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Mucin expression profile of benign and malignant cervical tissues and correlation with clinical-pathologic parameters

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Summary

Purpose: To detect the expression of mucins in diverse benign and malignant cervical tissues of cervical disease. *Materials and Methods:* 158 cases of cervical tissues were collected. Sections were stained with monoclonal antibodies against MUC1, MUC2, MUC4, MUC5AC, and MUC20 by immunohistochemistry. *Results:* Normal cervical epithelium showed high expression of MUC1, MUC4, and MUC5AC, partial expression of MUC20, and no MUC2. With the development from chronic cervicitis, cervical intraepithelial neoplasia (CIN) to cervical squamous cell carcinoma (SCC), the expression of MUC1, MUC4, and MUC20 was statistically significant. The expression of MUC1 was related with the depth of invasion and clinical stage of SCC. The positive rates of MUC4 and MUC20 were associated with the degree of differentiation and clinical stage of SCC. There was a correlation between the expression of MUC4, MUC1, and MUC20 in cervical squamous lesions. *Conclusion:* Mucins may be involved in the development of cervical cancer.

Key words: Mucin; Cervical intraepithelial neoplasia (CIN); Squamous cell carcinoma (SCC); Adenocarcinoma; Clinical-pathologic parameters; Immunohistochemistry.

Introduction

Mucins are high-molecular-mass glycoproteins components of mucus, which are synthesized by epithelial cells and hematopoietic cell lineages. These are large, heavily O-glycosylated with an abundance of oligosaccharides attached through O-glycosidic linkages to serine and threonine residues in the core protein backbone [1]. Under normal physiological conditions, mucins can protect and lubricate the epithelia of respiratory, gastrointestinal, and urogenital tracts, provide a transport medium for cells, and play a key role in other important capabilities, such as epithelial cell proliferation, differentiation, and integrity [2, 3]. When an epithelium suffers malignant transformation, the mucin genes can experience abnormal expression and aberrant glycosylation, causing decreased production of an expectant mucin and/or increased production of a structurally different and inexpectant mucin within the transformed epithelium [4]. The human *MUC* gene family codes up at least 20 known proteins, which can be divided into two categories: secreted (e.g. MUC2 and MUC5AC) and membrane-associated (e.g. MUC1, MUC4, and MUC20) [5]. The expression of mucin core protein is relatively tissue-specific. For example, MUC1 is well known as a main component of gastric mucins, and shows aberrant expression in different kinds of tumor types such as colorectal neoplasia [6] and breast cancer [7]. MUC2 is predominantly present in the colorectal epithelium [6]. MUC4 is a crucial tumor marker overexpressed in lung cancer and inimitably expressed in pancreatic ductal adenocarcinoma (CA) [8]. MUC5AC is extensively expressed in the tracheobronchial tree and gastric surface mucosa [6]. MUC20 is a novel identified membrane-bound mucin protein which is upregulated in renal injuries [9]. The human female reproductive tract also covers many of these *MUC* genes [10, 11].

In the past decade, due to advances in human papillomavirus (HPV) and cytological screening, the incidence of squamous cell carcinoma (SCC) of the uterine cervix has been decreasing, while the incidence of cervical CA has been increasing [12]. It is widely assumed that most cervical squamous lesions are infected with high-risk HPV, while several unusual morphologic subtypes of CA, such as gastric type adenocarcinoma including minimal deviation adenocarcinoma (MDA) are not infected [13]. Current HPV-targeted screening commonly cannot detect these lesions and HPV vaccination will not prevent their germination [14]. It may be sometimes difficult to differentiate between benign and malignant endocervical lesions because of the overlap in morphology among these lesions. Therefore some new biomarkers are waited to assist in early detection of CA and in the identification of different types of cervical lesions and cancers. To date, only limited data have explored the expression of mucins in various benign and malignant glandular lesions of the uterine cervix [4, 15, 16]. The pattern of mucin expression in cervical squamous epithelium has not been documented in a series. The objective of this study was to evaluate the expression of MUC1, MUC2, MUC4, MUC5AC,

