Study on the Small Breast Epithelial Mucin Expression Difference and its Clinical Correlation in Breast Cancer Patients in Han, Miao and Buyi Ethnic

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Abstract

Objective: To investigate small breast epithelial Mucin (SBEM) expression difference in mammary tissues and relationship with clinical - pathological features in breast cancer patients in Han, Buyi and Miao ethnic. Methods: In our study, SBEM protein expressions were detected by means of immunohistochemistry in 297 patients diagnosed from 2014 to 2015, including 99 normal breast tissue specimens, 99 cases of breast benign tumor tissue specimens and 99 tissues specimens from breast cancer patients. Each set of tissue specimens contains 33 samples from Han, Miao and Buyi ethnics, respectively. We analyze the expression of SBEM in different mammary tissues in different ethnics and the association of different SBEM expression levels in tissue of breast cancer patients with clinical - pathological features in Han, Miao and Buyi ethnics, respectively. Results: SBEM positive detection rate was no significant difference in the han, miao and buyi ethnic groups in breast cancer tissue, breast benign tumor tissue and normal breast tissue. SBEM expression in breast cancer tumor cells were related to the ki67 in the Han, Miao and Buyi ethnic (P=0.034, 0.027, 0.047), respectively. There was a marked association between the SBEM expression level and lymphatic metastasis (P =0.042, 0.039) in the Han and Miao ethnic, while the same results were not found in the Buyi people (P=0.072). Conclusion: The SBEM positive detection rate was no significant difference in the han, miao and buyi ethnic groups. SBEM was associated with lymphatic metastasis in the Han and Miao ethnic, while no correlation in the Buyi ethnic.

Keywords: *Small breast epithelial mucin; Breast cancer; Han, miao and buyi ethnic; Clinical - pathological features; Ethnic difference*

1. Introduction

Breast cancer is the most common malignancy and is second only to lung cancer in mortality among women worldwide [1,2]. While the incidence rate of breast cancer continues to rise over the last few decades , its mortality rate, on the other other hand, declines every year thanks for the development of diagnosis and targeting medication [3,4]. Micro metastasis is

considered to be a key factor for poor prognosis of breast cancer patients [5]. Despite the improvement in detection of breast cancer, about 30% of patients are still detected with micro metastasis at their first visit. The micro metastasis of breast cancer before primary treatment is considered to be one of the recurrence reasons that will directly impact the survival of patients. The development of distant metastasis is the major cause of their death [6]. So, it is crucial to find specific markers to detected micro metastasis and provide useful information to guide early therapeutic methods of breast cancer patients.

Small breast epithelial mucin (SBEM) was a tissue specific protein and only expresses in mammary and salivary glands [7]. High SBEM expression was found to be strongly associated with the histopathological detection of lymph node metastasis [7]. SBEM could serve as a useful marker for breast micro metastasis, also as a specific targets for the treatment of breast cancer [7]. O 'brien [8] confirmed that rates of detection of SBEM in protein levels were 51% (52/103) and 4% (1/26) in breast cancer and non-breast cancer tissue, respectively, which further confirmed the SBEM expression was significantly higher in breast cancer tissue than non-breast tissue. Skliris GP [9] study showed that SBEM expression rate was 18% in 300 patients with breast cancer using the method of immunohistochemical analysis and the SBEM expression rate was significantly higher in ER negative (22%) than in ER positive (13%) breast cancer tissue. Several laboratories showed that SBEM expression correlated with higher tumor grade, TNM staging and lymph node metastasis at both mRNA and protein levels.

SBEM has significant guidance for clinical activities. Liu ZZ, [10] reported that SBEM has the potential to be a specific marker for predicting hematogenous micro metastasis and response to neo-adjuvant chemotherapy in breast cancer. Valladares-Ayerbes M et al. [11] studied the expression profiles of SBEM gene in silico and in vitro, and demonstrated that SBEM-mRNA could serve as a marker for bone marrow micro metastasis in breast cancer patients and SBEM-mRNA could serve as a marker for bone marrow micro metastasis in breast cancer patients [10]. Research of Liang Liu et al [12] suggested SBEM 3+ score was cut-off value of prognosis and significantly correlated with decreased DFS and OS in TNBC patients. SBEM is an independent risk predictor and may offer utility as a prognostic marker in TNBC patients [12].

