Low *EIF2B5* expression predicts poor prognosis in ovarian cancer

Lin Hou, PhD[a], Yan Jiao, PhD[b], Yanqing Li, PhD[c], Zhangping Luo, PhD[c], Xueying Zhang, PhD[d], Guoqiang Pan, MD[e], Yuechen Zhao, MD[f], Zhaoying Yang, PhD[g,*], Miao He, PhD[h,*]

Abstract

Ovarian cancer has the highest mortality among gynecological cancers. Although ovarian cancer usually responds well to chemotherapy, most patients still have a poor prognosis. EIF2B5 is a crucial molecule in posttranscriptional modifications involved in tumor progression, and here we investigated the prognostic role of EIF2B5 in ovarian cancer. We examined the differential expression of *EIF2B5* mRNA in ovarian cancer by exploring The Cancer Genome Atlas (TCGA) database. The chi square test was used to identify a clinical correlation. Survival analysis and Cox regression model were performed to determine the association between *EIF2B5* expression and overall survival (OS) in ovarian cancer patients. As a result, Low *EIF2B5* expression was found in ovarian cancer tissues and correlated with survival status. Survival analysis showed that ovarian cancer patients with low EIF2B5 expression had a short OS. Moreover, Cox regression analysis indicated that low EIF2B5 expression was an independent risk factor for a poor prognosis in ovarian cancer. Additionally, according to gene set enrichment analysis, mesenchymal transition, angiogenesis, coagulation, and bile acid metabolism were differentially enriched in ovarian cancer with high EIF2B5 expression. In conclusion, Low EIF2B5 expression is an independent risk factor for a poor prognosis in ovarian cancer patients.

Abbreviations: EIF2B5 = eukaryotic translation initiation factor 2B subunit 5, GSEA = gene set enrichment analysis, NES = normalized enrichment score, OS = overall survival, TCGA = the cancer genome atlas.

Keywords: data mining, EIF2B5, ovarian cancer, prognosis, TCGA

1. Introduction

Ovarian cancer is the most lethal gynecological cancer globally,[1,2] and despite rapid advancements in treatment methods, the prognosis of ovarian cancer patients remains poor, with few effective prognostic biomarkers available at present.[3–5] Therefore, there is an urgent need for new molecular markers that can be used to predict the prognosis of ovarian cancer patients for the purpose of guiding treatment planning.

As a crucial molecule in posttranscriptional modifications, eukaryotic translation initiation factor 2B subunit 5 (EIF2B5) is important in cancer progression.[6] Early research regarding *EIF2B5* mainly aimed to study the roles of its expression in multiple sclerosis,[7,8] ovarioleukodystrophy,[9] and vanishing white matter (VWM) syndrome.[10–12] Additionally, recent studies indicated that high *EIF2B5* expressed in few cancerous tissue (lung cancer,[13] breast cancer,[14] and liver cancer[15]) and serve as a prognostic biomarker in hepatocellular carcinoma.[15] Nonetheless, the role of *EIF2B5* expression in ovarian cancer remains unclear.

To evaluate the clinical significance of *EIF2B5* expression in the prognosis of ovarian cancer patients, we analyzed the prognostic value of *EIF2B5* mRNA expression in The Cancer Genome Atlas (TCGA) cohort of ovarian cancer patients. First, we analyzed the differential expression of EIF2B5 in ovarian cancer patients and then studied its correlation with overall survival (OS) among the patients.

2. Materials and methods

2.1. Data source

The clinical data for *EIF2B5* expression in normal ovarian tissues and ovarian cancer tissues were downloaded from the TCGA (https://cancergenome.nih.gov/) and GTEx (www.gtexportal. org/) databases in May 2018.