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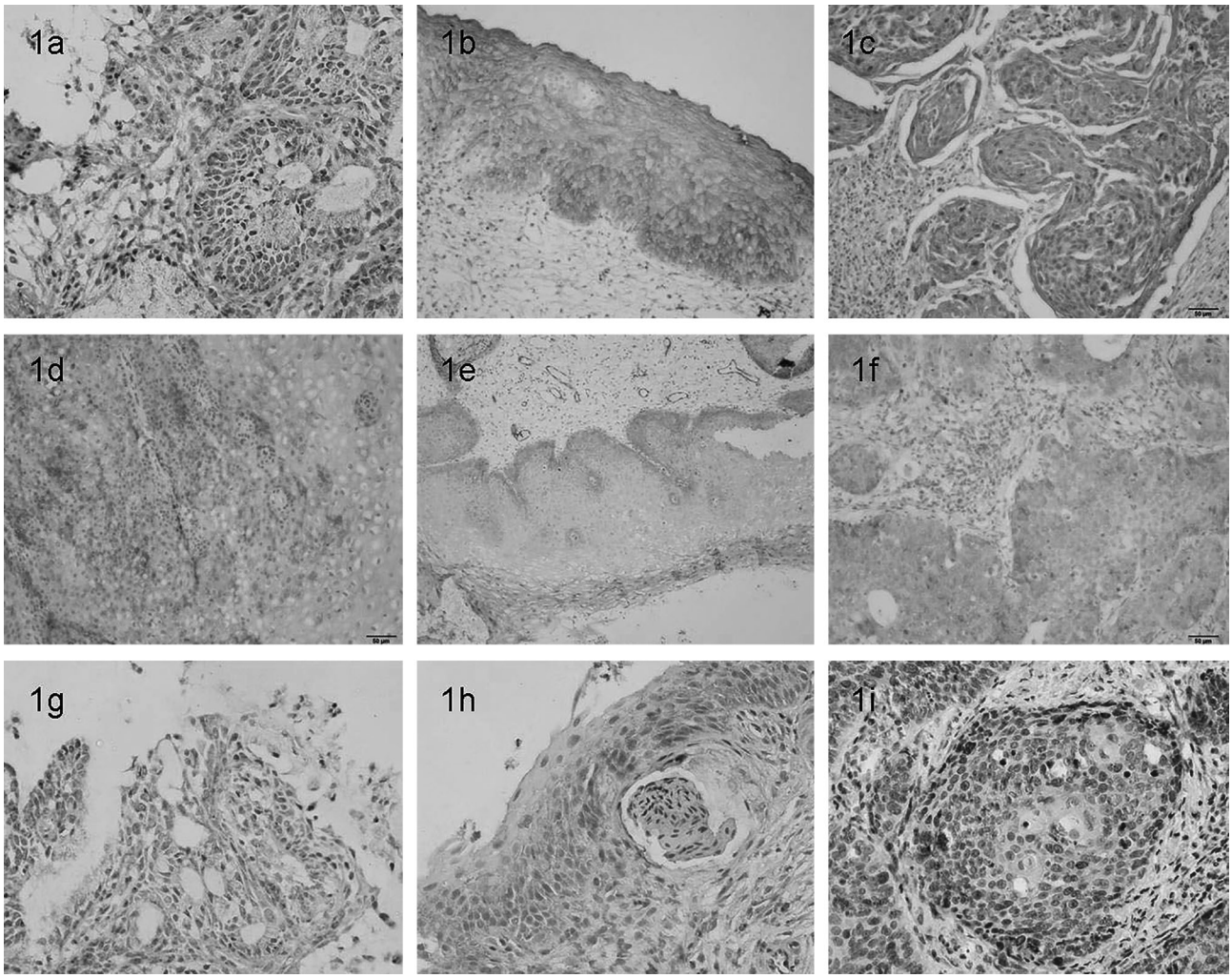

Figure 1. — MUC staining patterns in cervical squamous lesions: (a) to (c): MUC1 expressed in chronic cervicitis, CIN, and SCC; (d) to (f): MUC4 expressed in chronic cervicitis, CIN, and SCC; (d) to (f): MUC20 expressed in chronic cervicitis, CIN, and SCC.

and MUC20 in various lesions involving the uterine cervix, and to evaluate whether there was any correlation between the mucin profile of these lesions and clinical-pathologic factors.

