

ARTICLE

High level of SMN1 in esophageal cancer predicts poor prognosis: An analysis based on TCGA data

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Abstract

This case suggests that attention should be paid to the effect of physical exercise on the time of occurrence of cardiovascular events. Exercise is a double-edged sword. On the one hand, acute exercise may be the direct cause of thrombotic events; on the other hand, exercise training can reduce the potential risk of cardiovascular events. This case suggests that appropriate studies are needed to determine the effects of long-term and short-term exercise, exercise time and exercise intensity on platelet aggregation and activity, thereby reducing the occurrence of cardiovascular events.

Keywords: Esophageal cancer, Survival motor neurone gene, Prognosis, Biomarker, Gene Set Enrichment An

1. Introduction

There will be an estimated 18.1 million new cancer cases and 9.6million cancer deaths in 2018. esophageal cancer ranks seventh in terms of incidence (572,000 new cases) and sixth in mortality overall (509,000 deaths), Approximately 70% of cases occur in men. Esophageal cancer is highest incidence rates in Eastern Asians, where rates in Mongolia and China are in the top worldwide and it commons in several Eastern and Southern African countries(1,2). Squamous cell carcinoma (SCC) accounts for most of cases of esophageal cancer worldwide, whereas Esophageal adenocarcinoma less than SCC, some cases are

developed from Barrett's esophagus (BE)(3). The TNM classification system is used to stage esophageal carcinoma. Limited diseases are treated with surgery, Locally advanced diseases are treated with surgery combined with radiotherapy and chemotherapy, the diseases with distant metastasis receive palliative chemotherapy(4,5,6). Other targeted drugs include resumumab and trastuzumab, which primarily inhibit the VEGFR1 signaling pathway and the HER2 family signaling pathway(7,8). In recent years, immune checkpoint PD-1 and PD-L1 inhibitors have also been clinically used to treat esophageal cancer(9). Despite the therapy is improved in esophageal cancer, the prognosis is remain very poor, with a 5-year survival rate about 10% in non-surgical patients and about 15-20% in esophagectomy patients(10).

Therefore, it is significant to search for a new prognostic biomarker for the treatment of esophageal cancer. In our study, we found SMN1 is highly expressed in esophageal cancer than normal disease. SMN1 was first found in the disease of SMA, the Deletion or mutation of the SMN1 gene induced in the disease of SMA(11). At present, the relationship between SMN1 and tumor remains unclear. We have observed that high expression of SMN1 is associated with poor prognosis of esophageal cancer. Therefore, SMN1 may become a new and important prognostic biomarker of esophageal cancer.

2. Materials and methods

2.1 TCGA data download and bioinformatic analysis

The gene expression data and corresponding clinical data of esophageal carcinoma (project ID: TCGA-ESCA) were downloaded from TCGA database (<https://portal.gdc.cancer.gov/>). Expression data of SMN1 was extracted. Then the expression difference of SMN1 between normal and tumor samples were compared. Clinical information was extracted and patients with incomplete clinical information were excluded. Finally, there were 159 patients were included in survival analysis and 124 patients were included in to cox regression and multi-variate cox analysis. The media expression value of SMN1 were calculated. Patients with SMN1 expression lever higher than media value were distributed to the high expression group and others were distributed to the low expression group. Overall survival in high expression and low expression group were compared. Cox regression and multivariate cox analysis were used to analysis the influence of clinical characteristics on the survival.

2.2 Gene set enrichment analysis

Software GSEA 4.1.0 was used to perform gene set enrichment analysis (GSEA). The expression matrix file and phenotype file were prepared before GSEA. SMN1 expression level was used as a phenotype label. Gene set permutations number was set to 1000. Then the expression matrix file and phenotype file were imported and GSEA was performed.

