P-ISSN 0215-7284
e-ISSN 25279106
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Association of α-SMA and EpCAM Expressions with Recurrence Risk Based on Histopathological Subtypes of Basal Cell Carcinoma

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Received : 25-10-2022
Accepted : 18-11-2022
Published : 31-01-2024

ABSTRACT

Background
Basal cell carcinoma (BCC) is the most common skin malignancy in the world with a proportion 70%. Recurrent and aggressive variants are still challenging in diagnosis and treatment. Histopathologically, there are two group of BCC risk of recurrence, i.e low risk and high risk. Assessment the expression of alpha-smooth muscle actin (α-SMA) as a biomarker of c*ancer associated fibroblast* cell in stroma and the expression of epithelial cell adhesion molecule (EpCAM) in tumor cells probably have role in the pathomechanism of BCC progression, suggest it can distinguish the risk of BCC recurrence. This research aims to determine the assosiation between the expression of α-SMA and EpCAM with the recurrence risk group of BCC histopathological subtypes.

Methods
This was a cross sectional study using 48 samples, with 24 low and high risk groups each. Histopathological subtypes were determined from Hematoxylin and eosin slides. The expression of α-SMA and EpCAM was examined by the immunohistochemical method which was assessed semi-quantitatively. Statistical analysis was performed using the Chi-square test with $p<0.05$ was considered significant.

Results
Expression of α-SMA with the score 3 was found more frequent in high risk BCC (85.7%), while a score of 1 was more frequent in low risk BCC (100%). Loss of EpCAM expression was mainly found in high risk BCC (82.8%). Statistical analysis showed that there was a significant association between the expression of α-SMA and EpCAM and the recurrence risk group of BCC histopathological subtypes with p value = 0.000.

Conclusions
This study concluded that increased α-SMA expression and loss of EpCAM expression were associated with a high recurrence risk group of BCC histopathological subtypes.

Keywords: α-SMA, BCC, CAF, EpCAM, Stroma

P-ISSN 0215-7284
e-ISSN 25279106
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INTRODUCTION

Basal cell carcinoma (BCC) is the most common non-melanoma skin malignancy in the world with a proportion of 70%.[1] The incidence of BCC in the world tends to increase, including in several Asian countries.[2,3] The incidence of BCC is higher in the white race population with the highest incidence in Australia and the lowest in Africa. In the United States, it is estimated that there are around 2.8 million new cases of BCC each year, while in Europe there are as many as 700,000.[3] according to the data of the Singapore cancer registry, there were 8367 cases of BCC during the period 1968-2016.[4] KSB research data in Indonesia is still small. In 2014-2017 there were 176 cases of BCC at Dr. Cipto Mangunkusumo Jakarta.[5] Considerable progress has been made for BCC in terms of diagnostics and treatment options, but recurrent BCC variants still pose a significant challenge.

BCC recurrence rates were found to vary in some literature, range from 10-67%.[6] Histopathological subtype is one of the key risk factors for BCC recurrence and influences the prognosis.[3,7] There are ten histopathological subtypes of BCC which are grouped based on recurrence risk into low-risk and high-risk groups. Nodular, superficial, pigmented, fibro-epithelial, and infundibula cystic BCC are classified as low risk, while micronodular, infiltrative, morpheic, and basosquamous BCC are classified as high risk.[3] The causal mechanism for the differences in aggressiveness or the tendency for recurrence of the various BCC subtypes is not yet understood.

Stromal changes followed the change in BCC from a low-risk subtype to a high-risk group.[1] Fibroblasts, often known as cancer-associated fibroblasts (CAF), are a stromal component, has been shown to play a role in triggering tumor growth, progression, and invasion. This role is carried out through the remodeling of the extracellular matrix and the production of various cytokines and growth factors that promote tumor cell progression.[8] Alpha-smooth muscle actin (α-SMA) is the most common marker for all origins of CAFs.[9,10] CAF expression with α-SMA markers correlates with the risk of recurrence of colorectal carcinoma and histopathological grade in cholangiocarcinoma.[11,12] Different expression of CAF was found between BCC and other skin malignancies.[13] The study found that CAFs with the α-SMA biomarker were highly expressed in aggressive variant BCCs.[14,15] However, other studies did not find α-SMA expression in BCC.[13] Identification of CAF with α-SMA markers may be useful as a new prognostic marker of BCC.