Based on the resules above, SBEM might play an important role in progression and metastasis of breast cancer indifferent races. But humans have genetic diversity and every race and nationality's genome has its own characteristics. Only to figure out what each nation's genetic structure characteristics and the change rule, we can analyze their origins and the genetic relationship with other ethnic groups. This kind of population genetics research is a difficult project. The genetics investigation need to rely on all sorts of genetic markers, especially those with racial specificity and individual specific genetic markers, which can provide enough valuable genetic information.

The Southern GuiZhou is a multi-ethnic area of China, of which Han, Miao and Buyi ethnics are its main components. The breast cancer incidence here is increasing year by year as well as other regions in China. Because the different ethnic groups have different genetic background and ways of life, we are not sure whether there is difference between different ethnic groups. And to the best of our knowledge, this is the first study to investigate the differences of the expression of SBEM in mammary tissue of Han, Miao and Buyi ethnic and the association of SBEM expression in tissue of breast cancer patients with clinical- pathological features in different ethnics.

2. Materials and Methods

The study examined cases from 297 patients diagnosed from 2014 to 2015 in Third Affiliated Hospital of Guizhou Medical University, including 99 normal breast tissue specimens, 99 cases of breast benign tumor tissue specimens and 99 available formalin-fixed paraffin-embedded tissues specimens from breast cancer patients by means of immunohistochemistry (IHC). Each set of tissue specimens contains 33 samples from Han, Miao and Buyi ethnics, respectively. Clinical data of all the cases were reviewed retrospectively from medical records in our hospital. All the breast cancer patients underwent operational treatment according to clinical practice guidelines of National Comprehensive Cancer Network (NCCN) of the United States. None of the patients received neoadjuvant therapy.

Statistic and analysis of clinicopathological parameters, including age at diagnosis, disease stage, tumor size, tumor grade, lymph node status, P53 and Ki67, were listed in TABLE 2-4. Based on the past research results, SBEM expression with SBEM 3+ score is the SBEM cut-off value of prognosis. We divided the cases into two groups, one is the SBEM <3+ group defined as a high expression group, the other is SBEM =3+ group defined as a high expression group.

2.1 SBEM expression and evaluation of IHC

All tissues were collected surgically under the supervision of an experienced pathologist. SBEM expression was measured by IHC. Streptavidinperosidase (S-P) IHC staining was performed using SBEM antibody of mouse monoclonal (diluted 1/800, Abcam plc. Cambridge, UK). The detailed procedures were done as described by Jennbacken [13]. PBS was used to replace the primary antibody in negative controls. SBEM was a secreted protein and it was mainly located in cell membrane, secondly in cytoplasm. Normal breast tissues were in general weakly or negative. So it was evaluated and scored if cell membrane and/or cytoplasm reactivity were observed.

According to our data and TMA IHC grading method by Serrero G [14] Pan [15] and Liu Liang [12], our scoring was semi quantitatively categorized as: ≤ 5% of tumor cells staining with/without weakly stained was negative (0), followed by a score of 1 (>5% of tumor cells and with weak/focal positive staining or ≤ 5% of tumor cells with strongly stained), 2 (>5% of tumor cells and with moderate/focal positive staining), 3 (>5% of tumor cells and with strong/diffuse positive staining).

2.2 Statistical analysis

The correlation between SBEM, Clinicopathological characteristics and survival outcomes was compared by Pearson's X^2 test. Statistical significance was defined as *P* value <0.05. SPSS17.0 software package was used for all statistical analyses.