2.3 Statistical analysis

Statistical analyses were conducted by R software (R x64 3.6.2). The comparison of SMN1 expression between normal and tumor sample was analyzed by Wilcoxon signed-rank test. Clinical characteristics associated to survival was analyzed by Cox regression and Kaplan-Meier method. To further compare the effect of SMN1 expression on overall survival along with other clinical factors, multivariate Cox analysis was performed.

3. Result

3.1 Patient characteristics

As shown in Table 1, 124 primary esophageal cancers with gene expression data were downloaded from TCGA data. In our study cohort, the cancer status included 105(84.7%) males and 19(15.3%) females. Stage I disease was found in 12 patients(9.7%), Stage II in 63(50.8%), Stage III in 41(33.1%), Stage IV in 8(6.4%). T1 disease was found in 23(18.6%)T2 in 35(28.2%),T3 in 63(50.8%),T4 in 4(2.4%). Sixty-seven of 124 (54.1%) cases had lymph node metastases. Eight of 124 (6.5%) cases had distant metastases.

Table 1 esophageal carcinoma patient characteristics

characteristic		Total(N=124)	Percentage(%)
gender	male	105	84.7
	female	19	15.3
stage	I	12	9.7
	II	63	50.8
	III	41	33.1
	IV	8	6.4
T	1	23	18.6
	2	35	28.2
	3	63	50.8
	4	3	2.4
N	0	57	45.9
	1	56	45.2
	2	8	6.5
	3	3	2.4
M	1	116	93.5
	2	8	6.5

124 primary esophageal carcinoma patients were characterized with 12 patients(9.7%) in Stage I disease , 63(50.8%) in Stage II , 41(33.1%) in Stage III ,8(6.4%) in Stage IV. T1 disease was found in 23(18.6%), T2 in 35(28.2%), T3 in 63(50.8%), T4 in 4(2.4%). Sixty-seven of 124 (54.1%) cases had lymph node metastases. Eight of 124 (6.5%) cases had distant metastases.

In our study, the expression of SMN1 between normal and tumor samples were different, tumor samples were high expression compared with normal samples ($P=0.026$), which had statistical significance , as shown in Figure 1.

3.2 high-expression SMN1 influenced survival outcomes in esophageal cancers

A total of 124 esophageal samples with SMN1 expression were analyzed from TCGA data. Kaplan-Meier survival analysis showed that esophageal patients with SMN1-high had a worse prognosis than that with SMN1-low ($p = 0.011$),as shown in Figure 2.

3.3 Univariate analysis and multivariate analysis

The univariate analysis revealed that SMN1-high correlated significantly with a poor OS (hazard ratio [HR]: 1.39; 95% confidence interval [CI]: 0.96–1.99; $p = 0.078$),but the P value