Materials and Methods

The authors retrieved 158 cases of tissue samples from the Department of Pathology files of Clinical Medical College of Yangzhou University. The study was approved by ethics committee in clinical medical college. Patients were confirmed using the ICD-9 codes for cervical tissue in the period from December 1, 2009 to December 1, 2014. These cases included 52 cervical SCC (14 well differentiated, 26 moderately differentiated, and 12 poorly differentiated), 16 cervical CA, 60 cervical intraepithelial neoplasia (20 CIN III, 20 CIN II, 20 CIN I),15 chronic cervicitis, and 15 normal cervical tissues. According to the International Federation of Gynecology and Obstetrics (FIGO) 2009 Stage, SCC was divided as: ten Stage I, 13 Stage II, 17 Stage III, and 12 Stage IV. The patients ranged from 29 to 71 years old (median, 48.2 years).

All materials were fixed in 10% formalin before routine processing and embedded in paraffin, then they were sliced in four-μm sections. The sections were deparaffinized with xylene and rehydrated using graded alcohols. Antigen retrieval was performed by placing the sections in boiling citrate buffer (0.01 M/pH 6.0) for two minutes in a pressure cooker. After three washes in phosphate-buffered saline (PBS), 3% aqueous hydrogen peroxide was applied to the tissues to block for endogenous peroxidase. Non-specific binding proteins were blocked using 3% goat serum in PBE buffer. Primary antibodies were then added and incubated overnight for 4°C and washed three times with PBS containing 0.05% Tween 20. The primary antibodies applied included anti-MUC1 (clone Ma695), anti-MUC2 (clone CCP58), anti-MUC4 (clone 1G8), anti-MUC5AC (clone CLH2), and anti-MUC20 (clone RB13033). All antibodies except MUC20 were used at dilutions of 1:200 each and MUC20 was used at 1:25. Antigen-antibody reaction was then detected using the peroxidase polymerizing 3, 3-diaminobenzidine (DAB). The slides were then counterstained in a weak Mayers hematoxylin solution, dehydrated in graded alcohols, and mounted

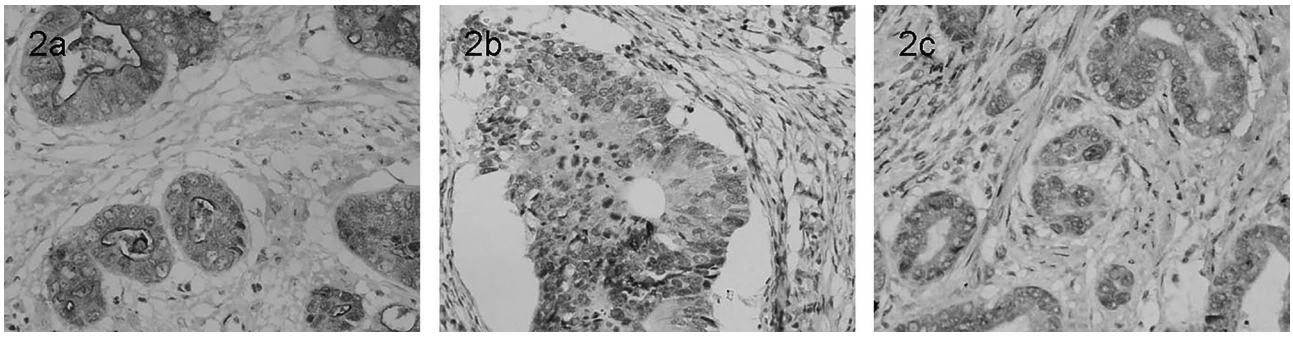

Figure 2. — MUC staining patterns in CA: (a) MUC1, (b) MUC2, (c) MUC5AC.