3. Results

FIG. 1 showed photomicrograph examples of SBEM expression with different scores in TNBC tissues determined by IHC. SBEM is mainly expressed in the cell membrane, the second expression in the cytoplasm, and few expressions in the nucleus;
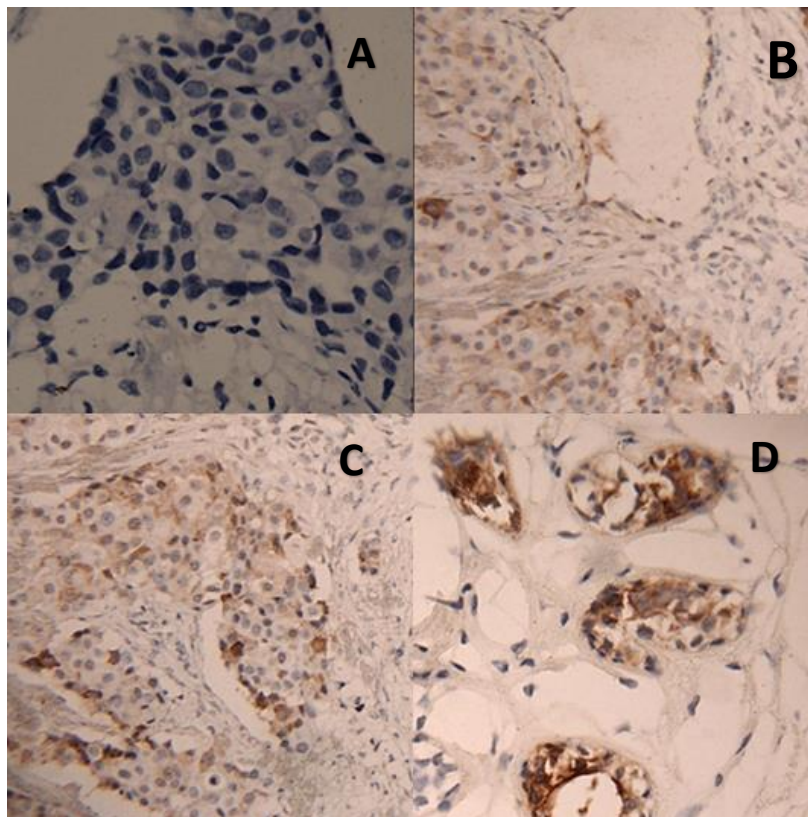
FIG. 1. **Immunohistochemical staining for SBEM. A: 0+: ≤ 5% of tumor cells staining with/without weakly stained was negative (original magnification × 200); B: 1+: >5% of tumor cells and with weak/focal positive staining or ≤ 5% of tumor cells with strongly stained (original magnification × 100); C: 2+: >5% of tumor cells and with moderate/focal positive staining (original magnification × 200); D: 3+: >5% of tumor cells and with strong/diffuse positive staining (original magnification × 200).**

TABLE 1 showed the SBEM positive detection rate in the breast cancer tissue, breast benign tumor tissue and normal breast tissue in the han, miao and buyi ethnic (TABLE 1). SBEM positive detection rate of breast cancer tissue is significantly higher than the breast benign tumor tissue and normal breast tissue (P =0.005). But there was no significant difference in the han, miao and buyi ethnic in breast cancer tissue, breast benign tumor tissue and normal breast tissue (P >0.05) (TABLE 1).

TABLE 1. **SBEM positive detection rate in the breast cancer tissue, breast benign tumor tissue and normal breast tissue in the han, miao and buyi ethnic.**

Tissue type	SBEM positive detection rate (%)			
	Han ethnic	**Miao ethnic**	**Buyi ethnic**	***P*-Value**
Breast cancer tissue	49	47	45	0.982
Breast benign tumor tissue	4	3	3	0.923
Normal breast tissue	3	2	2	0.889
***P*- Value**	0.005	0.005	0.005	

The clinicopathological characteristics of patients were described in TABLE 2-4. SBEM expression in TNBC tumor cells were related to ki67 in the Han, Miao and Buyi ethnic (*P* =0.034, 0.027, 0.047), respectively. There was a marked association between the low expression of SBEM group and high expression group in lymphatic metastasis (*P* =0.042, 0.039) in the Han and Miao ethnic, while the same results were not found in the Buyi people (*P* = 0.072) (TABLE 2-4).

TABLE 2. **Clinicopathological characteristics of patients in Han ethnic.**

Parameter	Number (n)	Subgroup cut-offs	SBEM<3		SBEM=3		P-valuer
			Number (n)	%	Number (n)	%	
Age	33		26		7		0.614
		X>35	19	73.1	4	57.1	
		X ≤ 35	7	26.9	3	42.8	
TNM staging	33		26		7		0.075
		I(1)	6	23.1	1	14.3	
		II(2)	14	53.8	3	42.8	
		III(3)	6	23.1	3	42.8	
P53	31		24		7		0.801
		Mutated	11	45.8	4	57.1	
		No-mutated	13	44.2	3	42.8	
Lymph Node	33		26		7		0.042
		+	10	38.5	5	71.4	
		_	16	61.5	2	28.6	
Grade	30		23		7		0.212
		Low (1)	9	39.1	2	28.6	
		Mod(2)	8	34.8	3	42.8	
		High(3)	6	26.1	2	28.6	
Size	33		26		7		0.872
		X>20mm	14	53.8	4	57.1	
		X ≤ 20mm	12	46.2	3	42.8	
Ki67	32		26		6		0.034
		X>35	11	42.3	5	83.3	
		X ≤ 35	15	57.7	1	16.7	