2.2. Data mining

All data mining was conducted using R (version 3.5.1).[16] The differences in *EIF2B5* expression according to clinical features

[a] Cancer Center, [b] Department of Hepatobiliary and Pancreatic Surgery, The First Hospital of Jilin University, [c] Department of Pathophysiology, College of Basic Medical Sciences, Jilin University, [d] Reproductive Medical Center, Department of Obstetrics and Gynecology, The Second Hospital of Jilin University, [e] Department of Gastrointestinal Surgery, First Hospital of Jilin University, [f] Department of Radiation Oncology, The Second Hospital of Jilin University, [g] Department of Breast Surgery, China-Japan Union Hospital of Jilin University, [h] Department of Anesthesia, The Second Hospital of Jilin University, Changchun, Jilin, PR China.

[*] Correspondence: Zhaoying Yang, Department of Breast Surgery, China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Changchun 130033, PR China (e-mail: zhaoyingyang@163.com); Miao He, Department of Anesthesia, The Second Hospital of Jilin University, Changchun 130022, PR China (e-mail: hemiao_2019@126.com).

are shown in boxplots drawn using the ggplot2 package.[17] To determine the high and low EIF2B5 expression groups, and the optimal cutoff value was obtained from ROC curve. Possible clinical correlations between *EIF2B5* expression and the clinical characteristics of ovarian cancer patients were evaluated by the chi square test. The survival curves were drawn using Survival Package.[18] The log-rank test was applied to examine the survival difference. Univariate Cox analysis was performed to select relevant variables, and a multivariate Cox model was used to evaluate the independent prognostic role of *EIF2B5* expression separate from other clinical characteristics.

2.3. GSEA

Gene set enrichment analysis (GSEA) uses predefined gene sets to rank target genes according to the degree of differential expression between the two types of samples, and then to test whether the pre-defined gene sets are at the top or bottom of the sorting table.[19] In the present study, we used GSEA 3.0 software to analyze the data of ovarian cancer patients. We obtained standardized enrichment fractions (NESs) by using permutation analysis 1000 times.

2.4. Ethical approval

Ethics committee approval was not necessary because all clinical data used in this study were obtained from a public database and are available for research.

3. Results

3.1. Differential expression of EIF2B5 in ovarian cancer

The data for EIF2B5 expression and clinical features including age, subdivision of cancer, cancer stage, longest dimension, lymphatic invasion, histologic grade, occurrence type, sample type, vital status, and *EIF2B5* expression are presented in Table 1 and Figure 1A. From the prepared boxplots, *EIF2B5* expression was low in ovarian cancer tissues compared with that observed in normal ovarian tissues. Additionally, low *EIF2B5* expression was observed in deceased patients, suggesting a potential connection between the survival status and *EIF2B5* expression (Fig. 1 and Table 2).

3.2. Correlation of EIF2B5 expression and survival

To explore possible correlations of *EIF2B5* expression with clinical factors, we completed the chi square test and found a specific correlation between vital status and expression of *EIF2B5* (Table 2). Moreover, patients with shorter OS time had much lower expression of *EIF2B5* (Fig. 2, $P = .034$), which is consistent with results of subgroup analysis, especially among the elderly patients (Fig. 2, $P = .022$).

The univariate Cox model revealed several potential survival-related variables including age, occurrence type, and *EIF2B5* expression. The Multivariate Cox model suggested that low *EIF2B5* expression was an independent risk factor for a poor prognosis in ovarian cancer patients, based on its association with a shorter OS (hazard ratio [HR] = 1.82, $P = .008$, Table 3).

As shown in Table 4, GSEA revealed significant differences in the enrichment of the MSigDB Collection (NOM $P < .05$, false discovery rate [FDR] < 0.25). We chose the most essential

Table 1

Demographic and clinical characteristics of TCGA ovarian cancer cohort.

Characteristic	n(%)
Age	
<55 yr	113(36.69)
≥55 yr	195 (63.31)
Subdivision	
NA	17 (5.52)
Bilateral	212 (68.83)
Left	37 (12.01)
Right	42 (13.64)
Stage	
NA	2 (0.65)
I	1 (0.32)
II	22 (7.14)
III	245 (79.55)
IV	38 (12.34)
Longest dimension	
large	124 (46.1)
small	145 (53.9)
Lymphatic invasion	
NA	180 (58.44)
No	44 (14.29)
Yes	84 (27.27)
Histologic grade	
NA	2 (0.65)
G1	1 (0.32)
G2	37 (12.01)
G3	261 (84.74)
G4	1 (0.32)
GB	2 (0.65)
GX	4 (1.3)
Occurrence type	
NA	145 (47.08)
Locoregional	4 (1.3)
Metastatic	1 (0.32)
Progression	12 (3.9)
Recurrence	146 (47.4)
Sample type	
Primary tumor	303 (98.38)
Recurrent tumor	5 (1.62)
Vital status	
Deceased	184 (59.74)
Living	124 (40.26)
EIF2B5 expression	
High	102 (33.12)
Low	206 (66.88)