Table 1. — *Expression of mucin in benign and malignant cervical tissues.*

Histologic type	Cases	MUC1 (%)	MUC2 (%)	MUC4 (%)	MUC5AC (%)	MUC20 (%)
Normal	15	15 (100.0)	0 (0.0)	14 (93.3)	15 (100.0)	8 (53.3)
Chronic cervicitis	15	1 (6.7)	0 (0.0)	5 (33.3)	3 (20.0)	4 (26.7)
CIN I	20	7 (35.0)	0 (0.0)	11 (55.0)	6 (30.0)	7 (35.0)
CIN II	20	9 (45.0)	0 (0.0)	15 (75.0)	5 (25.0)	9 (45.0)
CIN III	20	12 (60.0)	1 (5.0)	17 (85.0)	4 (20.0)	14 (70.0)
SCC	52	45 (86.5)	3 (5.8)	47 (90.4)	11 (21.2)	43 (82.7)
CA	16	6 (37.5)	10 (62.5)	13 (81.3)	7 (43.8)	7 (43.8)

with Vectamount permanent mounting media. Sections of normal breast (MUC1), normal colonic mucosa (MUC2 and MUC4), CA of the colon (MUC5AC), and normal kidney (MUC20) were used as positive controls. Negative controls were obtained by omitting the primary antibodies.

All tissue sections were examined by two pathologists independently. The degree of expression of each marker was evaluated according to the distribution, intensity, and pattern of staining. The distribution of staining was based on the percentage of the lesion involved by staining from 0% to 100%; positivity was considered staining in more than 5% of the cells. The intensity of staining was estimated by semiquantitative analysis as follows: 0, negative; 1, weak; 2, moderate; 3, strong. The pattern of staining was categorized characterized as cytoplasmic, membranous, or luminal/apical. If more than one staining pattern was seen in the same lesions, all patterns identified were recorded.

To compare MUC expression with individual surgical-pathologic variables, Chi-square analysis and Fisher's exact test were used for statistical analyses. Pearson analysis was done to investigate relationships among MUCs. For statistical purposes, Stage was reduced to two categories: Stages I and II versus Stages III and IV. The level of significance was set at 0.05.

Results

Sections of almost all normal cervical epithelium stained strongly for MUC1, MUC4, and MUC5AC; some stained for MUC20. MUC2 staining was completely negative in all normal cervical epithelium and benign tissues. MUC1 staining occurred in the cytoplasm of the cells as well as in the luminal aspect of the endocervical glands. The normal glandular epithelium was strongly positive for MUC4 with a predominately luminal pattern. In cases with associated squamous dysplasia (ranging from CIN I to CIN III), MUC4 and MUC20 showed intense staining of the dysplastic epithelium as opposed to the parabasal staining of uninvolved epithelium. MUC5AC staining was present in the cytoplasm of normal endocervical epithelium.

Chronic cervicitis→CIN→SCC, the expression of MUC2 and MUC5AC were not statistically significant ($p > 0.05$), while the positive rates of MUC1, MUC4, and MUC20 expression showed an increasing trend, their expression was statistically significant ($p < 0.05$) (Figure 1). Normal cervical tissues→ cervical CA, the positive rate of MUC2 expression was increased ($p < 0.05$); cervical mucinous CA especially stained strongly and diffusely, while the expression of MUC1 and MUC5AC was significantly reduced ($p < 0.05$) (Figure 2, Table 1).

In the present study, the cases of CA were small, and many previous studies have evaluated the relationship between mucin expression and clinical-pathological features and prognosis of CA. Furthermore, among the five mucin factors in the present study, only MUC1, MUC4, and MUC20 expression in SCC was statistically significant, therefore only the relationship between MUC1, MUC4, and MUC20 expression and clinicopathologic features of SCC were analyzed. The results are summarized in Table 2.

In SCC, MUC1 expression was associated with the depth of invasion ($p = 0.009$) and clinical Stage ($p = 0.049$), and there was no statistically significant correlation with age, the region of invasion, tumor size, differentiated degree, and lymph node metastasis ($p > 0.05$). MUC4 and MUC20 were frequently observed in poorly differentiated ($p =$

Table 2. — *Correlation between expression of mucin and clinicopathological features of SCC*