Another factor that may play a role in BCC invasion is interference with the adhesion between cells and the extracellular matrix.[16] One of the adhesion components is a transmembrane protein epithelial cell adhesion molecule (EpCAM) which plays a role in cancer cell adhesion, proliferation, migration, and differentiation. EpCAM expression and prognostic value were found to vary between different cancer cell types and organs.[16,17] EpCAM expression is associated with histopathological subtypes in several types of carcinomas including ovarian, thyroid, and breast carcinoma.[17] EpCAM protein has the monoclonal antibody BerEp4 which has been known as a sensitive marker for BCC. EpCAM expression was assessed in central and peripheral invasive BCC tumor cells.[3,18] Study by Gaiseret revealed a loss of EpCAM expression at the front of the aggressive BCC invasive area, whereas other studies found EpCAM expression that did not differ significantly between BCC subtypes.[15,19]

Based on the description above, the authors are interested in conducting research to study the association of α-SMA and EpCAM expression with recurrence risk groups based on histopathological subtypes of basal cell carcinoma. It is expected to provide benefits in determining the pathomechanism of differences in the risk of BCC recurrence as well as being useful as an alternative prognostic marker in both groups of histopathological subtypes of basal cell carcinoma.

METHOD

This research is cross-sectional study of all BCC that was diagnosed in the Anatomical Pathology Laboratory of RSUP Dr. M. Djamil Padang and Diagnostic Center for Anatomical Pathology, Faculty of Medicine, Andalas University for the 2019-2021 period. The sample was all cases with complete medical record data, slides, and paraffin blocks in good condition. Based on the statistical formula, [24] samples were obtained for each recurrence risk, bringing the total to 48 samples.

Re-evaluation of the Hematoxylin and eosin slides was carried out with results in the form of BCC histopathological subtypes. Assessment of the risk of recurrence of BCC based on histopathological subtype according to WHO and NCCN 2018 criteria, divided into 2

Maj Patol Indones 2024; 33(1): 749-756
DOI: 10.55816/mpi.v33i1.613

P-ISSN 0215-7284
e-ISSN 25279106
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groups, low and high risk.[12] The paraffin blocks were sectioned into 2 slides, and immunohistochemical staining with anti-α-SMA primary antibody (Biosys; Mob001;1:300) and BerEp4 primary antibody (Cell Marque; 248M-94;1:300) was performed. Immunohistochemical staining used Streptavidin Biotin Complex (SBC) method.

The immunohistochemical stains were observed using an Olympus CX23 binocular light microscope at 100-400 times magnification for all representative field areas. The assessment was carried out on each IHC slide for the expression of α-SMA in the cytoplasm, as well as EPCAM expression in the cell membrane. The α-SMA expression is positive if brown stains are found in the cytoplasm of fusiform cells in the peritumoral stroma, whereas EpCAM expression is positive if brown stains are seen on the tumor cell membrane. There are four tire scoring levels for α-SMA based on the intensity and area of staining. The immunostaining measurement was classified semiquantitatively into four categories as follows: score=0 (if the intensity is 0), score=1 (if the intensity is weak, 0%<staining area ≤50% or moderate intensity, 0%<staining area≤20%), score=2 (weak intensity, 50%< staining area ≤100% or moderate intensity, 20%<staining area≤50% or strong intensity, 0%< staining area ≤20%), score=3 (moderate intensity, 50%<staining area≤100% or strong intensity, 20%<staining area ≤100%).[13] Loss of EpCAM expression was defined as a marked decrease (staining intensity less than 50% compared with intensity in the rest of the tumor) in the intensity of EpCAM staining that occurred in tumor cells in border areas or tumor nests infiltrating the dermis.[19] The CPI assessment was carried out by two pathologist.

Univariate analysis in the form of descriptive data distribution based on age, sex, tumor location, and recurrence risk group of histopathological subtypes of BCC. The Chi-squared test was used to determine the association between α-SMA and EpCAM expression with the recurrence risk group of BCC histopathological subtypes. The statistical test is considered significant when the value is $p<0.05$.

