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Review Article

Musashi-2 and SCC-Ag expressions in patients with cervical cancer and their correlations with tumor malignancy

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Abstract: This paper aimed to investigate the clinical significance of Musashi-2 and SCC-Ag expressions in patients with CC. The relative expressions of Musashi-2, SCC-Ag, CC-related proliferation genes (FOXP3, eIF4E3), invasion genes (GOLPH3, GRP94),and autophagy genes (Beclin1, p62) in patients with CC were measured. The clinical significance of Musashi-2 and SCC-Ag were assessed. The expressions of Musashi-2, SCC-Ag, FOXP3, GOLPH3, GRP94, and p62 rose with disease severity, but the expressions of eIF4E3 and Beclin1 declined. The areas under the ROC curves (AUCs) of Musashi-2 and SCC-Ag for diagnosing the patients were 0.895 and 0.931, respectively. Musashi-2 and SCC-Ag were positively correlated with FOXP3, GOLPH3, GRP94, and p62, but negatively correlated with eIF4E3 and Beclin1. In conclusion, Musashi-2 and SCC-Ag are highly expressed in patients with CC, and can indicate tumor malignancy such as proliferation, invasion, and autophagy.

Keywords: Musashi-2, SCC-Ag, cervical cancer, tumor malignancy

Introduction

Cervical cancer (CC) is a malignant tumor mainly caused by human papilloma virus (HPV) infection and is also a major cause of cancer deaths among women around the world [1, 2]. Data indicate that the disease is the most common type of cancer among women after breast cancer, with approximately 530,000 new cases worldwide each year, with 80% of them occurring in developing countries [3, 4]. Its histological types are mainly composed of squamous cell carcinoma (SCC) and adenocarcinoma, with SCC accounting for 82.9%, adenocarcinoma accounting for 10.5%, and the rest being other or uncertain types [5]. At present, its main therapeutic method is surgery assisted by concurrent chemoradiotherapy, and its mortality rate in developing countries lacking treatment is 1 in 1 million [6]. Although its screening tools are continuously updated, the incidence is still on the rise in some developing countries and getting higher in young people [7, 8]. Therefore, we will conducted research on CC-related early diagnosis in order to provide indicative tools for young women to predict the disease's early conditions.

As a member of the Musashi family and an RNA binding protein that regulates mRNA translation, Musashi-2 is related to the metastasis of invasive tumors [9, 10]. Its abnormal upregulation usually occurs in breast cancer, ovarian cancer, and bladder cancer, and its overexpression activates cancer-promoting pathways or substances, thereby promoting cancer cells to migrate and invade [10-12]. Squamous cell carcinoma associated antigen (SCC-Ag), a tumor regulatory protein for SCC of various organs, inhibits cancer cell apoptosis induced by antitumor drugs [13]. It also guides the initial treatment of early squamous CC and predicts the pelvic lymph node metastasis of squamous CC [14]. This antigen can be applied to serum detection, so as to evaluate the early diagnosis and recurrence of patients with stage I or II squamous CC [15]. Studies have shown that abnormal Musashi-2 and SCC-Ag expressions in CC tumor tissues are related to shorter overall survival (OS) and progression-free survival

times (PFS), suggesting that both Musashi-2 and SCC-Ag may be involved in CC malignant progression [16, 17].

There is currently little research on the correlations of Musashi-2 and SCC-Ag with tumor malignancy, correlations analyzed in this study by measuring the expressions of Musashi-2, SCC-Ag, and the CC-related malignant genes of the tumors, and the potential of Musashi-2 and SCC-Ag to diagnose CC was verified.

Materials and methods

General information

One hundred and sixty-five patients with CC admitted to our hospital from March 2018 to June 2019 were enrolled in the study, of which 50 were in the benign group, 60 were in the early group, and 55 were in the middle and advanced group. Those in the benign group were aged 29-72 years, and their average age was 53.07±8.73 years. Those in the early group were aged 33-75 years, and their average age was 53.43±9.31 years. Those in the middle and advanced group were aged 35-75 years, and their average age was 54.55±9.20 years. This study was approved by the Ethics Committee of our hospital. The research objects and their families were fully informed, and each signed an informed consent form.

