의학석사 학위논문

피부혈관육종환자에서 Endoglin과 Survivin 발현의 임상적 의미

Clinical Implications of expression of Endoglin and Survivin in Patients with Primary Cutaneous Angiosarcoma in an Asian Population

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이 논문을 의학석사 학위 논문으로 제출함

2022년　02월

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ABSTRACT

Background: Targeted therapy and immunotherapy such as PD-1 targeting have been introduced for treating many types of cancers, including primary cutaneous angiosarcoma (CA). However, studies that examined other targeted molecules in CA are scarce.

Methods: We evaluated the expression of endoglin and survivin in addition to PD-1 and assessed the clinicoprognostic correlation between the expression of these molecules and clinical variables, overall survival (OS), and progression-free survival (PFS) in CA. We identified 51 patients diagnosed with CA at Asan Medical Center over the last 14 years, based on the staining results of paraffin sections of tissue samples for endoglin, survivin, and PD-1 that were reviewed by two dermatologists.

Results: Statistical analysis for the correlation between results and clinical data of CA revealed that whereas 35 (63.6%) and 30 (54.5%) samples were positive for endoglin and survivin respectively, only nine samples were positive for PD-1 (16.4%). Co-expression of endoglin and survivin was detected in 24 lesions ($P = 0.013$) and was significantly correlated to head, neck, face and scalp (HNFS) lesions in CA ($P = 0.005$, $P = 0.038$, respectively). However, the expression of these target molecules did not correlate with the OS or PFS of CA.

Conclusion: Considering that HNFS type CA is associated with unfavorable clinical outcomes in similar populations, our findings can be helpful in matching patients with CA with effective targeted therapy, and endoglin or survivin can be promising diagnostic and predictive biomarkers of HNFS type CA.

Keywords: cutaneous angiosarcoma, endoglin, survivin, targeted therapy

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INTRODUCTION

Cutaneous angiosarcoma (CA) is a rare malignant tumor associated with poor prognosis and frequently affects elderly patients. CA is typified by early metastasis and follows an aggressive clinical course of disease progression. Conventional chemotherapy alone is not adequate to inhibit the progression of inoperable or metastatic CA.(1) Effective treatment strategy for CA remains scarce, and the effectiveness of recently developed novel therapeutic approaches remains inconclusive.(2, 3)

Among the known immune checkpoint molecules, the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) axis is the most well-known prognostic biomarker. Immunotherapy with PD-1 inhibitors have achieved dramatic success in some cases of CA,(4) but unsatisfactory results have been reported in other patient group.(5) Although many studies on immunotherapy are still ongoing, including those identifying relevant immune checkpoint molecules and other targeted molecules, due to its rare occurrence, only limited data of CA are available.

Recently, molecular markers, such as endoglin and survivin, have been reported to be novel therapeutic targets of angiosarcoma.(6) Endoglin, a coreceptor for transforming growth factor β-1 (TGFβ-1), is overexpressed in the proliferating endothelial cells lining the activated vessel wall.(7) A study has shown that inhibition of endoglin in angiosarcoma suppresses tumorigenesis through non-Smad TGFβ-1 signaling.(8) Additionally, survivin, an important element for the regulation of Hippo pathway, is significantly expressed in the nucleus of almost all angiosarcomas.(9)

We hypothesized that these tumor-associated molecules may be used as biomarkers of primary CA for the Korean patient population. To better understand the pathogenesis of CA, we examined the expression of endoglin, survivin, and PD-1 in CA, and their correlation with the clinicopathologic characteristics and prognosis of primary CA in the Korean population.

MATERIALS AND METHODS

Study population and variables of interest

Upon obtaining approval from the Institutional Review Board of the Asan Medical Center, we performed an electric medical record database search to identify all patients diagnosed with primary cutaneous angiosarcoma (CA) based on skin biopsy results between January 1997 and December 2020. Only patients for whom all clinical data and biopsy slides were available were included in the study. Clinical data were retrieved from the patients' medical records: age at diagnosis, sex, tumor location, tumor size, multiplicity, presence of lymphedema, and the date of last follow-up. Overall survival (OS) was calculated from the date of initial diagnosis to the time of death from any cause or the last follow-up. Progression-free survival (PFS) was calculated from the date of initial diagnosis to the first day of disease progression, recurrence, or the last follow-up.