RESULTS

The clinicopathological characteristics of the subjects are presented in Table 1. Most cases were found in the age group of 40-70 years (70.8%) with an average 62.35 years. The youngest patient is 26 years old and the oldest is 91 years old. Based on gender, it was found that there were slightly more women than men with a result of 52.1%. Most tumor (77.1%) occurred in area H (especially the nose and cheeks). Mixed-type basal cell carcinoma was the most common histopathological subtype of high-risk BCC, with 15 cases (31.3%), this type was dominated by mixed nodular infiltrative subtype (86.6%). The most common histopathological subtype of low-risk BCC was nodular BCC, with 22 cases (45.8%). Perineural invasion was found in 5/48 cases (Figure 1).

Table 1. Clinicopathological characteristics of basal cell carcinoma.

Characteristics	f(n=48)	%
Age group		
<40 years old	3	6.3
40-70 years old	34	70.8
>70 years old	11	22.9
Mean	62,35±12.0	
Gender		
Man	23	47.9
Woman	25	52.1
Location[a]		
Area L	2	34.2
Area M	9	18.8
Area H	37	77.1
Recurrence risk group		
Low risk		
Nodular BCC	22	45.8
Pigmented BCC	2	4.2
High risk		
Micronodular BCC	1	2.1
Infiltrative BCC	4	8.3
Basosquamous carcinoma	4	8.3
Mixed BCC (nodular infiltrative and nodular micronodular)	15	31.3

[a]Location is according to NCCN 2018; Area L: trunk and extremities, area M: chin, forehead, scalp, and pretibial area H: area of the "mask" on the face (middle face, nose, periorbita, cheeks, lips, mandible) as well as pre and post auricles, ears, genitals, hands and feet.

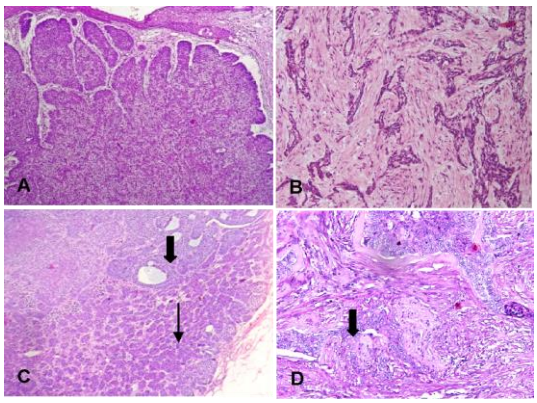

Figure 1. Histopathological picture of BCC A. Histopathology of nodular BCC, B. Infiltrative BCC, C. Mixed nodular (thick arrow) and micronodular (thin arrow) BCC, D. Perineural invasion of BCC (arrow). (H&E staining; A and B. 100 times original magnification, C. 40 times original magnification and D. 100 times original magnification).

Maj Patol Indones 2024; 33(1): 749-756
DOI: 10.55816/mpi.v33i1.613

P-ISSN 0215-7284
e-ISSN 25279106
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The α-SMA expression in the peritumoral stroma of both BCC risks in Figure 2 shows expression with a score 0-3. High risk BCC has high α-SMA expression in the peritumoral stroma with scores of 2 and 3 and none has a score of 0 or 1. Statistically, there was a significant association between α-SMA expression and the recurrence risk group based on the histopathological type of basal cell carcinoma (p=0.000) (Table 2).

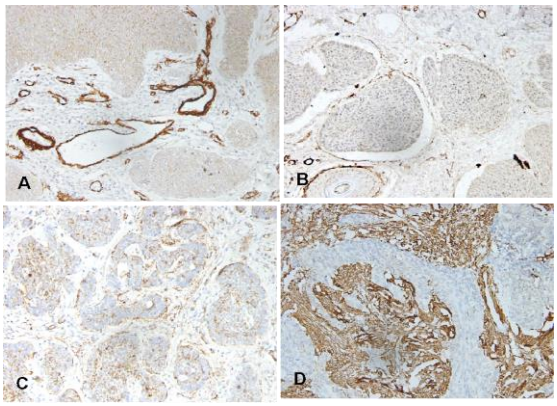

Figure 2. Description of α-SMA expression in BCC. A. scores 0 in low-risk BCC (nodular subtype), positive stain in internal muscle and vascular wall pericytes (positive control) B. Score 1 in low-risk BCC (nodular subtype), C. Score 2 in high-risk BCC, D. Score 3 in high-risk BCC (infiltrative subtype.