TABLE 3. **Clinicopathological characteristics of patients in Miao ethnic.**

Parameter	Number (n)	Subgroup cut-offs	SBEM<3		SBEM=3		P-valuer
			Number (n)	%	Number (n)	%	
Age	33		26		7		0.523
		X>35	22	84.6	5	71.4	
		X ≤ 35	4	15.4	2	28.6	
TNM staging	33		26		7		0.092
		I(1)	7	26.9	1	14.3	
		II(2)	13	53.8	4	63.6	
		III(3)	6	19.3	2	28.6	
P53	27		21		6		0.901
		Mutated	10	47.6	3	50.0	
		No-mutated	11	52.4	3	50.0	
Lymph Node	33		26		7		0.039
		+	11	42.3	6	85.7	
		_	15	57.7	1	14.3	
Grade	30		24		6		0.361
		Low (1)	9	37.5	2	33.3	
		Mod(2)	8	33.3	2	33.3	
		High(3)	7	29.2	2	33.3	
Size	33		26		7		0.849
		X>20 mm	17	65.4	4	63.6	
		X ≤ 20 mm	9	34.6	3	36.4	
Ki67	31		24		7		0.027
		X>35	7	19.2	5	71.4	
		X≤35	17	70.8	2	28.6	

TABLE 4. **Clinicopathological characteristics of patients in Buyi ethnic.**

Parameter	Number (n)	Subgroup cut-offs	SBEM<3		SBEM=3		P-valuer
			Number (n)	%	Number (n)	%	
Age	33		25		8		0.806
		X>35	21	72.4	6	63.6	
		X ≤ 35	4	27.6	2	36.4	
TNM staging	33		25		8		0.101
		I(1)	6	24.0	1	12.5	
		II(2)	12	48.0	3	37.5	
		III(3)	8	32.0	4	50.0	
P53	29		22		7		0.678
		Mutated	13	59.1	4	63.6	
		No-mutated	9	40.9	3	36.4	
Lymph Node	33		25		8		0.072
		+	10	40.0	5	62.5	
		_	15	60.0	3	37.5	
Grade	26		20		6		0.201
		Low (1)	8	40.0	3	50.0	
		Mod(2)	7	35.0	2	33.3	
		High(3)	5	25.0	1	16.7	
Size	33		25		8		0.615
		X>20mm	14	56.0	5	62.5	
		X ≤ 20mm	12	44.0	3	37.5	
Ki67	30		23		7		0.047
		X>35	9	39.1	4	63.6	
		X≤35	14	60.9	3	36.4	

4. Discussion

Breast cancer is pushed into first place in the United States and many other parts of world. Breast cancer alone is expected to account for 29% (226,870) of all new cancer cases among women [16]. Although incidence rate of breast cancer remains relatively stable in recent 5 years, its death rate declines by 34% because of the development of diagnosis and targeting medication [4,5]. The micro metastasis of breast cancer before primary treatment is considered to be one of the recurrence reasons that will directly impact the survival of patients. So, it is crucial to find specific markers to detected micro metastasis and provide useful information to guide early therapeutic methods of breast cancer patients.

Although various biological markers had been proposed for the detection of breast cancer cells, they were often affected by tumor differentiation, lower specificity and detection rate. But almost all the current clinical application of tumor markers can not reach ideal level in the identification of the tumor specificity and sensitivity.