Histopathology and immunohistochemistry

The hematoxylin and eosin-stained biopsy slides of 55 cases were reviewed. Formalin-fixed paraffin-embedded tumor samples were stained with antibodies against PD-1 (mouse, monoclonal, 1:1000 dilution; Cell Marque, Rocklin, CA, USA), PD-L1 (mouse, monoclonal, pre-diluent; Agilent, Santa Clara, CA, USA), endoglin (rabbit, monoclonal, 1:800 dilution; Abcam, Cambridge, UK), and survivin (rabbit, monoclonal, 1:800 dilution; Cell Signaling Technology, Inc, Danvers, MA, USA).

Evaluation of endoglin, survivin, and PD-1

Two experienced dermatologists (HJK, YJK) assessed the expression of each protein on the CA lesions. Slides were reviewed using a Nikon microscope at ×200 magnification. The cutoff value for expression scoring of endoglin, survivin, PD-1, and PD-L1 has not been standardized and was previously reported to be 1% or 5%.(10, 11) Therefore, all cases in this study were scored as negative (0~5%), or positive (over 5% of the tumor cells). The cutoff values for high expression were selected based on values with the maximum significant differences in OS or values obtained from previous studies.(8, 9)

Statistical analysis

The clinicopathological features and disease outcomes were estimated according to the different expressions of endoglin, survivin, and PD-1. Between-group comparisons were performed using a chi-squared or Fisher's exact test for categorical variables, and a t-test or Mann–Whitney test for continuous variables, as appropriate.

In addition, a univariable Cox regression analysis was performed to identify factors associated with OS and PFS and to estimate the association between the target molecules (endoglin, survivin, and PD-1) and prognosis (OS and PFS). Statistical analyses were performed using R software (version 3.1.2; R Foundation for Statistical Computing) and SPSS (version 18.0; SPSS Inc., Chicago, IL). P-values of < 0.05 were considered to indicate significance.

RESULTS

In this study, our retrospective review identified 51 patients with primary cutaneous angiosarcoma (CA) in a Korean population in our center. The demographic data of patients with CA are summarized in Table 1. There were four patients, in whom two biopsies were performed due to multiple lesions or recurrence; a total of 55 lesional sections were included in the study.

Of the total 55 slides, endoglin, survivin, and PD-1 were positive in 9 (16.4%), 35 (63.6%), and 30 (54.5%) slides, respectively. When PD-L1 was stained, the staining intensity was not evident. Thus the expression of PD-L1 was not included in this study. Co-expression of endoglin and survivin was found in 24 (43.6%) slides. There was a significant correlation between endoglin and survivin ($P = 0.013$). Whereas, we found no significant association between PD-1 and endoglin, as well as PD-1 and survivin.

Clinical variables were stratified based on the tumor expression of endoglin, survivin, and PD-1 (Table 1). Co-expression of both endoglin and survivin was associated with head, neck, face, and scalp (HNFS) lesion ($P = 0.005$ and $P = 0.038$). To compare sun-exposed and non-exposed skin areas, the difference in the expression pattern was observed in three patients with multiple lesions. All of them were found to have stronger expressions in scalp or head and neck lesions than in other anatomical lesions. However, neither exhibited significant differences in the protein expression at other anatomical lesions nor were there any correlations between other clinical variables (age, sex, tumor size, multiplicity, and presence of lymphedema) and the expression of endoglin, survivin, and PD-1.

Analysis of the OS and PFS according to the clinical variables showed that old age and large tumor size were significantly associated with poor outcomes (Tables 2, 3). However, the location of the tumor did not correlate with prognosis. Additionally, there were no significant associations between the expression of target molecules (endoglin, survivin, and PD-1) and prognosis (OS or PFS).

DISCUSSION

The roles of immune molecules and other target molecules have become increasingly important in cancer biology and treatment strategies. Indeed, targeted therapy and immunotherapy including PD-1 target have been introduced for treating many types of cancers but studies that examined the expression of previously-known targeted molecules such as PD-1/PD-L1 in cutaneous angiosarcoma (CA) are very scarce. Moreover, the knowledge on the expression of the novel target molecules including endoglin and survivin in CA remains only limited.

