The Relationship Between Phospho-p38, Matrix Metalloproteinase 9, and Major Histocompatibility Complex Class I Chain-Related Molecule A Expression in Pituitary Adenomas Demonstrates a New Mechanism of Pituitary Adenoma Immune Escape

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■ BACKGROUND: **The major histocompatibility complex class I chain-related molecule A (MICA) is one of the natural killer group 2D ligands. Soluble major histocompatibility complex class I chain-related molecule A (sMICA) mediates tumor immune escape, but the mechanism is not fully understood. In this study, we examined the expression of phospho-p38, matrix metalloproteinase 9 (MMP-9), and MICA and their relationships among each other in pituitary adenoma tissues to provide a histologic basis for the mechanism of pituitary adenoma immune escape.**

■ METHODS: **We applied immunohistochemistry, real-time quantitative reverse-transcriptase polymerase chain reaction, and Western blot to detect phospho-p38, MMP-9, and MICA expression at the mRNA and protein levels in pituitary adenoma tissues. Enzyme-linked immunosorbent assay was used to examine the expression levels of MMP-9 and sMICA in peripheral blood serum from patients with pituitary adenoma.**

■ RESULTS: **We found that p38, MICA, and MMP-9 mRNA levels were greater in pituitary adenomas than in normal tissues. The phospho-p38, MMP-9, and MICA proteins were overexpressed in pituitary adenomas, and the expression of MMP-9 and MICA were positively correlated with the expression of phospho-p38. In addition, the serum levels of sMICA and MMP-9 proteins in pituitary adenoma patients were significantly greater than those in normal controls.**

■ CONCLUSIONS: **These findings suggest that activation of the p38/mitogen-activated protein kinase pathway may increase MICA expression and induce MMP-9 expression. MMP-9 is involved in the shedding of sMICA from MICA to promote tumor immune escape. Furthermore, p38/mitogen-activated protein kinase could potentially represent a novel target for inhibiting pituitary adenoma immune escape.**

Pituitary adenoma is a common intracranial tumor that accounts for 10%–20% of all primary intracranial tumors, and its incidence rate is second only to glioma and meningioma.[1] Moreover, according to epidemiologic investigations, the morbidity of pituitary adenoma increases annually.[2] The occurrence and development of tumors are complex processes that involve many aspects, such as immune status, cell differentiation, and apoptosis. Therefore, exploring the relevant molecular mechanisms that contribute to the clinical diagnosis and treatment of pituitary adenoma is important.

Natural killer (NK) cells and T lymphocytes can activate cytotoxicity by binding to the major histocompatibility complex (MHC)

Key words
- Immune escape
- MICA
- MMP-9
- p38/MAPK
- phospho-p38
- Pituitary adenoma

Abbreviations and Acronyms
ELISA: Enzyme-linked immunosorbent assay
IHC: Immunohistochemistry
MAPK: Mitogen-activated protein kinase
MHC: Major histocompatibility complex
MICA: Major histocompatibility complex class I chain-related molecule A
MMP: Matrix metalloproteinase
MMP-9: Matrix metalloproteinase 9
mMICA: Membrane-anchored major histocompatibility complex class I chain-related molecule A
NK: Natural killer
NKG2D: Natural killer group 2D
qRT-PCR: Real-time quantitative reverse-transcriptase polymerase chain reaction
sMICA: Soluble major histocompatibility complex class I chain-related molecule A

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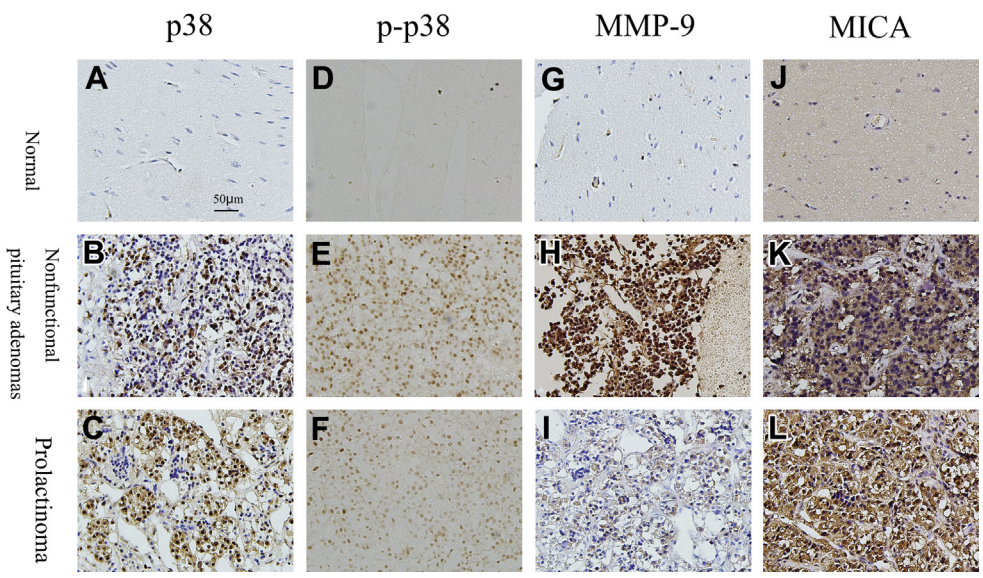