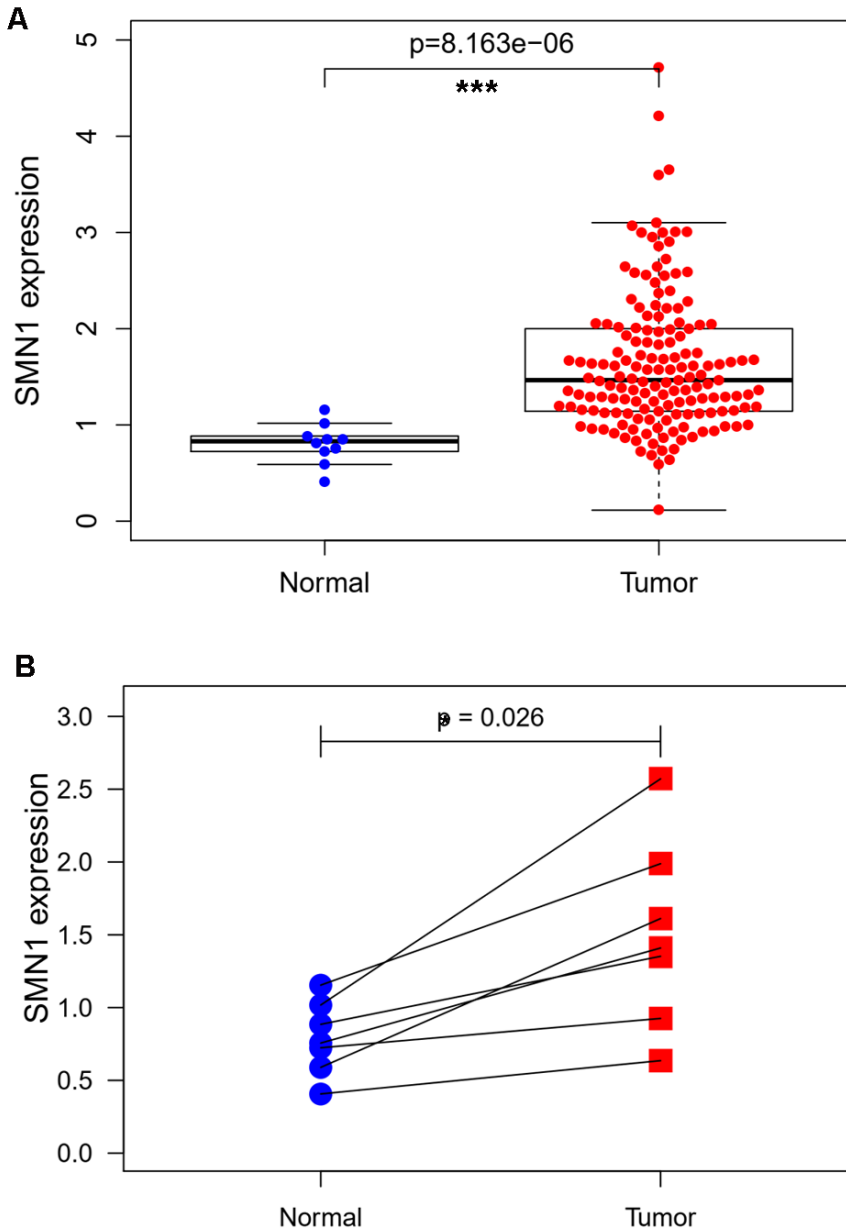


Figure 1. The expression of SMN1 between normal and tumor samples

had not statistical significance, maybe which was influenced by other clinicopathologic Variables, that associated with poor survival include advanced stage, lymph nodes and distant metastasis. Advanced stage, lymph nodes and distant metastasis were poor OS (HR):2.61; (CI):1.76-3.87; p=0.000, HR:4.69; CI:(2.14-10.27);p=0.000, HR:1.78;CI: (1.26-2.50); p=0.001 Relatively.(Table 2)

As showed in Figure 3, at multivariate analysis, SMN1 remained independently associated