In this study, loss of EpCAM expression was found in the invasive front areas of BCC as well as in tumor nests (Figure 3). Loss of EpCAM expression was higher in high risk recurrence BCC (82.8%) than low risk recurrence BCC (17.2%). Statistically, there was a significant association between EpCAM expression and the recurrence risk group based on BCC histopathological subtypes with a p=0.000 (Table 3).

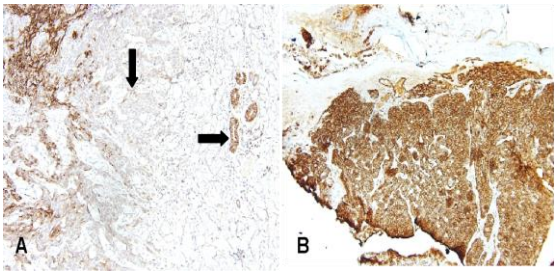

Figure 3. A. Display of loss of EpCAM expression at the edge of the invasive area (arrow) of high-risk BCC with an intensity <50% compared to the positive intensity in other tumor cells, expression in the internal positive control (eccrine gland) was stained strongly positive on the membrane (horizontal arrow). (B) Features of non-removal EpCAM expression in low-risk BCC. All tumor cells express EpCAM with brown color on the membrane (A. Original magnification 100 times, B. 40 times).

Table 2. Association of α-SMA expression with recurrence risk groups based on histopathological subtypes of BCC.

Characteristics	Recurrence risk		Total f (%)	*p-value*
	Low risk f (%)	High risk f (%)		
Expression of α-SMA				
Score 0	5 (100%)	0 (0%)	5 (100%)	0.000
Score 1	14 (100%)	0 (0%)	14 (100%)	
Score 2	2 (25%)	5 (75%)	8 (100%)	
Score 3	3 (14.3%)	18 (85.7%)	21 (100%)	

Table 3. Association between EpCAM expression and recurrence risk groups based on BCC histopathological subtypes.

Characteristics	Recurrence risk		Total f (%)	*p-value*
	Low risk f (%)	High risk f (%)		
EpCAM expression				
Loss of expression	5(17.2%)	24(82.8%)	29(100%)	0.000
Without losing expression	19(100%)	0(0.0%)	19(100%)	

DISCUSSION

In this study, it was found that the age range of the cases was 26-91 years, most of the cases were in the age group 60-70 years, with an average age of 62 years. Research by Mawardi et al, showed that most cases of BCC were in the fifth decade of life.[20] Various epidemiological studies on BCC have noted that most cases of BCC occur in the fifth to seventh decade of life, although it can also occur at a younger age. Age is a risk factor for BCC since the pathogenesis of BCC is associated with intermittent and prolonged exposure to UV light. Decreased biological function in old age promotes the accumulation of DNA damage and chronic inflammation, which leads to modification of the integrity of the dermal matrix.[21]

The incidence of BCC in many previous studies was reported to be higher in

Maj Patol Indones 2024; 33(1): 749-756
DOI: 10.55816/mpi.v33i1.613

P-ISSN 0215-7284
e-ISSN 25279106
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males than females. This is believed to be related to outdoor activity factors, where men tend to be exposed to repeated UV rays compared to men and women, especially in childhood.[1,22] In this study, it was found that the incidence of BCC was slightly higher in women compared to men, 52.1% and 47.9% respectively. Mawardi et al, reported a higher incidence of BCC in women.[20] Research on BCC in South Korea, Japan, and Singapore also noted a higher incidence in women.[4,6,23] The causes of this trend change have not been thoroughly investigated. The tendency of young women to pay attention to the facial area and aesthetics can lead to increased visits to dermatologists, which increases BCC findings.[1,24]