Category		Cases	MUC1			MUC4			MUC20		
			Pos.	Neg.	*p*	Pos.	Neg.	*p*	Pos.	Neg.	*p*
Age (years)	< 50	30	25	5	0.704	26	4	0.558	26	4	0.607
	≥ 50	22	20	2		21	1		17	5	
Depth of invasion	< 3	18	12	6	0.009	17	1	0.82	14	4	0.767
(mm)	≥ 3	34	33	1		30	4		29	5	
Region of invasion	< 7	18	13	5	0.076	14	4	0.08	12	6	0.066
(mm)	≥ 7	34	32	2		33	1		31	3	
Tumor size (cm)	< 4	24	18	6	0.064	21	3	0.856	17	7	0.085
	≥ 4	28	27	1		26	2		26	2	
Differentiated degree											
Well or moderately		33	26	7	0.083	33	0	0.009	31	2	0.001
Poorly		19	19	0		14	5		12	7	
Clinical Stage	I or II	23	17	6	0.049	18	5	0.030	15	8	0.002
	III or IV	29	28	1		29			29	0	
Lymph node status	Negative	40	33	7	0.282	38	2	0.133	34	6	0.713
	Positive	12	12	0		9	3		9	3	

Pos. = positive; neg. = negative.

Table 3. — *Correlation between expression of MUC4, MUC1, an MUC20 in CIN.*

Group	Expression	MUC4		r	*p*
		Positive	Negative		
MUC1	Positive	21	7	0.069	0.599
	Negative	22	10		
MUC20	Positive	27	3	0.280	0.030
	Negative	16	14		

Table 4. — *Correlation between expression of MUC4, MUC1, and MUC20 in SCC.*

Group	Expression	MUC4		r	*p*
		Positive	Negative		
MUC1	Positive	43	2	0.045	0.001
	Negative	4	3		
MUC20	Positive	41	2	0.368	0.007
	Negative	6	3		

0.009 and 0.001, respectively) and advanced cancer (p = 0.030 and 0.002, respectively). However, the expression of MUC4 and MUC20 was not related to age, the depth and region of invasion, tumor size, and lymph node metastasis ($p > 0.05$).

In the present study, chronic cervicitis → CIN → SCC, only MUC1, MUC4, and MUC20 expressions were statistically significant, therefore the relationships among MUC1, MUC4, and MUC20 were just analyzed by Pearson analysis. In CIN, the expression of MUC4 and MUC20 was correlated ($p < 0.05$), while there was no significant correlation of MUC4 with MUC1 ($p > 0.05$, Table 3). In SCC, the expression of MUC4 and MUC1, MUC20 was closely correlated (MUC1, $p < 0.01$; MUC20, $p < 0.01$, Table 4).

Discussion

In the current study, the authors observed that nearly all endocervical epithelium expressed MUC1, MUC4, and MUC5AC, and partly expressed MUC20. MUC2 was not appreciated in any of the normal or benign lesions of the cervix. The present findings with MUC1, MUC2, MUC4, and MUC5AC agreed with the previously published results [4, 10, 15-17]. Only a few studies are available with regards to the expression of MUC4 in normal endocervical epithelium, ranging from 40% to 75%, using various techniques including polymerase chain reaction, in situ hybridization, and immunohistochemistry [10, 11, 15]. There is no study on MUC20 expression in cervical epithelium.

In the present study, cervical CA was more likely to express MUC2, especially the mucinous CA, but the expression of MUC1 and MUC5AC in CA was significantly reduced when compared to the normal endocervical epithelium. Other studies [4,16] showed that endocervical CA *in situ* (AIS) expressed more MUC2: about 25% and 46%, respectively. Previous study[18] has indicated that MUC2 positive expression can be found regularly in a high percentage of carcinomas with predominantly mucinous features, regardless of the site of origin. Furthermore, Reithdorf *et al.* [16] observed MUC2 expression in neoplastic endocervical glands and postulated that MUC2 expression may accompany neoplastic transformation of endocervical glands. The present results support these theories. The possibility of *MUC2* gene expression preceding neoplastic transformation may be helpful to study CA and differentiate CA from SCC. Because the number of cases in this category is limited, it is difficult to generalize the results, and further investigations are warranted.