SBEM was a tissue specific protein, a member of the MUC family, and its expression is highly specific to mammary gland tissue. High SBEM expression was found to be strongly associated with the histopathological detection of lymph node metastasis [7]. However, as the genomes of different races and nationalities have their own characteristics, it is of guiding significance to explore the genetic characteristics of different ethnics in the diagnosis and treatment of breast cancer. The Buyi and Miao ethnic groups are the main components in Guizhou province in China. With different genetic backgrounds and lifestyles from those of the Han ethnic. As far as we knew the difference in the expression level of SBEM among different ethnic groups has not been reported. The aim of this research was to analyze whether there is the same expression in different mammary tissues in different ethnics and the association of SBEM expression in tissue of breast cancer patients with clinical- pathological features and to identify whether there is the same the clinical meaning for SBEM in Han, Miao and Buyi ethnics.

Researchers have conducted many studies on SBEM as a tumor marker of breast cancer micro metastasis worldwide. The research of O'Brien et al. [8] has shown that SBEM are expressed relatively exclusively in breast tissue than non-breast tissue and are potential new markers for breast cancer [9]. Zhong Lei et al. [17] study showed that SBEM expression rate was 53.3% and SBEM expression in TNBC tumor cells were related to TNM staging and axillary lymph node metastasis. The similar results were found by Yang Hua-wei et al. [18]. This research shows that SBEM is mainly expressed in the cell membrane, the second expression in the cytoplasm, few expression in the nucleus. SBEM positive detection rate of breast cancer tissue is significantly higher than the breast benign tumor tissue and normal breast tissue. But there was no significant difference in the han, miao, buyi ethnic in breast cancer tissue, breast benign tumor tissue and normal breast tissue.

Many factors can affect the prognosis of breast cancer recurrence and death, including tumor histologic features, clinical and pathological features of primary tumor, lymph node status, tumor hormone levels, Her-2 state, complications, age, menopausal status, tumor size, tumor grade, TNM staging, Ki67 and P53, which can evaluate DFS and OS [19-20]. Ki67 is also an important marker of cell proliferation, and it has important significance for prognosis judgment in breast cancer [21]. In our study, SBEM expression in breast cancer tumor cells were related to the ki67 in the Han, Miao and Buyi ethnic (*P*=0.034, 0.027, 0.047), respectively. There was a marked associations between the low expression of SBEM group and high expression group in lymphatic metastasis (*P*=0.042, 0.039) in the Han and Miao ethnic, while the same results were not found in the Buyi people (*P*=0.072)).Thus it can be seen that there is difference in different ethnic groups between SBEM different expression levels and metastatic tumor recurrence and the prognosis.

In conclusion, this research shows that SBEM positive detection rate was no significant difference in the han, miao and buyi ethnic in breast cancer tissue, breast benign tumor tissue and normal breast tissue.The SBEM expression level is related to the prognosis of breast cancer patients and its clinical significance is not exactly the same in different ethnics. However, it needs more centers to participate in further study because this is just a retrospective single-center study and the small sample size limits to some extent the generalization of the findings made in the study.

5. Conclusion

We have demonstrated that the SBEM positive detection rate was no significant difference in the han, miao and buyi ethnic groups in breast cancer tissue, breast benign tumor tissue and normal breast tissue.There was a marked associations between the SBEM expression level and lymphatic metastasis in the Han and Miao ethnic, while the same results were not found in the Buyi people. The distinction in different ethnic groups shall certainly provide a more reasonable choice for clinical individualized treatment and prognostic judgement in breast cancer patients.

6. Consent

Written informed consent was obtained from the patient for publication of this report and any accompanying images.

7. Abbreviations

SBEM: Small breast epithelial mucin; TNBC: Triple-negative breast cancer; HER2: Human epidermal growth factor receptor-2; TNM: Tumor node metastasis; IHC: immunohistochemical; RT-PCR: Reverse transcription polymerase chain reaction; NCCN: National comprehensive cancer network.

8. Compliance with Ethical Standards

Approval for this study was obtained from Third Affiliated Hospital of Guizhou Medical University, Guizhou, China.

9. Funding

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10. Conflict of Interest

The authors declare that they have no competing interests.

11. Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

12. Informed Consent

Informed consent was obtained from all individual participants included in the study.Log onto Editorial Manager as an author.

13. Authors' Contributions

LL contributed to the study design, data acquisition and analysis and drafted the manuscript; WL was involved in data acquisition and revision of the manuscript; QL and YY worked on aspects of data acquisition and analysis; HY contributed to the study design and data-analysis, developed the algorithm and coordinated the study; LH conceived and coordinated the study. All authors read and approved the final manuscript.

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