In other different types of cancers, there have been multiple studies that have investigated the expression of endoglin, survivin, and PD-1 and their correlation with prognosis. For example, high expression of endoglin and survivin was reported to be associated with poor prognosis in laryngeal carcinoma and hepatocellular carcinoma.(12, 13) Overexpression of survivin was also observed in head and neck squamous cell carcinoma, which predicts shorter OS.(14) In agreement with these findings, various molecules have been reported to be novel tumor targets for developing immunotherapy-based next-generation anticancer treatments.

In endothelial cells, survivin has been found to control apoptosis during tumor angiogenesis.(15) Survivin is expressed in almost all angiosarcomas, which was suggested to be a quite distinctly differentiating point from other vascular tumor lesions in a study.(9) In a recent genomic functional analysis, survivin was identified as a potential marker and therapeutic target of CA.(16)

As such, endoglin was overexpressed in endothelial cells that undergo active angiogenesis, forming microvessel environment of tumors.(17, 18) Furthermore, endoglin is also expressed in cancer-associated fibroblasts and immune cells residing in the tumor microenvironment.(19) In another study examining the expression and crosstalk of endoglin and survivin in CA, knockdown of endoglin led to a significant decrease in the expression of survivin.(8) TRC105 (anti-endoglin antibody) and YM155 (survivin suppressant) have been employed to treat advanced angiosarcoma as well as various types of cancers.(6, 9)

Interestingly, expression of PD-1 was undesirably elevated in the cytotoxic T cells of patients treated with TRC105(anti-endoglin antibody), suggesting an immune-dependent

effect of TRC105.(19, 20) Thus, combination therapy using PD-1 inhibitor and anti-endoglin antibody was tried and was demonstrated to synergistically enhance tumor regression rate in vivo.(19, 20) However, our study found no significant difference between the expression of endoglin and PD-1 in CA.

Thus far, the body of evidence on the use of these candidate biomarkers in designing effective immunotherapy and targeted therapy that will benefit patients with CA is still limited. As endothelial cells are the main tumor cells of CA, we tried to explore the role of endoglin and survivin in the CA and overall survival (OS) in our center. Of note, in our present study, endoglin and survivin were concurrently expressed in tumor cells with statistical significance, suggesting a crosstalk between pathways regulating the expression of endoglin and survivin. We identified a significant association between endoglin and survivin in CA, which is consistent with the findings on the expression of endoglin and survivin in other types of cancer. For example, in laryngeal carcinoma, nuclear expression of survivin correlates with microvascular density which is determined by the expression of endoglin.(13) A study also reported the overexpression of endoglin and survivin in hepatocellular carcinoma cells compared with that in normal hepatocytes.(12)

Of note, in our study, we observed an overexpression of endoglin and survivin in the HNFS subtype of CA. Compared to non-HNFS type CA, HNFS subtype of CA has higher tumor mutation burden,(21) which could be caused by UV damage from sun exposure, a clinical manifestation similar to melanoma.(21, 22) Therefore, we inferred that endoglin and survivin may also be associated with high tumor mutation burden. As higher tumor mutation burden is generally related to be higher cell surface immunopeptides in other types of cancers,(23) we hypothesized that the expression of PD-1 would be high in HNFS type CA, but we found no significant association between expression of PD-1 and type of CA in this study.

According to a previous clinicopathological and survival analyses study performed in a Korean population, CA on the scalp was also suggested to be the possible indicator of poor prognosis.(24) Their study also revealed that nodular morphology and multiplicity were related to a poor prognosis of CA which was not the case in our study. Our study revealed that old age and large tumor size may indicate poorer outcomes in patients with primary CA

of the Korean race. This discrepancy could be attributed to the different patient groups, relatively small number of cases in a single tertiary medical center and inherently poor prognosis and rarity of CA.

The main limitation of this study is the relatively small Korean population of patients diagnosed with CA and the impossibility of sub-classification of prognosis (such as relapse-free duration, treatment response, size reduction, tumor regression rate, or symptomatic improvement) due to the retrospective design of this study. Furthermore, although the immunohistochemical results for target molecules are accurate measures at a single time point, clinical manifestation in patients can be highly dynamic. Therefore, there may be a disconnection between the interpretation of results and clinical progression, especially in terms of overall survival duration.