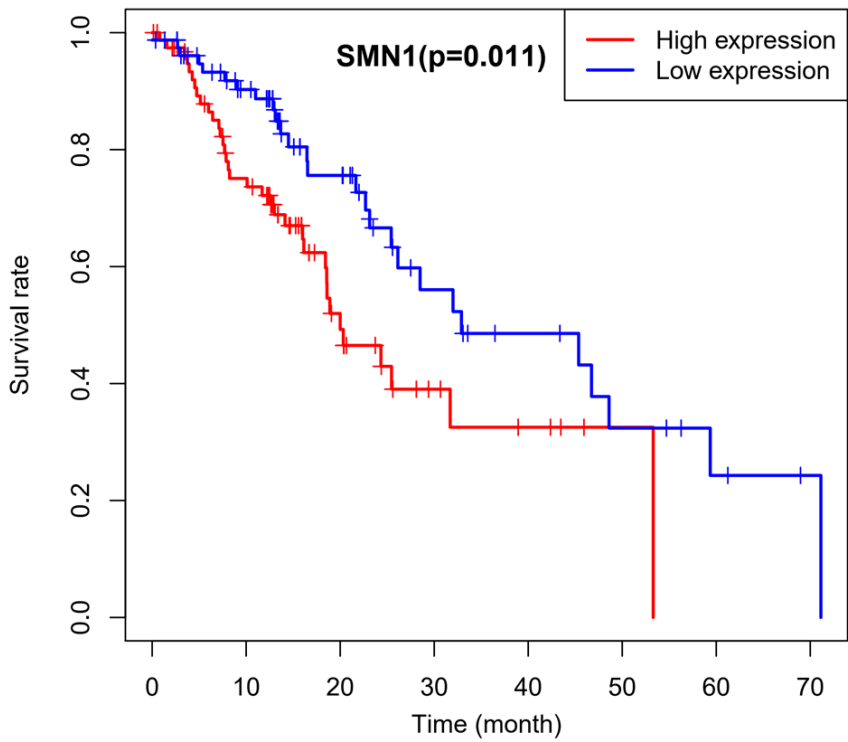


Figure 2. Kaplan-Meier survival analysis

Table 2 The univariate analysis revealed that SMN1-high correlated significantly with a poor OS

characteristics	HR(95%CI)	p-value
gender	2.38(0.74-7.70)	0.14
stage	2.61(1.76-3.87)	0.000
T	1.27(0.88-1.85)	0.203
M	4.69(2.14-10.27)	0.000
N	1.78(1.26-2.50)	0.00
SMN1	1.39(0.96-1.99)	0.078

SMN1-high was correlated significantly with a poor OS (hazard ratio [HR]: 1.39; 95% confidence interval [CI]: 0.96–1.99; $p = 0.078$),but the P value had not statistical significance, maybe which was influenced by other clinicopathologic Variables, that associated with poor survival include advanced stage, lymph nodes and distant metastasis. Advanced stage, lymph nodes and distant metastasis were poor OS (HR):2.61; (CI):1.76-3.87; $p=0.000$, HR:4.69; CI:(2.14-10.27); $p=0.000$, HR:1.78;CI: (1.26-2.50); $p=0.001$, Relatively

with overall survival, with a HR of 1.65 1.10–2.49, $p=0.016$.(Figure3 and Table 3)

3.4 GSEA identifies SMN1-related signaling pathway

To identify signaling pathways that are differentially activated in esophageal cancer,we conducted Gene Set Enrichment Analysis (GSEA) between low and high SMN1 expression

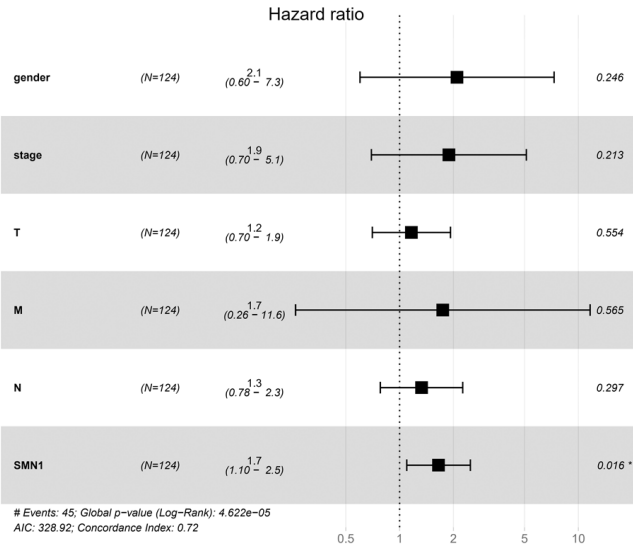


Figure 3. multivariate analysis

Table 3 and Figure 3 multivariate analysis

characteristics	HR(95%CI	p-value
gender	2.10 (0.60-7.32)	0.246
stage	1.89 (0.70-5.12)	0.213
T	1.16 (0.70-1.93)	0.554
M	1.75 (0.26-11.64)	0.565
N	1.33 (0.78-2.26)	0.297
SMN1	1.65 (1.10-2.49)	0.016