Inclusion and exclusion criteria

Inclusion criteria: Patients confirmed with CC by histopathologic examination (those in stage I in the benign group, those in stage II in the early group, those in stages III-IV in the middle and advanced group based on the FIGO 2009 stage) [18]; patients who had not received radiotherapy or chemotherapy before the surgical operation for cervical cancer; patients without systemic infection or serious organ dysfunction; patients without mental diseases or communication disorders. Exclusion criteria: Those who had received treatment or a surgical operation for cervical cancer; those with a history of surgery within half a year; those with complications such as other malignant tumors; those aged over 80 years; pregnant women.

qRT-PCR

The subjects' tumor tissues retained during their surgical operations for cervical cancer were frozen for later use. Total RNA was extracted from the collected tissues and tested using a TRIzol kit (Shanghai MingJin Biological Technology Co., Ltd., China, 5003050), and its purity, concentration, and integrity were determined using an ultraviolet spectrophotometer (Shanghai Puyuan Instrument Co. Ltd., China, Alpha-1860Plus) and agarose gel electrophoresis (Beijing Hongtao Foundation Technology Development Co., Ltd., China, HT-SCZ04). The reverse transcriptions of Musashi-2, SCC-Ag, forkhead box protein 3 (FOXP3), eukaryotic initiation factor 4E3 (eIF4E3), Golgi phosphoprotein 3 (GOLPH3), glucose-regulated protein 94 (GRP94), coiled-coil myosin-like Bcl-2 interacting protein (Beclin1), and EBI 3 associated protein of 60 kDa (p62) were strictly carried out based on the kit's instructions (Shanghai Yuduo Biotechnology Co., Ltd., China, YDJ2547). The amplification system was as follows: cDNA (1 μL), upstream and downstream primers (0.4 μL each), 2X TransScript® Tip Green qPCR SuperMix (10 μL), Passive Reference Dye (50X) (0.4 μL), and Nuclease-free water was finally added to supplement to 20 μL. The amplification conditions were as follows: pre-denaturation (94°C for 30 s), denaturation (94°C for 5 s), annealing and extension (60°C for 30 s), and cycling 40 times. There were 3 duplicate wells for each sample. Three repeated experiments were performed. The primers used in the PCR were designed by Shanghai Yingbio Technology Co., Ltd., China. GAPDH was used as an internal reference of mRNA. The standard value of the CC-related gene expression was 100. 2-ΔΔct was used to analyze the data.

Statistical analysis

GraphPad Prism 6 (GraphPad Software, San Diego, USA) was used to plot the figures. The count data were expressed as the number of cases/percentage [n (%)], and chi-square tests were used for the comparison between groups. The measurement data were expressed as the mean ± standard deviation (mean ± SD), and F tests were used for the comparisons between three groups. Receiver operating characteristic (ROC) curves were plotted to assess the diagnostic values of Musashi-2 and SCC-Ag in the patients with CC. The Pearson correlation coefficient (PCC) was used to analyze the correlations of the two with FOXP3, eIF4E3, GOLPH3, GRP94, Beclin1, and p62. When $P<0.05$, the

Table 1. Comparison of the general and pathological data [n (%), mean ± SD]

Categories	Benign group (n=50)	Early group (n=60)	Middle and advanced group (n=55)	χ^2/F value	*P* value
Age (Years)				6.308	0.958
<50	19 (38.00)	35 (58.33)	21 (38.18)		
≥50	31 (62.00)	25 (41.67)	34 (61.82)		
Average age (Years)	53.07±8.73	53.43±9.31	54.55±9.20	0.386	0.680
History of smoking				9.811	0.776
No	22 (44.00)	42 (70.00)	25 (45.45)		
Yes	28 (56.00)	18 (30.00)	30 (54.55)		
History of alcoholism				8.147	0.882
No	21 (42.00)	39 (65.00)	23 (41.82)		
Yes	29 (58.00)	21 (35.00)	32 (58.18)		
Place of residence				11.460	0.650
Countryside	11 (22.00)	32 (53.33)	20 (36.36)		
City	39 (78.00)	28 (46.67)	35 (63.64)		
Educational level				7.801	0.899
Primary school/junior high school	13 (26.00)	25 (41.67)	29 (52.73)		
Senior high school/university	37 (74.00)	35 (58.33)	26 (47.27)		
Degree of pathological differentiation				75.971	<0.001
Moderately + highly differentiated	47 (94.00)	48 (80.00)	10 (18.18)		
Lowly differentiated	3 (6.00)	12 (20.00)	45 (81.82)		
Tumor size (cm)				50.223	<0.001
<3	46 (92.00)	49 (81.67)	18 (32.73)		
≥3	4 (8.00)	11 (18.33)	37 (67.23)		
Pathological types				8.182	0.880
SCC	41 (82.00)	52 (86.67)	36 (65.45)		
Adenocarcinoma	9 (18.00)	8 (13.33)	19 (34.55)		
Serosal invasion				76.784	<0.001
No	46 (92.00)	42 (70.00)	6 (10.91)		
Yes	4 (8.00)	18 (30.00)	49 (89.09)		
Infiltration depth				103.008	<0.001
<1/2 mesenchyme	45 (90.00)	54 (90.00)	5 (9.09)		
≥1/2 mesenchyme	5 (10.00)	6 (10.00)	50 (90.91)		
Musashi-2	0.13±0.05	0.31±0.12	0.81±0.24	263.798	<0.001
SCC-Ag	0.50±0.19	1.11±0.32	2.4±0.49	390.710	<0.001