Based on anatomical location, the most common tumor in this study was in the H area, which was mainly in the nose (20.5%), followed by the cheek area (19%). This is in line with research conducted by Cercie et al, who also found that 56% of BCC to be located in the nose.[25] Bourlido et al, in a 15-year retrospective study, found that 34% of BCCs were located in the nose.[26] The face area is the main area that is exposed to UV rays, so it is an area with a high-risk category for BCC and tends to recur.[7] The presence of BCC in the area of the nose, eyes, lips, and ears is difficult to treat because it is related to technical problems in maintaining function and cosmetically.[27] Research by Mawardi et al, found BCC in the middle face area (including the nose) tends to be more aggressive than other areas of BCC.[28]

This study found that nodular BCC was the most common histopathological subtype in low-risk BCC (91.6%) and mixed BCC was the most common histopathological subtype in high-risk BCC (62.5%). This result is consistent with Iwulska et al, who obtained 45 high-risk BCC and 34 low-risk BCC, the majority of which were mixed and nodular subtypes, respectively.[15] Kiely et al, in a study of 694 BCC in England, found that the most histopathological type was the nodular type (49%), followed by a mixed type (39%).[22] Research by Ghanadanet et al, obtained the nodular type as the most common (43%), followed by the mixed type (32.4%).[29] Nodular BCC is the most common histopathological type, with a percentage of 70% of all types.[1,24]

All mixed histopathological subtypes had a nodular component, of which 86.7% were mixed nodular infiltrative subtype, and the remaining 13.3% were mixed nodular-micronodular subtype. This is in line with research by Cerciet et al, who found that 80% of mixed type BCC had nodular components, and the nodular infiltrative subtype is the most common subtype.[25] Kiely et al., found that 95% of mixed BCC had nodular components.[22] Ghana et al found consistent results, which nodular infiltrative subtype is the most common, followed by mixed nodular-micronodular.[29] A mixture of nodular with infiltrative and micronodular BCC can indicate the existence of a continuum of histological processes from low-risk to high-risk subtypes.[1]

This study assessed the expression of α-SMA in the peritumoral stroma. The results of this study indicate that there is a significant association between α-SMA expression in the peritumoral stroma and the recurrence risk group based on the histopathological subtype of BCC. Various studies have provided consistent but varied results in terms of α-SMA expression assessment techniques in the peritumoral stroma of BCC. Said et al, obtained a significant association ($p<0.001$) between the expression of α-SMA in the peritumoral stroma and aggressive BCC (76%).[30] Iwulskaet et al, obtaining positive expression of α-SMA is associated with a high recurrence rate in primary BCC.[15] Expression of α-SMA assessed in the stroma can be used as a marker in determining the aggressiveness of BCC. In this study, nodular BCC generally had a weak expression of α-SMA, whereas mixed nodular and infiltrative BCC had a strong expression. Moisejenkoet et al, found weak and negative expression of α-SMA in the nodular type BCC stroma and strong expression in the mixed type.[6] Yearset et al, found high expression of α-SMA in myofibroblasts stroma in the mixed nodular infiltrative subtype.[14].

Alpha smooth muscle actin is a marker of myofibroblast phenotype, which in the cancer environment is known as CAF.[10] Differences in α-SMA expression scores in this study indicate differences in the CAF population level in the two groups at risk of recurrence of BCC. Myofibroblast phenotype is more proliferative, migratory, and metabolically active.[8] Cancer-associated fibroblast secretes paracrine and juxtracrine signals that increase BCC growth which causes extracellular matrix remodeling. Through the production of the protein collagen, fibronectin, and matrix metalloproteinase (MMP) proteolytic enzymes, CAFs can form a fibrotic stroma that becomes an invasive pathway and provides a stroma pathway for cancer cell migration.[8,12] The presence of CAF with certain markers has been a predictor of

Maj Patol Indones 2024; 33(1): 749-756
DOI: 10.55816/mpi.v33i1.613

P-ISSN 0215-7284
e-ISSN 25279106
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recurrence and prognosis in colorectal, endometrial, urothelial, breast, and naso-pharyngeal carcinomas.[10] Alpha-smooth muscle actin as one of the reliable CAF markers, was found to be associated with a high recurrence risk in this study so that α-SMA expression in BCC stromal cells can be used as a marker in assessing the risk of BCC recurrence.