NA = not available.

signaling pathways based on NES (Table 4; Fig. 3). Figure 3 shows that mesenchymal transition, angiogenesis, coagulation, and bile acid metabolism were enriched in low *EIF2B5* expression phenotype, respectively.

4. Discussion

Although many advances in treatment strategies for ovarian cancer have been explored, the OS of these patients has not been improved. Thus, novel biomarkers that can be used to predict the prognosis of these patients remain urgently needed.[20–26] According to the results of the present study, low *EIF2B5* expression is an independent risk factor for a poor prognosis among ovarian cancer patients.
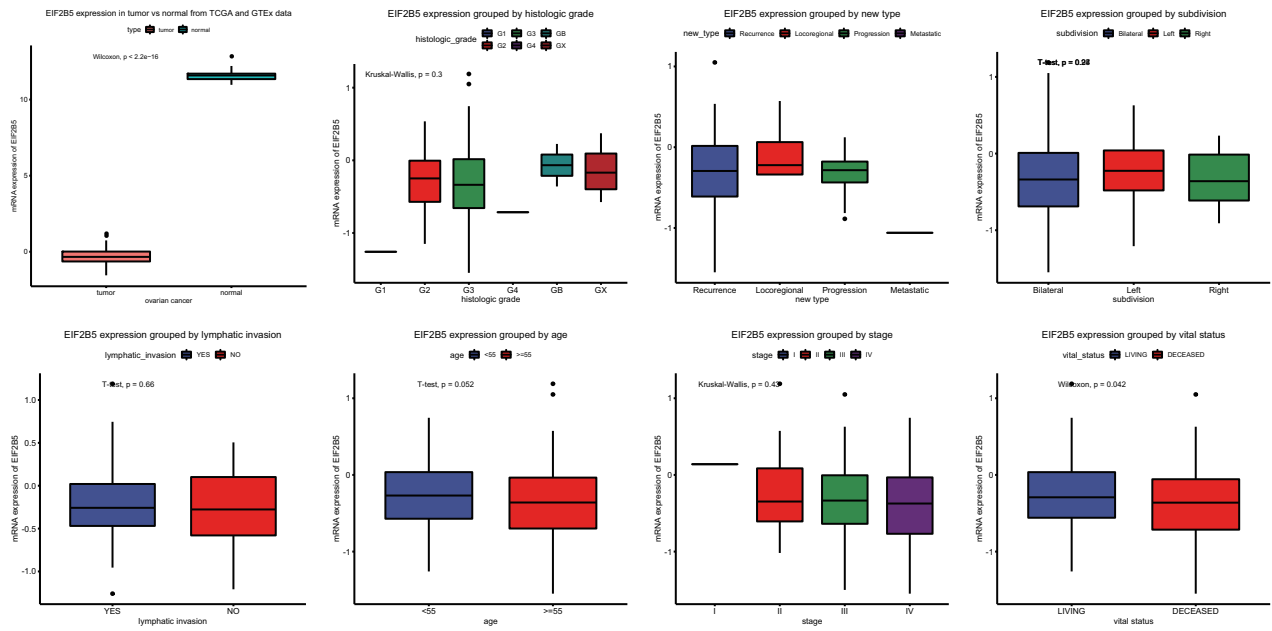
Figure 1. Differential *EIF2B5* expression in ovarian cancer. The *EIF2B5* expression in all ovarian cancer cases and different groups according to histologic grade, occurrence type, subdivision, lymphatic invasion, patient age, stage, and vital status.