difference was considered statistically significant.

Results

Comparison of the pathological data

There were significant differences between the benign, early, and middle and advanced groups with respect to the degree of pathological differentiation, tumor size, serosal invasion, infiltration depth, Musashi-2, and SCC-Ag (P<0.05), but not in age, average age, history of smoking, history of alcoholism, place of residence, educational level, or pathological types (P>0.05). See **Table 1**.

Musashi-2 and SCC-Ag expressions

The Musashi-2 expressions in the benign, early, and middle and advanced groups were (0.13± 0.05), (0.31±0.12), and (0.81±0.24), respectively. The SCC-Ag expressions in the three groups were (0.50±0.19), (1.11±0.32), and (2.4± 0.49), respectively. Their expressions in the three groups showed a significant upward trend, with a significant difference between the groups (P<0.001). See **Figure 1**.

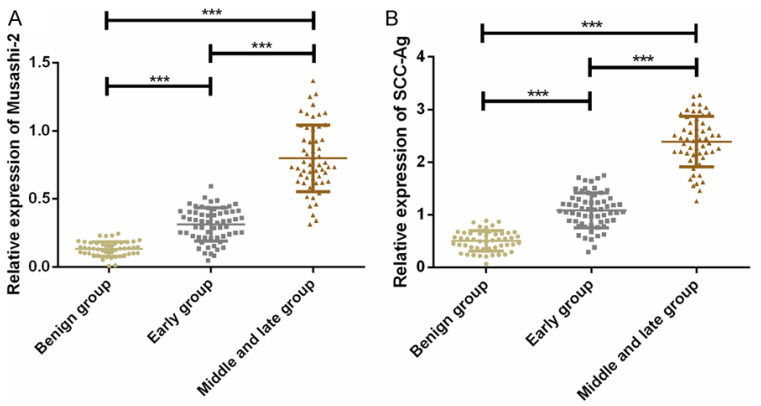

Figure 1. Musashi-2 and SCC-Ag expressions. A. The Musashi-2 expression in the benign, early, and middle and advanced groups showed a significant upward trend. B. The SCC-Ag expression in the benign, early, and middle and advanced groups showed a significant upward trend. Note: ***indicates P<0.001.

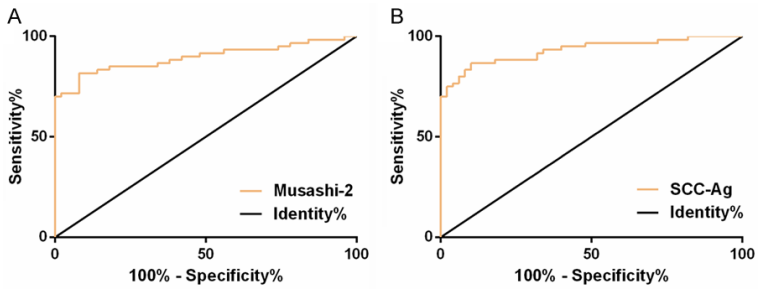

Figure 2. The ROC curves of Musashi-2 and SCC-Ag for diagnosing patients with CC. A. The ROC curve of Musashi-2 for diagnosing patients with CC. B. The ROC curve of SCC-Ag for diagnosing patients with CC.

