Int J Clin Exp Med 2016;9(2):3069-3075
www.ijcem.com /ISSN:1940-5901/IJCEM0015882

Original Article

Clinical significance of NGAL and MMP-9 protein expression in epithelial ovarian cancers

Jian Wu[1], An-Quan Shang[2], Wen-Ying Lu[2]

[1]*Department of Laboratory Medicine, Yancheng People's First Hospital, Yancheng 224005, Jiangsu Province, China;* [2]*Pathological Science Laboratory, The Sixth People's Hospital of Yancheng, Yancheng 224000, Jiangsu, China*

Received March 13, 2015; Accepted January 7, 2016; Epub February 15, 2016; Published February 29, 2016

Abstract: Objective: To investigate the significance of neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase-9 (MMP-9) protein expression in epithelial ovarian cancers. Methods: NGAL and MMP-9 serum and ovarian tissue levels were detected in 150 ovarian epithelial cancers. 42 benign ovarian tumors and 30 healthy women as controls by immunohistochemistry technique and reverse transcriptase-polymerase chain reaction (RT-PCR). Results: NGAL and MMP-9 expression in tissue and serum in ovarian cancer group were significantly higher than that in benign ovarian tumor group and normal control group (both $P<0.05$). Two high expression and clinical staging, lymph node metastasis positive correlation (both $P<0.05$). Moreover, in the serum and ovarian tissue of patients with epithelial ovarian cancer, the expression of NGAL had positive correlation with histological differentiation (both $P<0.05$) and the expression level of MMP-9 had negative correlation with histological differentiation ($P<0.05$). In patients with ovarian carcinoma tissue or serum, NGAL expression were positive expression of MMP-9 ($r=0.740$, $r=0.676$, both $P<0.05$). Conclusions: The high expression of NGAL and MMP-9 in epithelial ovarian cancer may be associated with the initiation and progression of epithelial ovarian cancer.

Keywords: Epithelial ovarian cancers, neutrophil gelatinase-associated lipocalin, matrix metalloproteinase-9

Introduction

The molecular biological characteristics of the latest to epithelial ovarian cancer (EOC) is a highly heterogeneous disease, its clinical characteristics is the disease starts without symptoms until advanced disease [1, 2], generally diagnosed. In the past few decades, mortality statistics has almost no change in patients with EOC. According to the latest cancer statistics, EOC (including the fallopian tube and primary peritoneal carcinoma) become the cancer of the female in western countries the fifth most common cause of death, the top four is lung cancer, breast cancer, colon cancer, pancreatic cancer.

As a new protein [3, 4], neutrophil gelatinase-associated lipocalin (NGAL) is found when Kjeldsen et al. study matrix metalloproteinase-9 in neutrophil in 1993. High expression of NGAL in inflammatory diseases and various tumor is cancer research field in recent years. As a secreted protein, relative molecular mass of NGAL was about 25000 bp. In the late stage of young and immature little synthesis in bone marrow neutrophil cells under physiological conditions. Matrix metalloproteinase-9 (MMP-9) is the Zn2+ dependent activation of proteolytic enzyme catalysis [5, 6].

The main degradation of type IV collagen and gelatin, which can promote the formation of malignant tumor growth and angiogenesis. MMP-9 can reduce the extracellular matrix and basement membrane and the influence of tissue remodeling, thereby promoting tumor invasion and metastasis.

In this experiment, detection the expression of NGAL and MMP-9 in ovarian cancer tissue and serum, which aim is to understand the relationship between the clinical and pathological features of ovarian cancer, and to explore the correlation between the expression of NGAL and MMP-9 in epithelial ovarian cancer.

Table 1. Primer sequences for RT-PCR analysis

Primers	Sense sequences	Antisense sequences	Product size
NGAL	5'-TCACCTCCCTCCTGTTTA-3'	5'-CTCCTTGGTTCTCCCGTA-3'	233 bp
MMP-9	5'-GCTCTTCCCTGGAGACCTGA-3'	5'-CTGCCTAACCCTGGACCT-3'	203 bp
β-action	5'-CTGGGACGACTGGAGAAAA-3'	5'-AAGGAAGGCTGGAAGAGTGC-3'	564 bp

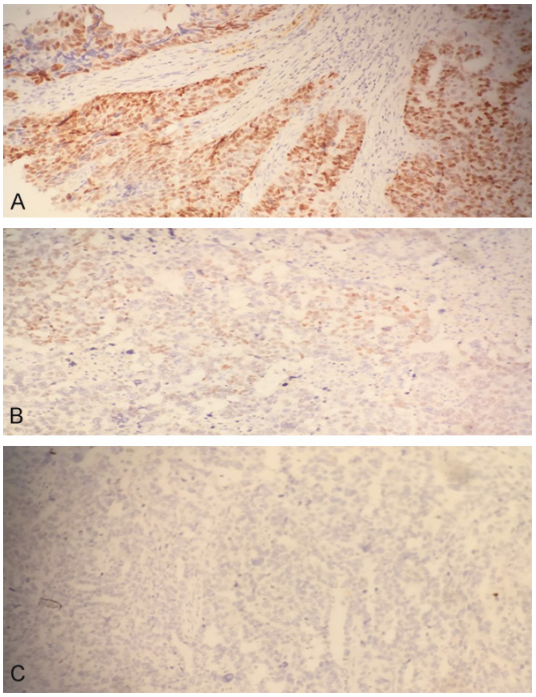

Figure 1. The staining result of EnVision immunohistochemistry for NGAL in ovary tissues and normal tissues. A: Staining strong positive result in ovary tissues; B: Staining positive result in ovary tissues; C: Staining negative result in normal tissues.