SMN1 remained independently associated with overall survival, with a HR of 1.65 (1.10-2.49) , p=0.016

data sets. GSEA reveal significant differences (FDR b 0.05, NOM p-val b 0.05) in enrichment of MSigDB Collection (c2.cp.biocarta and h.all. v6.1. symbols). We selected the most significantly enriched signaling pathways based on their enrichment score (ES) and normalized enrichment score (NES).(Figure 4 and Table 4). The figure 4 shows that spliceosome, proteasome pathway, RNA polymerase, RNA degradation and nucleotide excision repair are differentially enriched in SMN1 high expression phenotype and phosphatidylinositol signaling system enriched in SMN1 low expression phenotype.

4. Discussion

Survival motor neuron(SMN) is associated with the disease of spinal muscular atrophy(SMA), which is an -motoneurone disorder caused by SMN protein levels(12-15). The human SMN gene has two subtypes, SMN1 and SMN2,but the amount of functional SMN protein

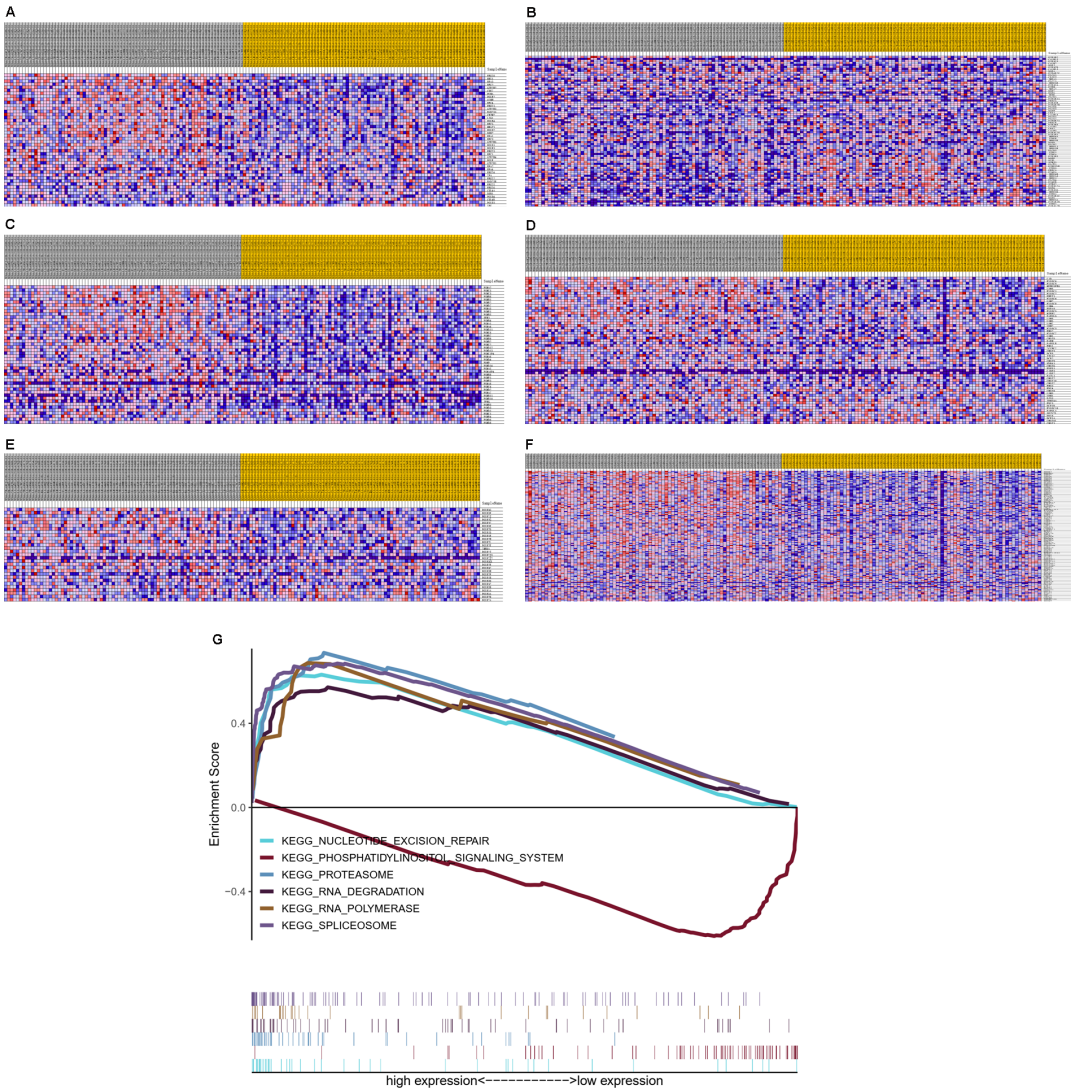


Figure 4. GSEA identifies SMN1-related signaling pathway

produced by SMN2 is not sufficient to prevent progressive-motoneurone degeneration. Therefore, SMN1 plays a major role in the occurrence of disease(16,17).

To our knowledge, we do not know the role of SMN1 expression and its potential prognostic relationship in esophageal cancer, therefore, our current major research focuses on the meaningful role of SMN1 in esophageal cancer.

In our study bioinformatics analysis from TCGA showed that high-express SMN1 in esophageal cancer associated with survival time and poor prognosis. To further investigate the functions of SMN1 in esophageal cancer, GSEA showed that spliceosome, proteasome pathway, RNA polymerase, RNA degradation and nucleotide excision repair are differentially enriched in SMN1 high expression phenotype and phosphatidylinositol signaling system

Figure 4 and Table 4 GSEA identifies SMN1-related signaling pathway

NAME	ES	NES	NOM p-val	FDR q-val
KEGG_SPLICEOSOME	0.685	2.172	0.000	0.005