Table 2. The ROC diagnostic parameters of Musashi-2 and SCC-Ag

Groups	AUC	95%CI	S.E	Cut-off	Sensitivity (%)	Specificity (%)
Musashi-2	0.895	0.832-0.957	0.032	0.20	81.67	92.00
SCC-Ag	0.931	0.884-0.978	0.024	0.76	86.67	90.00

The diagnostic values of Musashi-2 and SCC-Ag

The ROC curves of Musashi-2 and SCC-Ag for diagnosing patients with CC were plotted. The area under the curve (AUC) of Musashi-2 was 0.895 (95% CI: 0.832-0.957), the cut-off value was 0.20, the sensitivity was 81.67%, and the specificity was 92.00%. The AUC of SCC-Ag was 0.931 (95% CI: 0.884-0.978), the cut-off value was 0.76, the sensitivity was 86.67%, and the specificity was 90.00%. See **Figure 2** and **Table 2**.

The expressions of the CC-related proliferation genes

The CC-related proliferation genes (FOXP3 and eIF4E3) were explored. The FOXP3 expression showed a significant upward trend, but the eIF4E3 expression showed a significant downward trend in the benign, early, and middle and advanced groups, with a significant difference between the groups (P<0.001). See **Figure 3**.

The expression of the CC-related invasion genes

The CC-related invasion genes (GOLPH3 and GRP94) were explored. Their expressions in the benign, early, and middle and advanced groups showed a significant upward trend, with a significant difference between the groups (P<0.001). See **Figure 4**.

The expressions of the CC-related autophagy genes

The CC-related autophagy genes (Beclin1 and p62) were explored. The Beclin1 expression showed a significant downward trend, but the p62 expression showed a significant upward trend in the benign, early, and middle and advanced groups, with a significant difference between the groups (P<0.001). See **Figure 5**.

Correlation analysis

PCC was used to analyze the correlations of Musashi-2 and SCC-Ag with the CC-related proliferation, invasion, and autophagy genes. Musashi-2 and SCC-Ag were positively correlated with FOXP3 (r=0.604, P<0.001; r=0.618, P<0.001), negatively correlated with eIF4E3 (r=-0.605, P<0.001; r=-0.572, P<0.001), positively correlated with GOLPH3 (r=0.634, P< 0.001; r=0.688, P<0.001) and GRP94 (r= 0.624, P<0.001; r=0.606, P<0.001), negatively correlated with Beclin1 (r=-0.556, P<0.001;

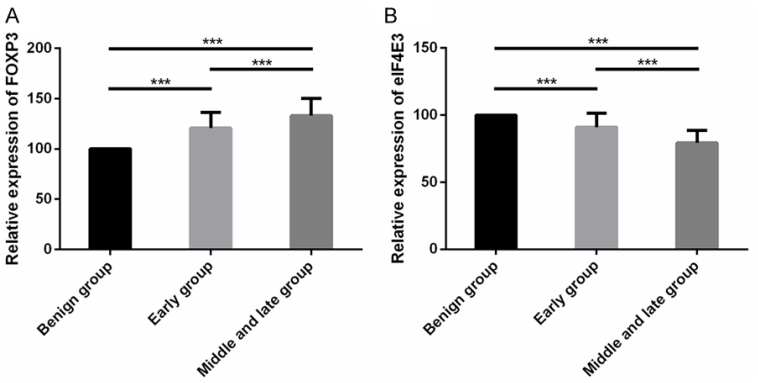

Figure 3. FOXP3 and eIF4E3 expressions. A. The FOXP3 expression showed a significant upward trend in the benign, early, and middle and advanced groups. B. The eIF4E3 expression showed a significant downward trend in the benign, early, and middle and advanced groups. Note: ***indicates P<0.001.