Figure 1. The expression levels of phospho-p38, matrix metalloproteinase 9 (MMP-9), and major histocompatibility complex class I chain-related molecule A (MICA) protein in pituitary adenoma tissues and controls. p38, phospho-p38 (p-p38), MMP-9, and MICA expression were determined by immunohistochemistry. (**A**–**C**) p38 protein was scarcely detected in normal brain tissues but positively expressed in pituitary adenoma tissues. (**D**–**F**) Phospho-p38 protein was absolutely not expressed in normal brain tissues but was strongly expressed in pituitary adenoma tissues. (**G**–**I**) The expression levels of MMP-9 between normal brain tissues and pituitary adenoma tissues. (**J**–**L**) Representative images of MICA, which was minimally expressed in normal brain tissues but was highly expressed in pituitary adenoma tissues. It was detected not only in pituitary adenoma cells but also in the stroma. Magnification: ×100.

class I chain-related molecule A (MICA) ligands on the surface of tumor cells, killing tumor cells.[3] However, there are multiple immune escape mechanisms in various tumor cells, primarily by avoiding recognition by cytotoxic cells, directly impairing the functioning of antigen-presenting cells or cytotoxic cells, or inducing suppressor cells.[3] It is known that tumor cells secrete a soluble MHC class I chain-related molecule A (sMICA) that can bind to natural killer group 2d (NKG2D) and downregulate its expression to promote immune escape.[4,5] Matrix metalloproteinase (MMP), a type of zinc-dependent enzyme, plays an important role in the degradation of extracellular matrix in the tumor microenvironment.[6] In osteosarcoma, matrix metalloproteinase 9 (MMP-9) promotes the release of sMICA from tumor cells, and MMP-9 inhibitors increase the expression of membrane-anchored MHC class I chain-related molecule A (mMICA) and decrease sMICA release without inhibiting MICA mRNA transcription.[7] Therefore, MMP-9 plays an important role in sMICA-mediated tumor immune escape.

p38/mitogen-activated protein kinase (p38/MAPK) is a major pathway among the MAPK signal transduction pathways. Activation of the p38/MAPK pathway activates downstream nuclear transcription factors and regulates the expression of related genes, which affect cellular responses such as cell proliferation, differentiation, inflammation, and apoptosis.[8] Recent studies have indicated that the p38/MAPK pathway significantly involves in tumor invasion and metastasis formation in various cell lines by regulating the expression of MMPs family.[9] The p38/MAPK pathway regulates the expression of MICA by activating the E2F transcription factor 1.[10] This study detects the expression levels of phospho-p38, MMP-9, and MICA proteins and evaluates the relationships between them in pituitary adenomas to provide a histologic basis for mechanistic research on the function of the NKG2D-MICA pathway in pituitary adenoma immune escape.

MATERIALS AND METHODS

Materials

Immunohistochemistry (IHC) case slice source was the pathology department of Binzhou Medical College Hospital, from which 38 paraffin-embedded nonfunctional pituitary adenoma tissue sections (20 male and 18 female) and 21 paraffin-embedded prolactinoma pituitary adenoma tissue sections (6 male and 15 female) were randomly selected.

A total of 39 patients were diagnosed with pituitary adenoma, including 21 nonfunctional pituitary adenomas (12 male and 9 female) and 18 prolactinoma pituitary adenomas (6 male and 12 female). All patients received trans-sphenoidal surgery at the Binzhou Medical University Affiliated Hospital between July 2015 and December 2017. These patients were diagnosed with pituitary adenoma by postoperative pathologic confirmation. Fresh tumor tissue samples from these patients were stored in liquid nitrogen for real-time quantitative reverse-transcriptase

polymerase chain reaction (qRT-PCR) and Western blot. We also collected 20 normal brain tissues from Binzhou Medical University Affiliated Hospital for immunohistochemistry, qRT-PCR and Western blot as controls. In addition, peripheral blood serum was collected from each patient with pituitary adenoma before surgery for enzyme-linked immunosorbent assay (ELISA). We also collected peripheral blood serum from 10 volunteers as controls. None of the patients had immune-related diseases or were treated with immunosuppressive drugs. The study was approved by the Binzhou Medical College Hospital Medical Ethics Committee. The study received the consent of all patients and volunteers.