KEGG_PROTEASOME	0.735	2.032	0.000	0.021
KEGG_RNA_POLYMERASE	0.687	2.024	0.000	0.015
KEGG_RNA_DEGRADATION	0.571	1.915	0.014	0.038
KEGG_NUCLEOTIDE_EXCISION_REPAIR	0.631	1.881	0.019	0.041
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	-0.618	-2.127	0.000	0.041

spliceosome, proteasome pathway, RNA polymerase, RNA degradation and nucleotide excision repair are differentially enriched in SMN1 high expression phenotype and phosphatidylinositol signaling system enriched in SMN1 low expression phenotype

enriched in SMN1 low expression phenotype. This may indicate that SMN1 serves as a potential prognostic indicator and therapeutic target in esophageal cancer.

SMN1 involved in multiple roles of RNA, including transcription, pre-mRNA splicing, snRNP assembly, the 3' end of histone mRNA processing, snoRNP assembly, telomerase activity, SG formation, translation, signal recognition particle (SRP) biogenesis and mRNA trafficking. Other roles included DNA recombination and repair, intracellular trafficking, endocytosis and autophagy(18).

In this study, we observe that SMN1 high expression phenotype was associated with spliceosome, proteasome pathway, RNA polymerase, RNA degradation and nucleotide excision repair, and low expression was associated with phosphatidylinositol signaling system. Some research was reported SMN1 participated in RNA functions, but proteasome pathway and phosphatidylinositol signaling system have not reported. We found that proteasome pathway is enriched in SMN1 high expression and phosphatidylinositol signaling system enriched in SMN1 low expression phenotype. Therefore, this regulatory mechanism needs further study.

In conclusion, SMN1 expression may be a potential prognostic molecular marker of poor survival in esophageal cancer, moreover, proteasome pathway and phosphatidylinositol signaling system may be the key pathway regulated by SMN1 in esophageal cancer. Further experimental studies are needed to prove the biological role of SMN1.

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