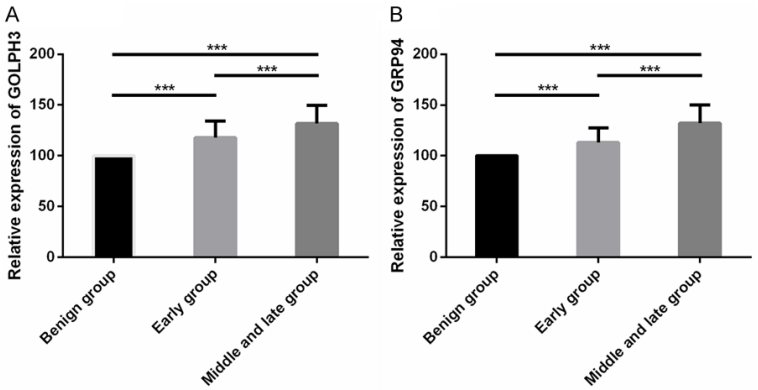

Figure 4. GOLPH3 and GRP94 expressions. A. The GOLPH3 expression showed a significant upward trend in the benign, early, and middle and advanced groups. B. The GRP94 expression showed a significant upward trend in the benign, early, and middle and advanced groups. Note: ***indicates P<0.001.

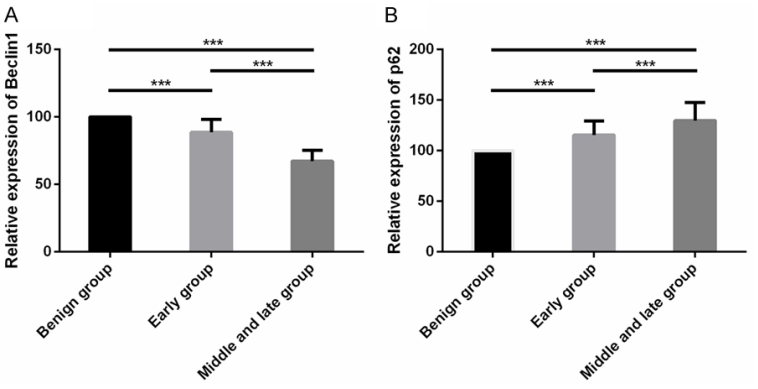

Figure 5. Beclin1 and p62 expressions. A. The Beclin1 expression showed a significant downward trend in the benign, early, and middle and advanced groups. B. The p62 expression showed a significant upward trend in the benign, early, and middle and advanced groups. Note: ***indicates P<0.001.

r=-0.586, P<0.001), and positively correlated with p62 (r=0.688, P<0.001; r=0.570, P<0.001). See **Figure 6**.

Discussion

More and more scholars have shown their interest in the roles of Musashi-2 and SCC-Ag in CC. According to Dong et al., Musashi-2 mRNA, which is highly expressed in CC tissues and regulated by miR-143 and miR-107, promotes CCC invasion, proliferation, and sphere formation [19]. According to Fu et al., SCC-Ag in combination with other clinical features can be used as a guiding tool for treating patients with CC, with a reference value for judging the occurrence of the postoperative indications of adjuvant radiotherapy and radiotherapy as well as judging the choice of concurrent radiotherapy and chemotherapy or surgery [20]. In our study, the Musashi-2 and SCC-Ag expressions showed an increasing trend in sequence in the benign, early, and middle and advanced groups, and their AUCs for diagnosing patients with CC were 0.895 and 0.931, respectively. This indicates that the two may be involved in the development and progression of CC, so they have a good diagnostic value for patient prognosis and have the potential to become biomarkers for the early diagnosis of patients with CC. In a study on the diagnostic value of SCC-Ag by Wang et al., the AUC, sensitivity, and specificity of SCC-Ag for predicting distant metastasis were 0.784, 80.00%, and 72.73%, respectively [21]. This suggests that SCC-Ag has a better diagnostic value for evaluating distant metastasis.