Immunohistochemistry

After formalin fixation and paraffin embedding of tissues, paraffin sections (4-μm thick) were dewaxed. Endogenous peroxidase was inactivated by quenching in a 3% hydrogen peroxide solution for 15 minutes at room temperature. Citrate buffer was used for thermal remediation followed by incubation with 1.5% normal goat serum at 37°C for 30 minutes to prevent nonspecific binding. The sections were then incubated with primary antibody at 4°C overnight: anti-p38 (1:100; Cell Signaling Technology, Danvers, Massachusetts, USA), antiphospho-p38 (1:50; Cell Signaling Technology), anti-MICA (1:100; Abcam, Cambridge, United Kingdom), and anti-MMP-9 (1:100; Abcam). The sections were subsequently incubated with the appropriate biotinylated secondary antibody (1:5000; Beijing Zhong Shangolden Bridge Technology, Co., Ltd.) at 37°C for 30 minutes. The reactions were developed using a DAB chromogenic kit (Beijing Zhong Shangolden Bridge Technology) at room temperature, and Harris hematoxylin was used to visualize nuclei (except for phospho-p38), followed by dehydration, transparentizing, and sealing.

Interpretation of the results are as follows. p38 was positively expressed in the cytoplasm and in the nuclei, and phospho-p38 was expressed in only the nuclei, whereas MMP-9 was cytoplasmic. In particular, MICA was not only expressed in the cytoplasm and nucleus but also in the stroma. Scores were calculated for the percentage of positive cells (0, <5%; 1+, 5%–25%; 2+, 26%–50%; and 3+, >50% cells positive) and for the staining intensity (0, no staining; 1+, pale yellow; and 2+, brown yellow). The final scores were tallied by adding the 2 scores and were classified according to the following standards: 0– (negative), 2+ (weakly positive), 3–4++ (moderately positive), and 5+++ (strongly positive).

Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction

Total RNA was extracted using a MiniBEST Universal RNA Extraction Kit (TaKaRa, Kusatsu, Japan) and was reverse-transcribed into complementary deoxyribonucleic acid using a RevertAid First-Strand cDNA Synthesis Kit (TaKaRa) according to the manufacturer's protocols. qRT-PCR was performed with specific primers (Sangon Biotech, Shanghai, China) on a CFX96TM Real-time PCR Detection System C1000. In every group of samples, the gene expression was measured in triplicate, and the results were analyzed using the $2-\Delta\Delta Ct$ formula. The following primers were used:

p38/MAPK, 5′-	TCGAGACCGTTTCAGTCCAT-3′	(forward)
5′-	CCACGGACCAAATATCCACT-3′	(reverse)
MMP-9,5′	-ACGGCAGAGAGCATTGTGTA-3′	(forward)
5′-	CCTGTAGCGTAAGAGCCAGAG-3′	(reverse)
MICA, 5′	-AACCCTGACTGCACAGATCC-3′	(forward)
5′-	ATCTTCCCTTTTGCACCTCC-3′	(reverse)
β-actin	5′-CCAACTGGGACGACAT-3′	(forward)
5′-	TCTGGGGTCATCTTCTCG-3′	(reverse)

Western Blot Analysis

Whole proteins from tissues were collected with RIPA lysis buffer (Solarbio Science & Technology, Beijing, China), and the protein concentrations were determined using a BCA assay. The isolated proteins were transferred to polyvinylidene difluoride membranes after electrophoresis. The membranes were blocked using 7% nonfat milk for 2 hours at room temperature. p38 (1:1000; Cell Signaling Technology), phospho-p38 (1:1000; Cell Signaling Technology), MICA (1:1000; Abcam), and MMP-9 (1:1000; Abcam) antibodies were added overnight at 4°C. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control. The membranes were then completely washed with Tris-buffered saline with Tween, and horseradish peroxidase–coupled secondary antibodies (1:2000; Affinity) were added for 2 hours at room temperature. The membranes were washed again with Tris-buffered saline with Tween for film chemiluminescence and exposure. Grayscale analysis was performed with Image J software.

Enzyme-Linked Immunosorbent Assay

Detection of sMICA and MMP-9 protein levels in peripheral blood serum was performed using a MICA ELISA kit (Mlbio, Shanghai, China) and an MMP-9 ELISA kit (Langton, Shanghai, China) according to the manufacturers' protocols. The optical density (450 nm) was read by a microplate reader (Thermo Multiskan FC; Thermo Fisher Scientific, Waltham, Massachusetts, USA), and the protein concentrations were calculated using a standard curve.