Taken together, the results on the correlation between prognosis (OS or PFS) and the immunohistochemical expression of endoglin, survivin, and PD-1 in CA of our patients were not found in our study. We have to be careful to interpret the positive correlation results of the past studies since racial differences should be considered. For example, a study has reported that the expression of PD-L1 is not associated with the prognosis of primary angiosarcoma.(25) Expression of PD-L1 has been a promising novel therapeutic predictor for cancer immunotherapy,(26) and PD-1 positive cells with tumor site expression of PD-L1 were reported to be an indicator of favorable prognosis in CA.(27) Whereas, in metastatic angiosarcoma, expression of PD-L1 was associated with shorter survival.(28) Similarly, although there was a correlation of endoglin with age and organ metastases, the association between endoglin and poor prognosis was not significant.(8) In another study, although most cases exhibited expression of survivin, it did not correlate with poor outcome.(9)

Although there are many studies indicating the association between endoglin, survivin, and PD-1 and poor prognosis in other carcinomas,(8, 9, 27) we suggest that these target molecules appear to be of little relevance in predicting the prognosis in Asian patients diagnosed with CA.

Despite negative results of correlation with prognosis and several limitations, our first attempt on the endoglin and survivin immunohistochemical study for clinicoprognostic correlation in CA is meaningful. Significant expression of endoglin and survivin was

confirmed and each molecule was significantly correlated to head, neck, face, and scalp (HNFS) lesions of CA. Our study also revealed that old age and large tumor size is a new prognostic indicator of poorer OS and PFS in patients with primary CA of the Korean race. Considering that HNFS type CA is associated with unfavorable clinical outcomes, our findings can be helpful in matching patients with CA with effective targeted therapy, and endoglin or survivin can be promising diagnostic and predictive biomarkers of HNFS type CA.

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국문요약

배경: 표적치료 및 면역치료가 일차성 피부 혈관육종을 포함한 여러 종류의 암종을 치료하기 위해 도입되고 있다. 하지만 피부혈관육종에서 표적 물질들이 연구된 것은 매우 드물다. 따라서 이번 연구에서는 Endoglin, Survivin과 PD-1 의 발현과 피부혈관육종의 예후 및 임상변수와의 관계를 분석하고자 하였다.

재료 및 방법: 14년간 서울아산병원에서 피부혈관육종으로 진단된 51명의 환자가 본 연구에 포함되었다. 전자의무기록을 통해 피부혈관육종 환자의 임상변수들과 예후를 분석하였고 Endoglin, Survivin과 PD-1으로 조직 샘플을 염색하여 두 명의 피부과 의사가 분석하였다.

결과: 총 55개의 샘플 중 Endoglin 양성은 35개 (63.6%), Survivin 양성은 30개 (54.5%) 였으며, PD-1 양성은 9개 (16.4%) 였다. 24개의 샘플에서 Endoglin 과 Survivin 이 동시에 양성이었고 (p = 0.013) 두경부, 얼굴과 두피 병변에서 유의미하게 양성 소견을 보였다 (각각 p = 0.005, p = 0.038). 하지만 Endoglin, Survivin 과 PD-1 모두 피부혈관육종의 전체생존율, 무진행생존율과의 연관성은 찾을 수 없었다.

결론: 비슷한 인구에서 두경부, 얼굴, 두피의 피부혈관육종은 안 좋은 예후와 연관이 있는 것을 감안하면, 이번 연구는 피부혈관육종으로 진단된 환자를 효과적인 표적치료 대상으로 지정하는 데 도움이 될 수 있을 것이다. 또한 Endoglin 과 Survivin 은 두경부, 얼굴, 두피의 피부혈관육종에서 유망한 진단적, 예측적 생물학적 표지자가 될 것으로 기대된다.