Togami *et al.* [1] reported that overexpression of MUC1 corresponded with a lower disease-free survival rate and lymph metastasis for cervical mucinous CA and deter-

mining the level of MUC1 antigen expression in punch-biopsied specimens preoperatively may have the potential to predict the risks on the link with CA and be helpful to indicate radical hysterectomy and lymphadenectomy. In addition, MUC1 expression status in hysterectomized specimens may afford some guidance about postoperative adjuvant therapy. Therefore they considered that the expression of MUC1 may be available as an independent prognostic factor for CA. Terada [19] presented a 32-year-old Japanese woman of simultaneous early microinvasive endometrioid adenocarcinoma (EMEA) and CIN III in the uterine cervix with an emphasis on immunohistochemical finding that MUC1 was present in CIN III but was absent in EMEA. The present findings supported their result. Overexpression of MUC1 can disrupt the adhesion of tumor cells to their surrounding stroma by lowering the activity of E-cadherin and integrin, and may have a role in hiding the tumorigenic antigen, hence, helping tumor cells escape immune surveillance and promote carcinoma invasion and metastasis.

Mitsuhashi *et al.* [20] examined the expression of MUC5AC on CA and normal endocervical epithelium using immunohistochemical technology and discovered that the former was significantly reduced. They reported that MUC5AC expression was suppressed in CA and associated with paracervical invasion and histological type. Patients with negative MUC5AC staining revealed worse survival than those with positive MUC5AC staining. Other prior studies [4, 18] reported that the phenotypic expression of MUC5AC may be of value in distinguishing endometrial CA from those CAs of endocervical origin. Furthermore, this discrimination may probably be useful in small biopsies such as endocervical curettage. It should be acknowledged, however, that the specificity of MUC5AC positive expression in the particular sites is not absolute, since MUC5AC positive expression was also noted in a small percentage of endometrial adenocarcinoma. Though different previous results have appeared, the relationship between MUC expression and different types of cervical CA and clinicopathological features, more clinical data needs to be accumulated.

Although there are some reports assessing the relationship between MUC antigen expression and SCC, only few assessed the relationship between MUC antigen expression and clinical pathologic features outcome in patients with SCC. In present study, chronic cervicitis→CIN→SCC, the positive rates of MUC1, MUC4, and MUC20 expression showed an increasing trend. The depth of invasion and clinical stage were associated with MUC1 expression, MUC4, and MUC20 expression was frequently observed in low differentiated and advanced cervical cancer.

Munro *et al.* [21] suggest that MUC4 could be a lineage biomarker in benign cervical tissues that may be abnormally expressed in squamous dysplasia and squamous carcinoma, which is conserved when cervix is HPV-infected and cells undergo dysplastic change. These changes may give occasion to permanent expression of MUC4, triggering the diffusely positive staining we see in invasive SCC. Lopez-Ferrer *et al.* [22] suggested that in benign endocervical tissues and squamous dysplasia, the increased MUC4 expression may give support to the hypothesis that endocervical gland act as a site for progenitor cells that may develop or differentiate into squamous dysplasia. All these discoveries provide more evidence to suggest that MUC4 expression may be constant or turned on during dysplastic transformation. Given the diffuse MUC4 staining in CIN, MUC4 staining in SCC cannot be used to make a correct diagnosis. Nonetheless, the pattern of MUC4 expression could still provide crucial information with reference to the development of cervical malignancies. These data demonstrate that MUC4 expression is coincidentally upregulated in malignant cervical lesions. By comparison, the MUC4 expression in CA is not remarkably different from expression in normal endocervical glands.

MUC20, a novel mucin protein, suppressed the hepatocyte growth factor-induced Grb2-Ras pathway [23]. Xiao *et al.* [24] examined the protein and mRNA levels of MUC20 in colorectal cancer tissues using immunohistochemistry and Real-Time quantitative PCR and revealed that MUC20 was significantly upregulated. MUC20 overexpression might promote migration and invasion abilities of colorectal cancer cells, which is related to recurrence and poor prognosis. MUC20 overexpression also predicted poor outcome in endometrial cancer and enhanced EGF-mediated invasive behavior through activation of EGFR-STAT3 pathway [25].

Conclusion

In conclusion, the present findings may suggest that the expression of MUC1, MUC4, and MUC20 is related to the occurrence and development of SCC, but the precise mechanism is unclear. One of the mechanisms may be that Toll-like receptors (TLRs) either directly activate various signaling molecules, such as NF-κB, MAP kinases, p38, and JNK pathways or via inflammatory molecules regulate MUC expression [26]. The mechanism requires further research.

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