Statistical Analysis

SPSS 21.0 statistical analysis software (IBM Corp., Armonk, New York, USA) was used to analyze the experimental data. All data were expressed as the mean ± standard deviation. The unpaired 2-tailed Student t-test was used to analyze the experimental data between the 2 groups. Correlations between indicators were assessed by Spearman rank correlation analysis. Values were considered significant when $P < 0.05$ and highly significant when $P < 0.01$.

Table 1. Expression in Pituitary Adenoma Tissues

		Expression				
Tissues	**N**	–	+	++	+++	***P****
p38 protein						
Control	20	16 (80%)	3 (15%)	1 (5%)	0 (0%)	
Nonfunctional pituitary adenomas	38	0 (0%)	4 (11%)	25 (66%)	9 (23%)	<0.01
Prolactin pituitary adenomas	21	0 (0%)	1 (5%)	14 (66%)	6 (29%)	<0.01
phospho-p38 protein						
Control	20	20 (100%)	0 (0%)	0 (0%)	0 (0%)	
Nonfunctional pituitary adenomas	38	0 (0%)	5 (13%)	15 (40%)	18 (47%)	<0.01
Prolactin pituitary adenomas	21	0 (0%)	4 (19%)	9 (43%)	8 (38%)	<0.01
MMP-9 protein						
Control	20	15 (75%)	5 (25%)	0 (0%)	0 (0%)	
Nonfunctional pituitary adenomas	38	0 (0%)	6 (16%)	14 (37%)	18 (47%)	<0.01
Prolactin pituitary adenomas	21	0 (0%)	3 (14%)	7 (33%)	11 (53%)	<0.01
MICA protein						
Control	20	19 (95%)	1 (5%)	0 (0%)	0 (0%)	
Nonfunctional pituitary adenomas	38	0 (0%)	8 (21%)	23 (60%)	7 (19%)	<0.01
Prolactin pituitary adenomas	21	0 (0%)	3 (14%)	14 (66%)	4 (20%)	<0.01

Values are n (%).
MICA, major histocompatibility complex class I chain-related molecule A; MMP-9, matrix metalloproteinase 9.
*$P < 0.01$.

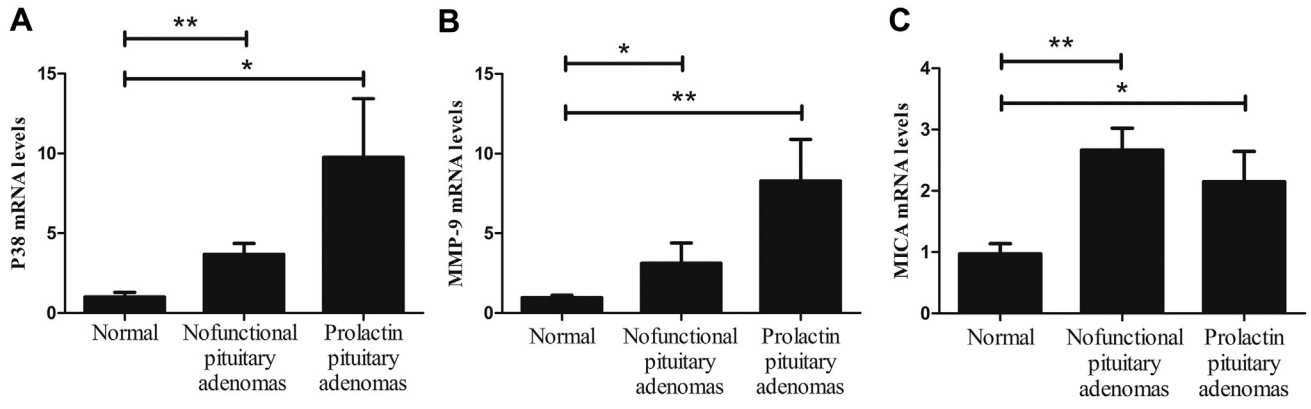

Figure 2. The levels of p38, matrix metalloproteinase 9 (MMP-9), and major histocompatibility complex class I chain-related molecule A (MICA) mRNA expression in pituitary adenoma tissues and controls. p38, MMP-9, and MICA mRNA expression was detected by real-time quantitative reverse-transcriptase polymerase chain reaction. (**A**) The expression levels of p38 mRNA were significantly greater in pituitary adenoma tissues than in normal brain tissues. (**B**) The expression levels of MMP-9 mRNA were significantly greater in pituitary adenoma tissues than in normal brain tissues. (**C**) The expression levels of MICA mRNA were significantly greater in pituitary adenoma tissues than in normal brain tissues. (*$P < 0.05$, **$P < 0.01$).