중요 단어: cutaneous angiosarcoma, endoglin, survivin, targeted therapy

FIGURES AND FIGURE LEGENDS

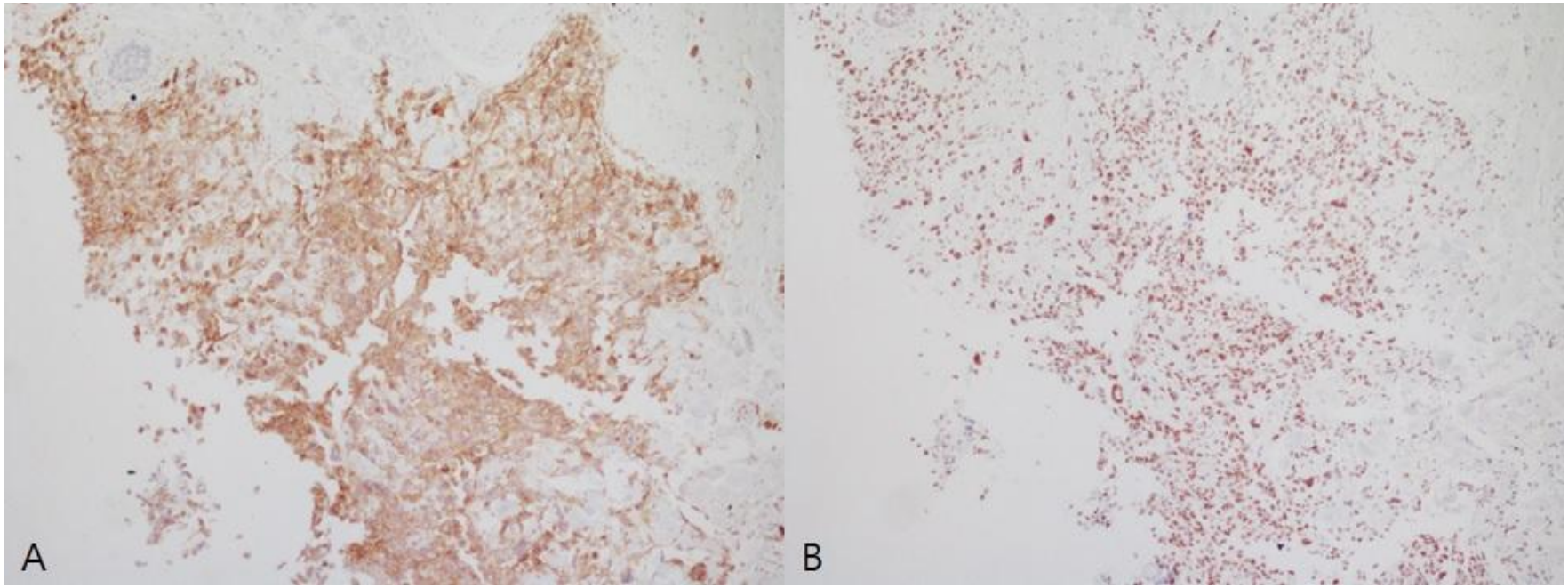

Figure 1. Coexpression of endoglin and survivin was detected in the scalp lesion in a 68 year-old patient. (A) endoglin (×100) (B) survivin (×100)