Early studies of EIF2B5 mainly focused on its role in VWM diseases, which involve downregulation of EIF2B5.[8,10,11] Recently, several studies began investigating the role of EIF2B5 in various cancers, including lung cancer,[13] breast cancer,[14]

and liver cancer.[15] In these studies, EIF2B5 was shown to be overexpressed at both mRNA and protein levels in the cancerous tissues. In contrast, in the present study we observed the opposite phenomenon in which *EIF2B5* expression was lower in ovarian

Table 2

Correlation between *EIF2B5* expression and clinicopathologic characteristics in ovarian cancer.

| Parameter | Variable | n | EIF2B5 mRNA expression | | | | χ^2 | P |
			high	%	low	%		
Age	<55 years	113	45	(44.12)	68	(33.01)	3.1614	.0754
	≥55 years	195	57	(55.88)	138	(66.99)		
Subdivision	Bilateral	212	68	(69.39)	144	(74.61)	2.8766	.2373
	Left	37	17	(17.35)	20	(10.36)		
	Right	42	13	(13.27)	29	(15.03)		
Stage	I	1	1	(0.99)	0	(0)	3.758	.2888
	II	22	10	(9.9)	12	(5.85)		
	III	245	78	(77.23)	167	(81.46)		
	IV	38	12	(11.88)	26	(12.68)		