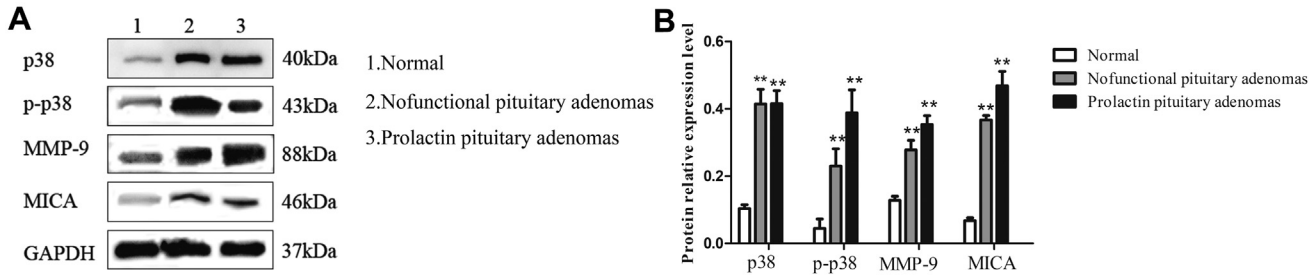

Figure 3. p38, phospho-p38, matrix metalloproteinase 9 (MMP-9), and major histocompatibility complex class I chain-related molecule A (MICA) protein expression in pituitary adenoma tissues and controls. p38, phospho-p38, MMP-9, and MICA expression were measured by Western blot.(**A**) The expression levels of p38, phospho-p38, MMP-9, and MICA proteins were all greater in nonfunctional pituitary adenoma and prolactin pituitary adenoma tissues than in normal brain tissues.(**B**) (**$P < 0.01$). GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

RESULTS

Detection of p38, Phospho-p38, MMP-9, and MICA Proteins Expression in Pituitary Adenoma and Normal Brain Tissues via IHC

To detect the expression of p38, phospho-p38, MMP-9, and MICA proteins, we studied 38 nonfunctional pituitary adenomas and 21 prolactinoma pituitary adenomas in postoperative pathologic slices. We also detected the expression of these proteins in normal brain tissues as controls. As a result, we found that the expressions of p38, phospho-p38, MMP-9, and MICA proteins were greater in nonfunctional pituitary adenoma and prolactin pituitary adenoma tissues than in normal brain tissues (Figure 1). The positive rates of p38, phospho-p38, MMP-9, and MICA proteins are described in Table 1. Through statistical analysis, the differences in p38, phospho-p38, MMP-9, and MICA expression between pituitary adenomas and normal brain tissue were shown to be statistically significant (Table 1; **P < 0.01).

The Levels of p38, MMP-9, and MICA mRNA Expression in Pituitary Adenoma and Normal Brain Tissues

In addition to the detection of p38, MMP-9, and MICA protein expressions, we also studied them at an mRNA level. qRT-PCR results showed that mRNA expression levels of p38, MMP-9, and MICA in nonfunctional pituitary adenoma and prolactin pituitary adenoma tissues were significantly greater than those in normal brain tissues (Figure 2; *P < 0.05, **P < 0.01).

The Protein Expression of p38, Phospho-p38, MMP-9, and MICA Assessed by Western Blot Analysis

To further test our findings through IHC, we used Western blot technology to detect the protein expressions of p38, phospho-p38,