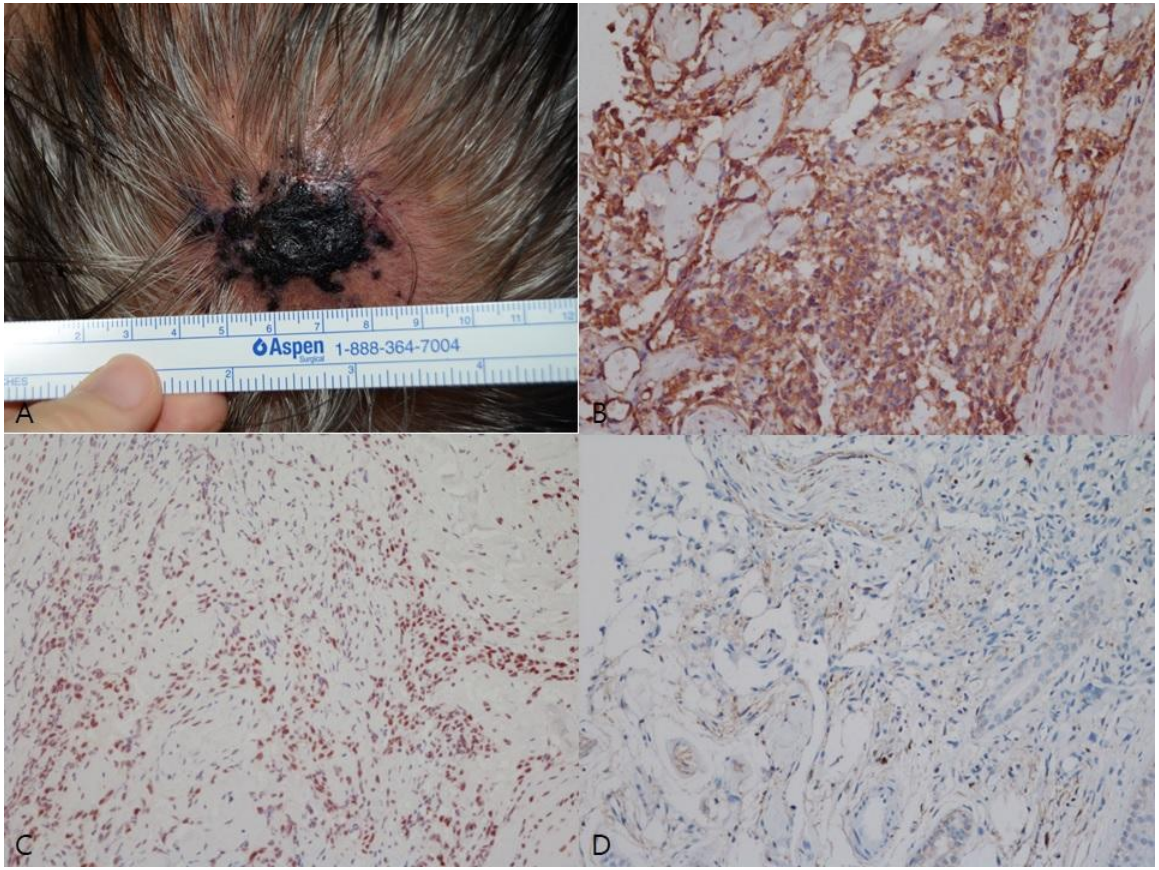

Figure 2 HNFS type CA is associated with unfavorable clinical outcomes. (A) About 5cm-sized mass in the scalp of a 74-year-old man. (B) Histopathology shows solid sheets of large pleomorphic cells with irregular vascular spaces. (H&E ×200) Coexpression of endoglin (×200) (C) and survivin (×100) (D) was also found. He expired 10-month after diagnosis of cancer despite surgery, chemotherapy and radiation therapy.

TABLE LEGENDS

Table 1. The expression of endoglin, survivin, and PD-1 in patients with cutaneous angiosarcoma