Longest dimension	Large	124	39	(45.35)	85	(46.45)	0.0014	.9701
	Small	145	47	(54.65)	98	(53.55)		
Lymphatic invasion	No	44	20	(37.04)	24	(32.43)	0.1248	.7239
	Yes	84	34	(62.96)	50	(67.57)		
Histologic grade	G1	1	0	(0)	1	(0.49)	5.0295	.4123
	G2	37	17	(16.83)	20	(9.76)		
	G3	261	81	(80.2)	180	(87.8)		
	G4	1	0	(0)	1	(0.49)		
	GB	2	1	(0.99)	1	(0.49)		
	GX	4	2	(1.98)	2	(0.98)		
Occurrence type	Locoregional	4	2	(3.39)	2	(1.92)	1.5881	.6621
	Metastatic	1	0	(0)	1	(0.96)		
	Progression	12	3	(5.08)	9	(8.65)		
	Recurrence	146	54	(91.53)	92	(88.46)		
Sample type	Primary tumor	303	98	(96.08)	205	(99.51)	3.1215	.0773
	Recurrent tumor	5	4	(3.92)	1	(0.49)		
Vital status	Deceased	184	51	(50)	133	(64.56)	5.4254	.0198
	Living	124	51	(50)	73	(35.44)		
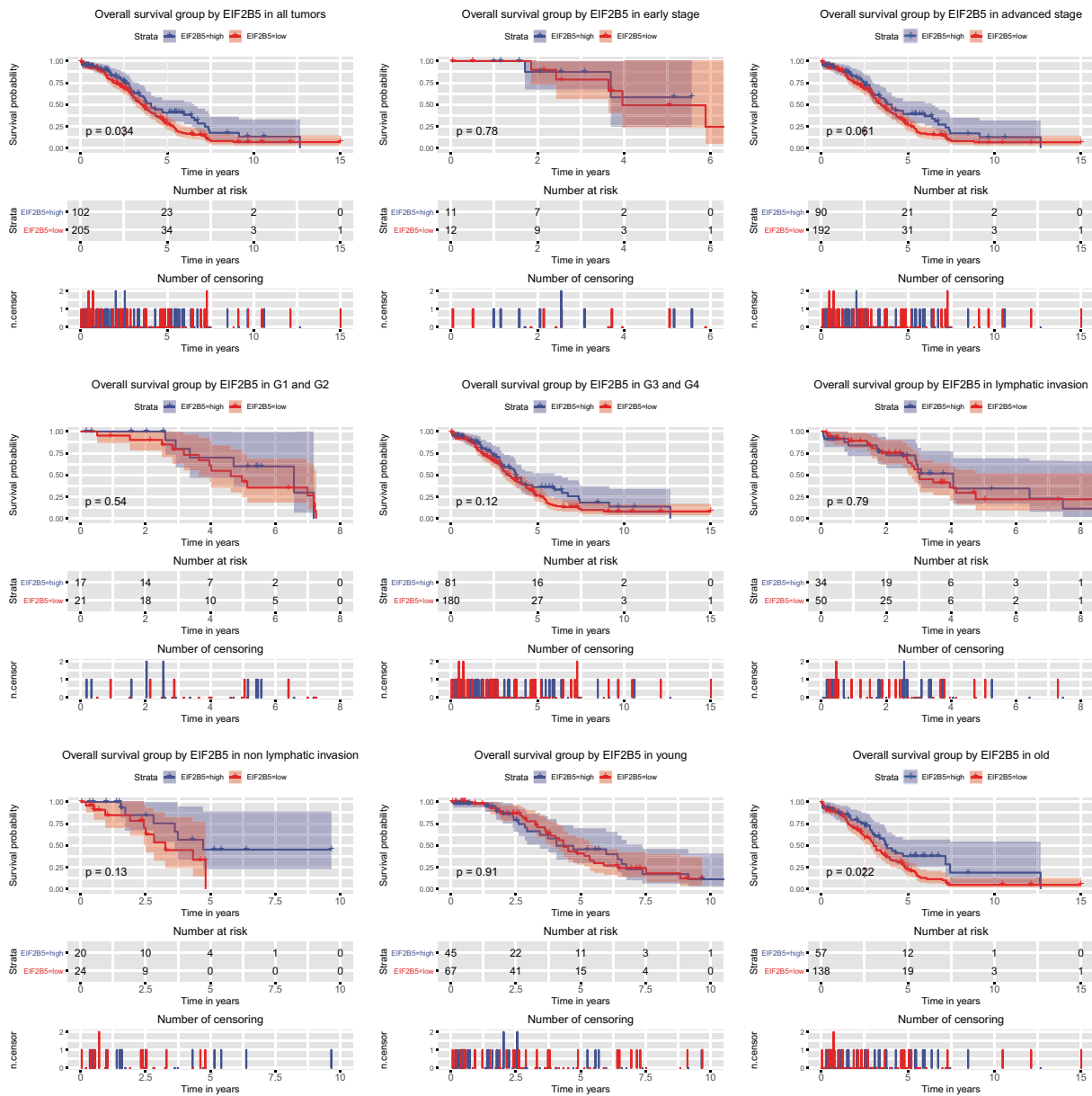
Figure 2. Survival analysis for groups of ovarian cancer cases with differing *EIF2B5* expression in ovarian cancer and subgroup analysis according to early stage, advanced stage, G1 and G2, G3 and G4, lymphatic invasion, non-lymphatic invasion, younger, and older.