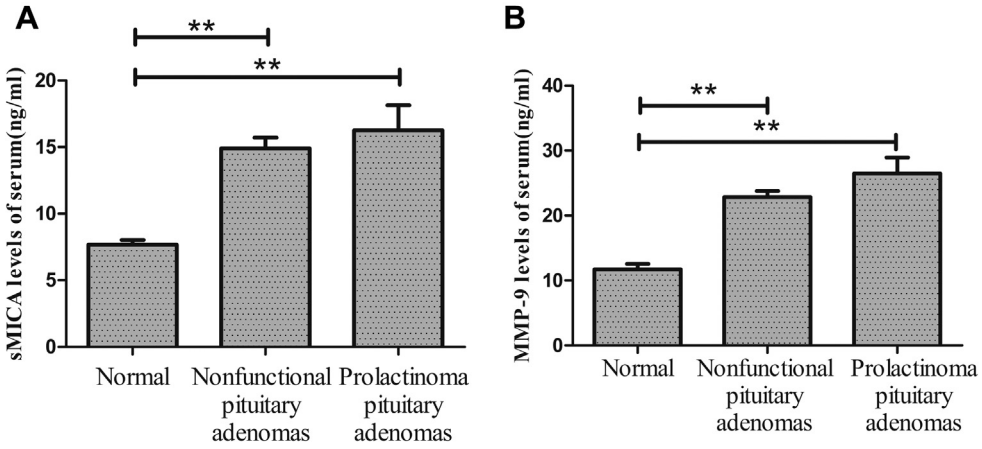

Figure 4. The expression levels of soluble histocompatibility complex class I chain-related molecule A (sMICA) and matrix metalloproteinase 9 (MMP-9) in peripheral blood sera. sMICA and MMP-9 expression were investigated by enzyme-linked immunosorbent assay. (**A**) The expression of sMICA in peripheral blood sera from pituitary adenoma patients was greater than that in controls. (**B**) The expression of MMP-9 in peripheral blood sera from pituitary adenoma patients was greater than that in controls. (**$P < 0.01$).

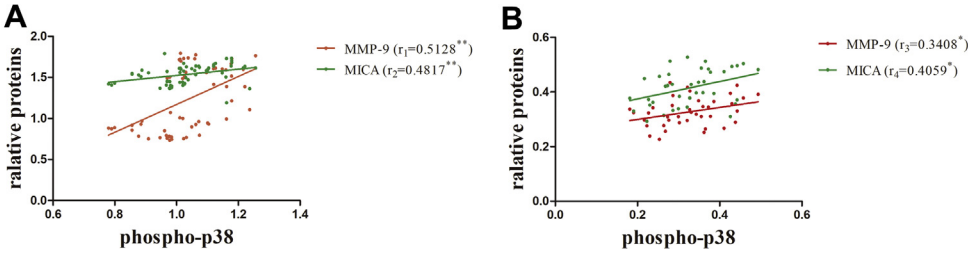

Figure 5. Relationships between phospho-p38, matrix metalloproteinase 9 (MMP-9), and major histocompatibility complex class I chain-related molecule A (MICA) expression in pituitary adenoma tissues. (**A**) Correlations between phospho-p38, MMP-9, and MICA were assessed by Spearman rank correlation analysis based on the immunohistochemistry results. The expressions of MMP-9 and MICA were positively correlated with the expression of phospho-p38 (**$P < 0.01$). (**B**) Correlations between phospho-p38, MMP-9, and MICA were assessed by Spearman rank correlation analysis based on the Western blot results. The expressions of MMP-9 and MICA were positively correlated with the expression of phospho-p38 (*$P < 0.05$).

MMP-9, and MICA. The results showed that the p38, phospho-p38, MMP-9, and MICA proteins were overexpressed in nonfunctional pituitary adenoma and prolactin pituitary adenoma tissues, and the levels were significantly greater than those in normal brain tissues (Figure 3; *P < 0.05, **P < 0.01).

sMICA and MMP-9 Protein Levels in Peripheral Blood Serum from Pituitary Adenoma Patients and Normal Controls

MMP-9 and sMICA, a type of MICA, exist in the blood of patients with tumor and healthy controls. We aimed to know whether there was any difference between the sMICA and MMP-9 expressions in the blood of patients with tumor and healthy controls. ELISA results showed that the serum levels of sMICA protein in patients with pituitary adenoma were significantly greater than those in normal controls (Figure 4A; **P < 0.01) and that the serum levels of MMP-9 protein in patients with pituitary adenoma were significantly greater than those in normal controls (Figure 4B; **P < 0.01).

Relationships Between Phospho-p38, MMP-9, and MICA Expression in Pituitary Adenoma Tissues

In our research, we found that the phospho-p38, MMP-9, and MICA proteins were overexpressed in pituitary adenomas. We speculated whether there were correlations between the expressions of phospho-p38 and MMP-9 or phospho-p38 and MICA. Therefore, we conducted the Spearman analysis based on the IHC and Western blot results.

Based on the IHC results, we found that phospho-p38 and MMP-9 protein expression levels were positively correlated in patients with pituitary adenoma ($r_1 = 0.5128$, **P < 0.01; Figure 5A, Table 2). Phospho-p38 and MICA protein expression levels were positively correlated in patients with pituitary adenomas ($r_2 = 0.4817$, **P < 0.01; Figure 5A, Table 2).

Based on the Western blot results, we found that phospho-p38 and MMP-9 protein expression levels were positively correlated in patients with pituitary adenoma ($r_3 = 0.3408$, *P < 0.05; Figure 5B). Phospho-p38 and MICA protein expression levels were positively correlated in patients with pituitary adenomas ($r_4 = 0.4059$, *P < 0.05; Figure 5B).

