for colorectal cancer. LASSO regression was used to optimize the candidate genes. We identified the prognostic value of the novel LASSO score for hepatic metastases in colorectal cancer. These findings provide a refined understanding of prognostic markers along with several unexpected observations. The results of the present study may help us understand the biological mechanisms underlying hepatic metastases in colorectal cancer and in developing targeted therapy for colorectal cancer. Although expression-based studies of LASSO Score have their own limitations, we suggest LASSO Score as a potential prognostic biomarker for hepatic metastases in colorectal cancer.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

Author Contributions

DSK, DL, and YHK initiated the study and guided the work. HJH, GHK, BYH, JK, IAW, and HSC collected and normalized the data. JY, HK, KS, and YN analyzed and interpreted the experimental data. All authors wrote the manuscript with input from all co-authors.

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