This study obtained three samples of nodular histopathological subtypes that expressed α-SMA with a score of 3, this could be due to the possibility of an infiltrative or micronodular component as a mixed type with nodular in this preparation which has not been seen due to the surgical method used. Differences in sampling techniques can cause failure to assess the aggressive type of BCC by 17% in the preoperative biopsy method compared Mohs micrographic surgery (MMS). In that study, the diagnosis of nodular to mixed nodular micronodular biopsy was diagnosed in MMS.[25] In this study, the sampling technique was not known.

Association between EpCAM expression and recurrence risk group based on the histopathological subtype of basal cell carcinoma. This study assessed EpCAM expression in BCC using BerEp4 monoclonal antibody. In this study, EpCAM was expressed in all research samples. In line with the study by Ansai et al and Sunjaya et al obtained EpCAM expression found in all KSB.[17,22] EpCAM protein was found to be overexpressed in epithelial progenitor cells and cancer cells, especially adenocarcinoma originating from the colon, prostate, breast and pancreas and low expression was found in squamous cell carcinoma.[17] Basal cell carcinoma is a malignancy that originates from mutations in keratinocyte progenitor cells.[31]

The results of this study indicate that there is a association between EpCAM expression and the recurrence risk group based on the histopathological subtype of BCC. Studies assessing the relationship between EpCAM expression and recurrence risk groups based on BCC histopathological subtypes have not been found to date. A study by Gaiser et al, found a greater loss of EpCAM expression in infiltrative BCC than nodular BCC with a ratio of 60.4% and 12.5% ($p<0.0001$), this loss was seen at the front of the invasive area. Iwulskaet et al, found that in the aggressive primary BCC group, more expression of EpCAM with the missing BerEp4 marker was found (27.8%) than without loss of expression (12.5%).[15] This study also found loss of EpCAM expression which is generally found in the front of the tumor invasive area, so it can be concluded that there is downregulation of EpCAM in the high-risk type BCC invasive frontal area.[19]

Loss of EpCAM expression in other malignancies is generally processing related to epithelial membrane transition (EMT).[17] As a marker of epithelial cells, loss of EpCAM expression marks the initial change in the loss of epithelial phenotype in tumor cells. Loss of EpCAM expression was found in anaplastic thyroid carcinoma associated with EMT. and in lobular breast carcinoma.[17] In this study, some loss of EpCAM expression was found in the leading invasive areas of high-risk BCC. These findings explain that there is a possibility that there is an EMT process in BCC that causes invasive tendencies. Other studies that assess the relationship between EMT and EpCAM in BCC should be carried out. The EpCAM protein participates in maintaining cell integrity through modification of the composition of tight junction functionally by regulating the amount and location of claudins due to direct binding of the EpCAM transmembrane domain to claudin-7.[16,17] Loss of EpCAM expression also disrupts cell integrity thereby initiating migration and invasion which increases the aggressiveness of tumor cells.[17] Loss of EpCAM expression plays a role in high-risk BCC.

Loss of EpCAM expression was also found in non-invasive tumor nests in this study. The same thing was obtained by Ansai et al, that EpCAM expression is weak in the morpheic type, but this loss of expression is generally found in areas with small nest patterns that are not front invasive areas. EpCAM expression is positively correlated with proliferation and negatively correlated with differentiation.[17] EpCAM is negatively expressed in the squamous epithelium but positively expressed in hair follicle follicular germ cells. EpCAM is expressed in all BCCs and negative in squamous cell carcinoma.[17] Basal cell carcinoma has a histopathology that differentiates into squamous cells and mature keratinized hair structures (keratotic variant). This differentiation of BCC can be found in nodular BCC.[31] Loss of EpCAM expression can also be found in the squamous component and the transition zone of the basosquamous carcinoma subtype, which is a high-risk BCC subtype.2 . Loss of EpCAM expression in high-risk BCCs can also be caused by differentiation.

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P-ISSN 0215-7284
e-ISSN 25279106
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The data from the results of this study indicate that increased α-SMA expression and loss of EpCAM expression accompany the formation of high-risk BCCs. The detection of high α-SMA expression in the stroma and loss of EpCAM expression especially at the leading front of the invasive area can be predictors for a high risk of recurrence of BCC.

CONCLUSION

There is an association between increased expression of α-SMA and loss of EpCAM expression with an increased risk of recurrence based on histopathological subtypes in BCC.

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