DISCUSSION

Pituitary adenoma is a common epithelial cell—derived tumor. The treatment strategies for pituitary adenoma primarily include radiotherapy, drug treatment, and surgery, which is the preferred treatment.[11] However, pituitary adenoma is still prone to recurrence and is poorly controlled, so discovering new treatment strategies is urgent. Infiltration of immune cells in pituitary adenoma suggest increased probability for immunotherapy based on the memory characteristic of immune cells and the specificity of immunotherapy, which may inhibit tumor recurrence.[12] With advances in research regarding the immunotherapy of pituitary adenoma, immunotherapy is expected to become another important treatment for pituitary adenoma.[13]

The NKG2D/NKG2DL system has an important role in tumor immune surveillance, and the NKG2D-activating receptor is expressed in various immune cells, including NK cells, NK T cells, and CD8+ T cells.[14] MICA is one of the NKG2D ligands and is rarely expressed on healthy cells; however, its expression may increase because of a variety of cellular stresses, such as viral infection and malignancy transformation.[15] MICA protein has 2 major isoforms in vivo, mMICA, which is located on the surfaces of cell membranes, and sMICA, which is secreted into tumor microenvironments and blood. MICA is highly expressed on a broad range of epithelial tumors, making them extremely susceptible to killing by NK cells.[14] In contrast, tumors can escape the surveillance of the immune system through MICA shedding. sMICA binds to NKG2D and reduces its expression on the surface of NK cells by facilitating NKG2D internalization, which then inhibits the cytotoxicity of NK cells, thus promoting tumor immune escape.[7] Chen et al.[16] found that nuclear factor-kappa B, MMP-9, and MICA play roles in the immune escape of pituitary adenomas.

Table 2. Relationship Among Phospho-p38, MMP-9, and MICA Expression Levels in Pituitary Adenoma Tissues Based on the IHC Results

	OD (MMP-9)	OD (Phospho-p38)	OD (MICA)
1	0.932	1.1036	1.6134
2	0.755	1.0231	1.6716
3	0.9273	1.0625	1.639
4	0.7364	0.9776	1.3657
5	0.7445	0.984	1.3938
6	0.8737	0.7897	1.4072
7	0.8579	0.856	1.5478
8	0.7528	0.9089	1.5564
9	0.9689	0.8877	1.5867
10	1.0722	0.9803	1.4002
11	1.7648	1.2559	1.6134
12	1.7281	1.0588	1.5847
13	1.6662	1.1765	1.639
14	1.5568	1.0967	1.5847
15	1.6118	1.1259	1.6716
16	1.7949	1.0127	1.6115
17	1.7301	1.179	1.6803
18	1.6024	1.1479	1.5541
19	1.4991	0.9696	1.4499
20	1.5198	1.0254	1.4167
21	0.9439	1.1007	1.5932
22	0.7692	1.0316	1.6293
23	0.9618	1.0719	1.6365
24	0.7665	0.9463	1.3691
25	0.7739	0.9839	1.3769
26	0.8826	0.7796	1.4194
27	0.9179	0.8558	1.5394
28	0.7612	0.9689	1.5729
29	0.7836	0.8769	1.5291
30	1.0012	1.0203	1.4036
31	1.7378	1.0376	1.4714
32	1.6981	1.0138	1.6013
33	1.6062	1.1196	1.6369
34	1.7764	1.0619	1.5319
35	1.5968	1.2159	1.6136
36	1.7309	1.0231	1.5765
37	1.2164	1.1783	1.7031
38	1.3894	1.1619	1.1931
39	1.4919	0.9697	1.4535
40	1.4989	1.0265	1.4263
41	0.7943	0.9669	1.4061
42	0.7832	0.9766	1.3695
43	0.8899	0.7995	1.4312
44	0.9302	0.8846	1.5099
45	0.8512	0.9613	1.7905
46	0.9948	1.1615	1.7312
47	1.0098	1.0105	1.4395
48	1.1081	1.2365	1.3645
49	1.7165	1.0087	1.6112
50	1.4137	1.1201	1.6554
51	1.5618	1.0301	1.4960
52	1.3872	1.2209	1.6336
53	1.7226	1.0109	1.5615
54	1.5153	1.1778	1.7319
55	1.5906	1.1596	1.5036
56	1.5038	0.9779	1.4496
57	1.4863	1.0197	1.4426
58	0.7964	1.0739	1.6226
59	0.8969	1.0493	1.6061

Continues

Spearman's rank correlation coefficient 1 (R1) (phospho-p38 and MMP-9) = 0.5128**.
Spearman's rank correlation coefficient 2 (R2) (phospho-p38 and MICA) = 0.4817**.
** $P < 0.01$.
MMP-9, matrix metalloproteinase 9; MICA, major histocompatibility complex class I chain-related molecule A; IHC, immunohistochemistry; OD, optical density.