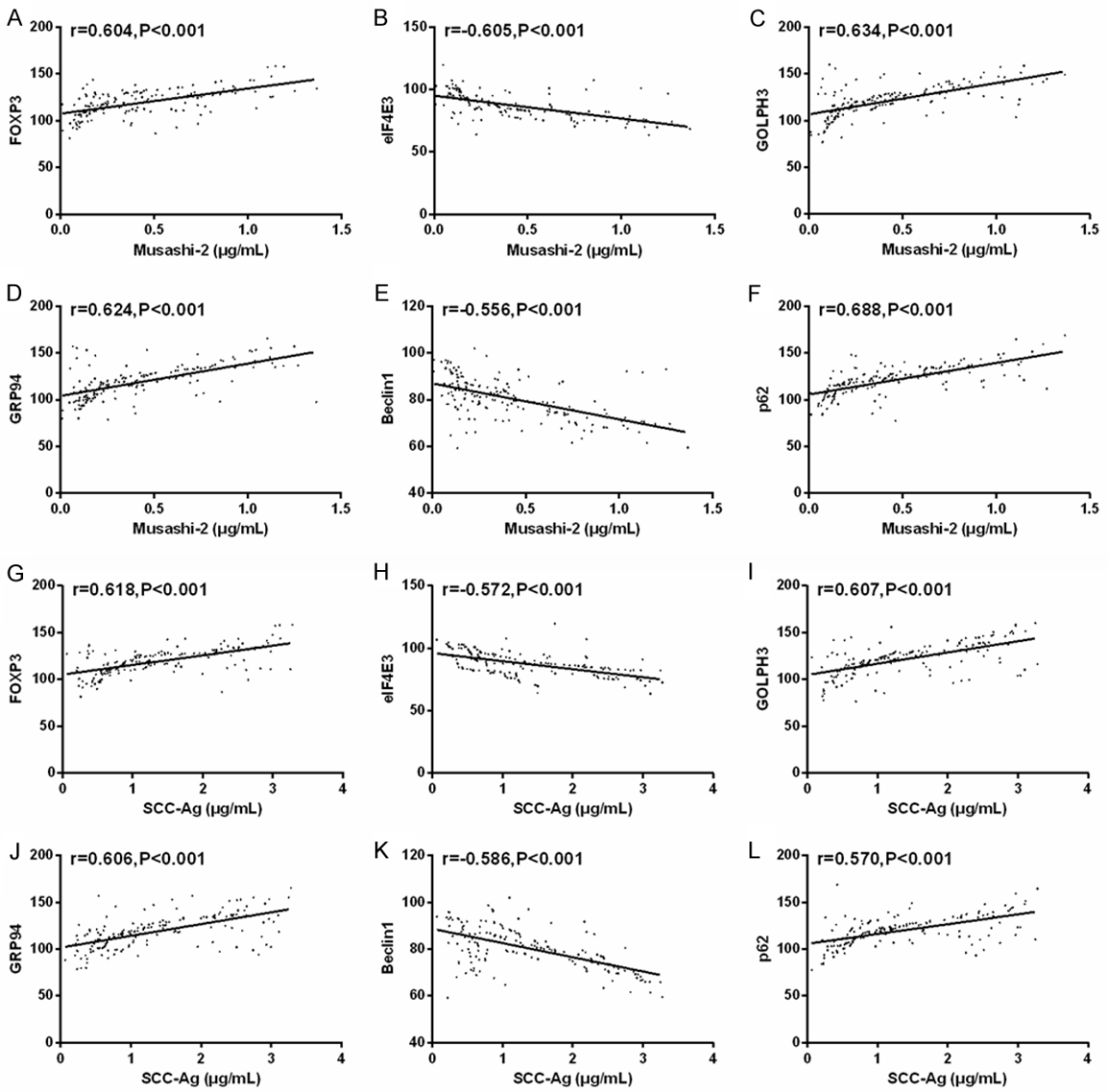

Figure 6. Results of the correlation analysis. A-F. Musashi-2 was positively correlated with FOXP3, negatively correlated with eIF4E3, positively correlated with GOLPH3 and GRP94, negatively correlated with Beclin1, and positively correlated with p62 (r=0.604, P<0.001; r=-0.605, P<0.001; r=0.634, P<0.001; r=0.624, P<0.001; r=-0.556, P<0.001; r=0.688, P<0.001). G-L. SCC-Ag was positively correlated with FOXP3, negatively correlated with eIF4E3, positively correlated with GOLPH3 and GRP94, negatively correlated with Beclin1, and positively correlated with p62 (r=0.618, P<0.001; r=-0.572, P<0.001; r=0.607, P<0.001; r=0.606, P<0.001; r=-0.586, P<0.001; r=0.570, P<0.001).