Table 3

Univariate and multivariate Cox regression analyses of overall survival duration.

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	*P*
Age	1.63	1.19–2.24	0.003	1.31	0.87–1.96	.194
Subdivision	0.84	0.67–1.04	0.101			
Stage	1.09	0.8–1.5	0.581			
Longest dimension	1.12	0.82–1.52	0.485			
Lymphatic invasion	1.02	0.85–1.23	0.798			
Histologic grade	1.12	0.88–1.42	0.349			
Occurrence type	144.5	9.04–2310.19	0	110.34	6.87–1771.23	.001
Sample type	0.43	0.11–1.73	0.235			
EIF2B5 expression	1.42	1.02–1.96	0.035	1.8	1.17–2.79	.008

CI = confidence interval; HR = hazard ratio.

Gene set enrichment with low *EIF2B5* expression.

Gene set name	NES	NOM *P* value	FDR *q* value
HALLMARK_EPITHELIAL_ MESENCHYMAL_TRANSITION	−1.79131	.02045	0.279636
HALLMARK_ANGIOGENESIS	−1.69705	.015968	0.34191
HALLMARK_COAGULATION	−1.61995	.025692	0.368721
HALLMARK_BILE_ACID_METABOLISM	−1.58339	.014257	0.352256

Gene sets with NOM *P* value <.05 were considered as significant.
FDR = false discovery rate; NES = normalized enrichment score; NOM = nominal.

cancer tissues than in normal ovarian tissues. This discrepancy may be due to differences in the cancer types, which might suggest exclusive functions and mechanisms of *EIF2B5* in ovarian cancer. Moreover, *EIF2B5* expression showed a decreasing trend from stage I to stage IV ovarian cancer, suggesting that the function of *EIF2B5* may change throughout different stages of ovarian cancer. To better understand the dynamics of *EIF2B5* expression in ovarian cancer, a subgroup analysis is necessary. Additionally, considering that the low *EIF2B5* expression continued to decline with disease progression, the relationship between *EIF2B5* and survival needs to be further studied.

Previous research also linked EIF2B5 expression with cancer patients' prognosis. A previous study demonstrated that high *EIF2B5* expression is associated with a shorter survival time in colorectal cancer cases.[27] Also, expression of minor alleles of *EIF2B5* was found to improve the prognosis of ovarian cancer
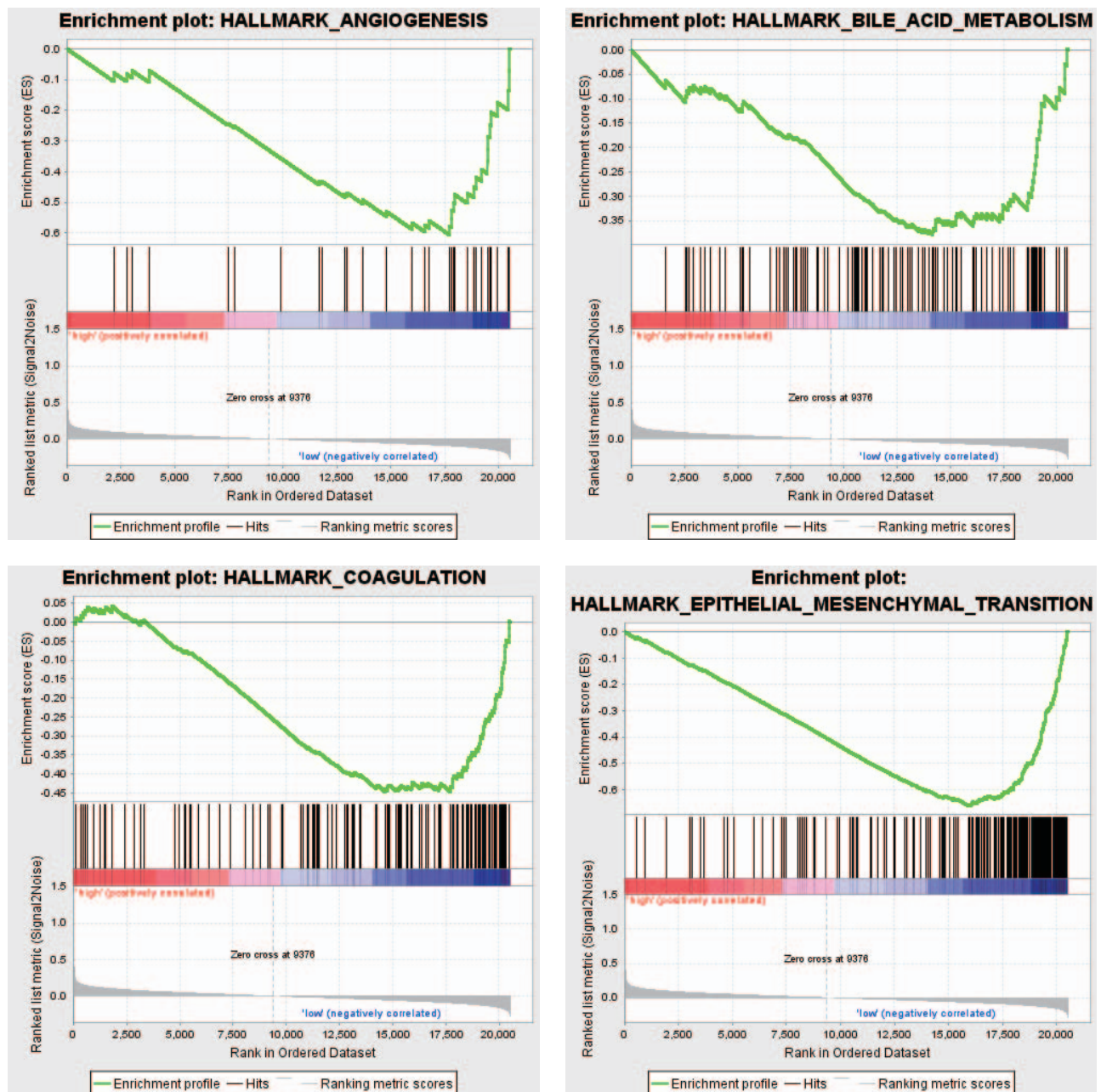