Abnormal activation of the p38/MAPK pathway is closely related to tumor occurrence and development. The mechanism of p38/MAPK pathway activation is mediated by dual phosphorylation at the Thr-Gly-Tyr motif.[17] In recent years, many studies have indicated that p38/MAPK-specific inhibitors could block MICA expression in endothelial cells, T cells, and thyroid cancer cells.[18-20] These data indicate that the p38/MAPK pathway may regulate MICA expression through multiple mechanisms. In our study, the phospho-p38-positive rate was 100% in pituitary adenoma tissues, where it was expressed significantly greater than in normal brain tissues. Therefore, we conclude that the p38/MAPK pathway may have an important role in the occurrence and development of pituitary adenoma. Furthermore, we also found that MICA was overexpressed in pituitary adenoma tissues and was positively correlated to the expression of phospho-p38. This result indicated that the p38/MAPK pathway may regulate MICA expression in pituitary adenoma.

MMP-9 is an important gelatin enzyme of the MMP family and is a downstream gene in the p38/MAPK pathway. Recent research

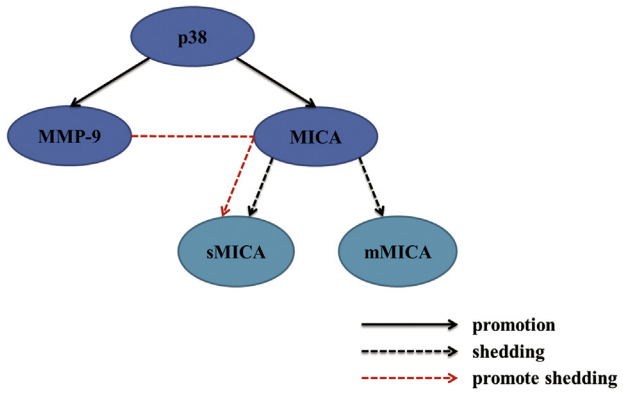

Figure 6. The pathway from p38 activation to matrix metalloproteinase 9 (MMP-9) expression and from major histocompatibility complex class I chain-related molecule A (MICA) to soluble histocompatibility complex class I chain-related molecule A (sMICA) transformation. The p38/mitogen-activated protein kinase pathway may increase MICA expression and induce the expression of MMP-9; MMP-9 mediates MICA shedding in pituitary adenoma cells.

demonstrated that the p38/MAPK pathway regulates the expression of many cytokines, transcription factors, and enzymes, for instance, tumor necrosis factor alpha, cyclooxygenase 2, matrix metalloproteinase 1, matrix metalloproteinase 2, and MMP-9.[21] The p38/MAPK pathway regulates MMP-9 expression in breast cancer and bladder cancer cell lines,[9,22] and specific inhibition of the p38/MAPK pathway decreases MMP-9 secretion in tumor cells.[23] We found that the expression of MMP-9 in pituitary adenomas was significantly greater than in normal brain tissues and that it was positively correlated with phospho-p38 expression, indicating that MMP-9 expression may be regulated by the p38/MAPK pathway in pituitary adenoma.

Leifler et al.[24] found that MMPs modify immune responses by cleaving a variety of receptors and adhesion molecules on cell surfaces, cytokines, and growth factors. Like the shedding of MICA, several studies showed that distinct MMPs are involved in different types of tumor cells.[25] For instance, membrane-type matrix metalloproteinase 14 directly mediates MICA shedding, and MMP-9 may be involved in MICA shedding in osteosarcoma.[7,26] In this study, MMP-9, MICA, and phospho-p38 were overexpressed in pituitary adenoma tissues, and the expression levels of MMP-9 and MICA were positively correlated with the expression level of phospho-p38. In addition, we also found that the expression levels of sMICA and MMP-9 were significantly greater in the sera of patients with pituitary adenomas. Therefore, we speculated that the p38/MAPK pathway may increase MICA expression and induce the expression of MMP-9. MMP-9 mediates MICA shedding in pituitary adenoma cells, and sMICA then causes a decrease in the cytotoxicity of NK lymphocytes, which promotes immune escape in pituitary adenoma (Figure 6).

Our research elucidated new phenomenologic observations that should be further validated at the cellular level in the future to provide mechanistic evidence for our hypothesis. In our further research, we plan to investigate cellular activity, cell cycle, and downstream protein expression by interfering with the expression of related proteins and genes.

CONCLUSIONS

In conclusion, activation of the p38/MAPK pathway may increase the expression of MICA and induce the expression of MMP-9. MMP-9 is involved in the shedding of sMICA from MICA to promote tumor immune escape. Furthermore, p38/MAPK could potentially represent a novel target for inhibiting tumor cell immune escape.

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