	Endoglin			Survivin			PD-1		
	Negative (n=20)	Positive (n=35)	P-value	Negative (n=25)	Positive (n=30)	P-value	Negative (n=46)	Positive (n=9)	P-value
Median age, years (IQR)	68 (64-73)	68 (64-76)	0.45	69 (65-75)	68 (62-75)	0.51	68 (62-75)	68 (66-74)	0.84
Sex			0.53			0.50			0.90
Female	5 (25.0)	5 (14.3)		6 (24.0)	4 (13.3)		9 (19.6)	1 (11.1)	
Male	15 (75.0)	30 (85.7)		19 (76.0)	26 (86.7)		37 (80.4)	8 (88.9)	
Anatomical site			**0.005**			**0.038**			0.28
Scalp, head and neck	**12 (60.0)**	**33 (94.3)**		**17 (68.0)**	**28 (93.3)**		36 (78.3)	9 (100)	
Others	**8 (40.0)**	**2 (5.7)**		**8 (32.0)**	**2 (6.7)**		10 (21.7)	0 (0)	
Size			0.16			0.65			1.00
< 5 cm	11 (55.0)	27 (77.1)		16 (64.0)	22 (73.3)		32 (69.6)	6 (66.7)	
≥ 5 cm	9 (45.0)	8 (22.9)		9 (36.0)	8 (26.7)		14 (30.4)	3 (33.3)	
Multiplicity			0.23			1.00			0.85
No	9 (45.0)	23 (65.7)		15 (60.0)	17 (56.7)		26 (56.5)	6 (66.7)	
Yes	11 (55.0)	12 (34.3)		10 (40.0)	13 (43.3)		20 (43.5)	3 (33.3)	
Lymphedema			0.96			0.48			0.83
No	18 (90.0)	33 (94.3)		22 (88.0)	29 (96.7)		42 (91.3)	9 (100)	
Yes	2 (10.0)	2 (5.7)		3 (12.0)	1 (3.3)		4 (8.7)	0 (0)	
PD-1			0.56			0.24			-
Negative	18 (90.0)	28 (80.0)		23 (92.0)	23 (76.7)		-	-	
Positive	2 (10.0)	7 (20.0)		2 (8.0)	7 (23.3)		-	-	
Endoglin			-			**0.013**			0.56
Negative	-	-		**14 (56.0)**	**6 (20.0)**		18 (39.1)	2 (22.2)	
Positive	-	-		**11 (44.0)**	**24 (80.0)**		28 (60.9)	7 (77.8)	
Survivin			**0.013**			-			0.24
Negative	**14 (70.0)**	**11 (31.4)**		-	-		23 (50.0)	2 (22.2)	
Positive	**6 (30.0)**	**24 (68.6)**		-	-		23 (50.0)	7 (77.8)	
Median survival, months	13.9	16.5	0.71[a]	11.1	19.8	0.06[a]	15.9	30.4	0.36[a]

IQR = interquartile range; PD = programmed death.

[a]Survival was compared using a univariable Cox regression test.

Table 2. Factors associated with overall survival in patients with cutaneous angiosarcoma

	Univariable analysis			Multivariable analysis		
	Unadjusted HR	**95% CI**	***P*-value**	**Adjusted HR**	**95% CI**	***P*-value**
Age	1.043	1.006–1.081	0.021	1.036	1.000–1.073	0.053
Sex						
Female	Reference category					
Male	1.394	0.642–3.026	0.401			
Anatomical site						
Scalp, head and neck	Reference category					
Others	0.997	0.475–2.090	0.993			
Size						
< 5 cm	Reference category			Reference category		
≥ 5 cm	2.892	1.443–5.796	0.003	2.699	1.323–5.508	0.006
Multiplicity						
No	Reference category					
Yes	0.967	0.532–1.756	0.911			
Lymphedema						
No	Reference category					
Yes	0.746	0.230–2.423	0.626			
Endoglin						
Negative	Reference category					
Positive	0.880	0.481–1.607	0.676			
Survivin						
Negative	Reference category			Reference category		
Positive	0.521	0.285–0.952	0.034	Eliminated		
PD-1						
Negative	Reference category					
Positive	0.696	0.307–1.576	0.384			

HR = hazard ratio; CI = confidence interval; PD-1 = programmed cell death-1.

Table 3. Factors associated with progression-free survival in patients with cutaneous angiosarcoma

	Univariable analysis			Multivariable analysis		
	Unadjusted HR	**95% CI**	***P*-value**	**Adjusted HR**	**95% CI**	***P*-value**
Age	1.038	1.003–1.075	0.035	1.035	1.000–1.072	0.050
Sex						
Female	Reference category					
Male	1.311	0.628–2.741	0.471			
Anatomical site						
Scalp, head and neck	Reference category					
Others	0.934	0.448–1.948	0.856			
Size						
< 5 cm	Reference category			Reference category		
≥ 5 cm	2.718	1.389–5.319	0.004	2.661	1.343–5.273	0.005
Multiplicity						
No	Reference category					
Yes	1.616	0.899–2.907	0.109			
Lymphedema						
No	Reference category					
Yes	0.562	0.173–1.823	0.337			
Endoglin						
Negative	Reference category					
Positive	0.859	0.478–1.542	0.610			
Survivin						
Negative	Reference category					
Positive	0.621	0.346–1.115	0.111			
PD-1						
Negative	Reference category					
Positive	0.806	0.356–1.825	0.606			

HR = hazard ratio; CI = confidence interval; PD-1 = programmed cell death-1.