Figure 3. Enrichment plots from GSEA.

patients via the inhibition of angiogenesis and tumor growth.[28] However, the association between *EIF2B5* expression and OS remains unknown in ovarian cancer. In the present study, we found that the overall survival time of ovarian cancer patients with low *EIF2B5* expression was short, while subgroup analysis revealed the same phenomenon with differences in the stage and histologic grade of ovarian cancer. Interestingly, we found that the survival difference was especially significant in older patients. However, this study doesn't contain the variables like race and cancer type, because the races information of TCGA is absent, and only epithelial type exists. Future study needs to explore these variables in other population. Moreover, mesenchymal transition, angiogenesis, coagulation, and bile acid metabolism may be signaling pathways of *EIF2B5* in ovarian cancer.

To the best of our knowledge, this is the first study analyzing the prognostic value of the *EIF2B5* expression in ovarian cancer. Together with other studies of *EIF2B5*, our study provides insight into the role of *EIF2B5* expression in various cancer types. However, as we did not explore the underlying mechanism of the function of EIF2B in ovarian cancer, future in vitro and in vivo experiments are needed to explore the mechanism in depth.

5. Conclusion

In the present study, we investigate the predictive value of *EIF2B5* expression for ovarian cancer patients' prognosis. We found that low *EIF2B5* expression was an independent risk factor for a shorter survival time among ovarian cancer patients. Our future research will include in vitro and in vivo experiments to explore the underlying mechanism of this relationship in depth.

Author contributions

Conceptualization: Lin Hou, Yan Jiao, Zhaoying Yang, Miao He.
Data curation: Yanqing Li.
Formal analysis: Yanqing Li.
Funding acquisition: Zhaoying Yang.
Investigation: Zhangping Luo.
Project administration: Zhangping Luo, Miao He.
Resources: Zhangping Luo, Miao He.
Software: Yanqing Li.
Supervision: Zhangping Luo, Yuechen Zhao.
Validation: Xueying Zhang, Yuechen Zhao, Zhaoying Yang, Miao He.
Visualization: Yanqing Li, Xueying Zhang.
Writing – original draft: Yan Jiao.
Writing – review & editing: Lin Hou, Guoqiang Pan, Zhaoying Yang, Miao He.