FOXP3 is a marker for inhibiting the immune defense of regulatory T (Treg) cell subsets. It is positively correlated with persistent HPV infection and squamous intraepithelial lesions by maintaining the host's state of immunosuppression [22]. In a study on FOXP3 in CC by Zhang and others, FOXP3 and toll-like receptor 4 were found to be highly expressed in CCCs and positively correlated significantly with each other [23], indicating that the two may have a synergistic effect on promoting the immune escape of CC. eIF4E3 is a eukaryotic translation initiation factor and a translation initiation protein that inhibits cancers [24]. According to Xu et al., it has a low expression in CCCs, and its phosphorylation level increases significantly after chemotherapy [25], showing that eIF4E3 can be used to evaluate the curative effect on patients with CC. As an evolutionarily conserved phosphatidylinositol 4-phosphate (PI4P)

binding protein, GOLPH3 is a tumor suppressor gene and has an anti-tumor effect during secretion and transportation by the Golgi apparatus [26, 27]. According to Feng and others, its high level is related to the poor prognosis of patients with squamous CC, and its low level is associated with the cell cycle arrest, an increase in the apoptotic level, the enhancement of cisplatin sensitivity, and a decrease in cell viability [28]. GRP94 is a paralog of endoplasmic reticulum-resident heat shock protein 90, closely related to the survival and progression of tumors such as melanoma and ovarian cancer [29]. Studies have shown that GRP94 is related to CCCs' radioresistance. Its high expression is a molecular mechanism that causes resistance to radioactive rays, so silencing its gene expression may contribute to the cells' radiotherapy resistance [30]. Beclin1 is an autophagy-related protein, and its overexpression can inhibit CCCs from proliferating, invading, and migrating. This indicates that knocking down its expression may become a potential therapeutic target for patients with CC [31]. According to Hu et al., its low level is a risk factor affecting patient survival, and its expression is negatively correlated with the differentiation, lymph node metastasis, recurrence, and patient death of the disease [32]. p62 is involved in autophagy and signal transduction, so it has the potential to become a new antigen [33]. It is upregulated in CCC lines and can reflect their autophagy levels [34]. In our study, the FOXP3, GOLPH3, GRP94, and p62 expressions showed a significant upward trend in sequence, while eIF4E3 and Beclin1 expression showed a significant downward trend in the benign, early, and middle and advanced groups. These findings suggest that the former four are positively correlated while the latter two are negatively correlated with the tumor malignancy of patients with CC.

Finally, PCC was performed to analyze correlations of Musashi-2 and SCC-Ag with the CC-related malignant genes. The two were positively correlated with FOXP3, negatively correlated with eIF4E3, positively correlated with GOLPH3 and GRP94, negatively correlated with Beclin1, and positively correlated with p62. The above results show that Musashi-2 and SCC-Ag are related to the proliferation, invasion, and autophagy of cancer cells in patients with CC, so they can reflect the tumor malignancy of the patients.

This study confirms the high expressions of Musashi-2 and SCC-Ag in patients with CC, their good diagnostic values for tumor lesions in the patients, and their correlations with tumor-related malignant genes, but there is still room for improvement. First of all, we can supplement the research on the patients' cell biological functions and the discussion on the relevant molecular regulatory mechanisms. Second, we can enlarge the sample size and histological types to increase the accuracy of the results. These deficiencies will be gradually addressed in the future.

Conclusion

Musashi-2 and SCC-Ag expressions are up-regulated in patients with CC, and the two have a good diagnostic value for reflecting tumor malignancy such as proliferation, invasion, and autophagy.

Disclosure of conflict of interest

None.

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tute on Deafness &Other Communication Disorders; Ontario Tumour Bank, London Health Sciences Centre; Ontario Tumour Bank, Ontario Institute for Cancer Research; Ontario Tumour Bank, The Ottawa Hospital; Oregon Health &Science University; Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center; SRA International; St Joseph's Candler Health System; Eli &Edythe L. Broad Institute of Massachusetts Institute of Technology &Harvard University; Research Institute at Nationwide Children's Hospital; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University; University of Bergen; University of Texas MD Anderson Cancer Center; University of Abuja Teaching Hospital; University of Alabama at Birmingham; University of California, Irvine; University of California Santa Cruz; University of Kansas Medical Center; University of Lausanne; University of New Mexico Health Sciences Center; University of North Carolina at Chapel Hill; University of Oklahoma Health Sciences Center; University of Pittsburgh; University of São Paulo, Ribeir ão Preto Medical School; University of Southern California; University of Washington; University of Wisconsin School of Medicine &Public Health; Van Andel Research Institute; Washington University in St Louis. Integrated genomic and molecular characterization of cervical cancer. Nature 2017; 543: 378-384.

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