References

[1] Fidler MM, Bray F, Soerjomataram I. The global cancer burden and human development: A review. Scand J Public Health 2018;46: 27–36.
[2] Torre LA, Islami F, Siegel RL, et al. Global cancer in women: burden and trends. Cancer Epidemiol Biomarkers Prev 2017;26:444–57.
[3] Eisenhauer EA. Real-world evidence in the treatment of ovarian cancer. Ann Oncol 2017;28(suppl_8):viii61–5.
[4] Mallen A, Soong TR, Townsend MK, et al. Surgical prevention strategies in ovarian cancer. Gynecol Oncol 2018;151:166–75.
[5] Pignata S, C Cecere S, Du Bois A, et al. Treatment of recurrent ovarian cancer. Ann Oncol 2017;28(Suppl 8):viii51–6.
[6] Brady LK, Wang H, Radens CM, et al. Transcriptome analysis of hypoxic cancer cells uncovers intron retention in EIF2B5 as a mechanism to inhibit translation. PLoS Biol 2017;15:e2002623.
[7] Zahoor I, Asimi R, Haq E. No evidence for a role of Ile587Val polymorphism of EIF2B5 gene in multiple sclerosis in Kashmir Valley of India. J Neurol Sci 2015;359:172–6.
[8] Zahoor I, Haq E, Asimi R. Multiple Sclerosis and EIF2B5: a paradox or a missing link. Adv Exp Med Biol 2017;958:57–64.
[9] Ibitoye RT, Renowden SA, Faulkner HJ, et al. Ovarioleukodystrophy due to EIF2B5 mutations. Pract Neurol 2016;16:496–9.
[10] Esmer C, Blanco Hernandez G, Saavedra Alanis V, et al. Association between homozygous c.318A>GT mutation in exon 2 of the EIF2B5 gene and the infantile form of vanishing white matter leukoencephalopathy. Bol Med Hosp Infant Mex 2017;74:364–9.
[11] Raini G, Sharet R, Herrero M, et al. Mutant eIF2B leads to impaired mitochondrial oxidative phosphorylation in vanishing white matter disease. J Neurochem 2017;141:694–707.
[12] Bektas G, Yesil G, Ozkan MU, et al. Vanishing white matter disease with a novel EIF2B5 mutation: A 10-year follow-up. Clin Neurol Neurosurg 2018;171:190–3.
[13] Xue D, Lu M, Gao B, et al. Screening for transcription factors and their regulatory small molecules involved in regulating the functions of CL1-5 cancer cells under the effects of macrophage-conditioned medium. Oncol Rep 2014;31:1323–33.
[14] Yang S, Zhang H, Guo L, et al. Reconstructing the coding and non-coding RNA regulatory networks of miRNAs and mRNAs in breast cancer. Gene 2014;548:6–13.
[15] Jiao Y, Fu Z, Li Y, et al. High EIF2B5 mRNA expression and its prognostic significance in liver cancer: a study based on the TCGA and GEO database. Cancer Manag Res 2018;10:6003–14.
[16] Team RDCJC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2009;14: 12-21.
[17] Wickham H. Ggplot2: elegant graphics for data analysis. J R Stat Soc 2011;174:245–6.
[18] Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model. Vol 97. New York: Springer; 2000.
[19] P. T, VK M, S M, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. A Subramanian A 2005;102:15545–50.
[20] Jiao Y, Fu Z, Li Y, et al. Aberrant FAM64A mRNA expression is an independent predictor of poor survival in pancreatic cancer. PloS One 2019;14:e0211291.
[21] Jiao Y, Li Y, Lu Z, et al. High trophinin-associated protein expression is an independent predictor of poor survival in liver cancer. Dig Dis Sci 2019;64:137–43.
[22] Jiao Y, Li Y, Liu S, et al. ITGA3 serves as a diagnostic and prognostic biomarker for pancreatic cancer. Onco Targets Ther 2019;12:4141–52.
[23] Jiao Y, Li Y, Jiang P, et al. PGM5: a novel diagnostic and prognostic biomarker for liver cancer. Peer J 2019;7:e7070.
[24] Li Y, Jiao Y, Fu Z, et al. High miR-454-3p expression predicts poor prognosis in hepatocellular carcinoma. Cancer Manag Res 2019;11: 2795–802.
[25] Sun Z, Sun L, He M, et al. Low BCL7Ax expression predicts poor prognosis in ovarian cancer. J Ovarian Res 2019;12:41.
[26] Hou L, Zhang X, Jiao Y, et al. ATP binding cassette subfamily B member 9 (ABCB9) is a prognostic indicator of overall survival in ovarian cancer. Medicine (Baltimore) 2019;98:e15698.
[27] Palaniappan A, Ramar K, Ramalingam S. Computational identification of novel stage-specific biomarkers in colorectal cancer progression. PloS One 2016;11:e0156665.
[28] Goode EL, Maurer MJ, Sellers TA, et al. Inherited determinants of ovarian cancer survival. Clin Cancer Res 2010;16:995–1007.