Patients and controls

From recently five years, samples of epithelial ovarian cancers were collected from 150 patients (93 cases of serous carcinoma and 57 cases of mucinous carcinoma) who received surgical resection in the First People's Hospital of Yancheng City (Yancheng, China) and had been diagnosed by pathological confirmation. There are 42 cases of benign ovarian epithelial tumors (30 patients with serous cystadenocarcinoma and 12 cases of mucinous cystadenocarcinoma). Each case had detailed clinical and pathological data and none received preoperative chemotherapy or radiotherapy. Cancer patients age 36 to 73 years (mean age 53.9±11.6 years). Select 18 cases in our hospital as control group with normal ovarian tissue resection of uterine fibroids surgery and blood specimens. Ages 35 to 69 years (mean age 49.5±10.4 years).

According to the International Federation of gynecology and obstetrics (FIGO) clinical staging criteria in 2000:51 cases of stage I-II, 99 cases of stage III-IV.

No statistically significant difference was detected in age between the two groups. All specimens were obtained under informed consent with approval by the Ethics Committee of our hospital (Identification No. HMU (Ethics) 20131103).

Immunohistochemical staining techniques

Immunohistochemical method to EnVision staining was used to detect the distribution of NGAL and MMP-9. Immunohistochemical procedures were performed strictly with the kit manual operation. The EnVision and DAB chromogenic reagent kit (Antibody Diagnostic Inc., USA) was used to immunohistochemical staining. All slice staining were operated under the same conditions, the tissue was sliced to 4 μm, dehydration, dewaxing and antigen repaired by using PH 6.0, 0.01 mol/L citric acid. Normal goat serum was dropped on the slice by incubating for 10 min at room temperature, then corresponding specific antibodies were dropped on the slice by incubating for 1.5 h at room temperature. It was washed with PBS for 3 min by 3 times. The second antibody was dropped on the slice by incubating for 30 min at room temperature. It was colored by DAB, nucleus was stained by hematoxylin, dehydrated by gradient ethanol, cleared by xylene, sealed by natural gum. Each batch dyeing all has positive control (with the known positive section reagent which was offered by reagent company) and negative control (the corresponding specific antibody was replaced by PBS).

The nucleus in yellow or tan reactant particles is positive. Four independent random were detected by optical microscope of Olympus (BH-2) at high magnification (×200). According to the positive staining degree and the percentage of tumor cells, the criteria for judgment was as follows. There is no express (-): Or a small amount of cell shading. Expression is less than

Table 2. The staining positive result of EnVision immunohistochemistry for NGAL in ovary, benign ovarian and normal tissue

Group	n	NGAL positive			Negative	Positive Rate (%)	Strong Positive Rate (%)
		+	2+	3+	(-)		
Normal	30	0	0	0	30	0	0
benign ovarian	42	6	4	2	30	28.6	11.9
ovary	150	30	45	55	20	86.7	36.7
P^* value					0.000		
$P^▲$ value					0.021		

Note: *The χ^2 test was used to compare staining positive result between normal and ovary tissues, P<0.05; ▲The χ^2 test was used to compare staining positive result between benign ovarian and ovary tissues, P<0.05.

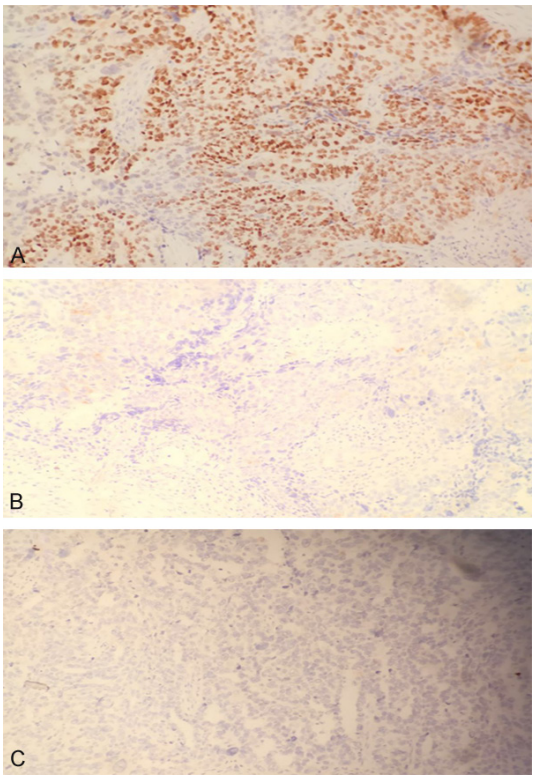

Figure 2. The staining result of EnVision immunohistochemistry for MMP-9 in ovary tissues and normal tissues. A: Staining strong positive result in ovary tissues; B: Staining positive result in ovary tissues; C: Staining negative result in normal tissues.

5%. Lower expression (+): Pale yellow or positive cells was 5% to 29%. Moderate expression (2+): Yellow or positive cells was 30% to 59%. High expression (3+): A tan or positive cells was more than 60%.

ELISA detection of serum NGAL and MMP-9

Extract of patient fasting venous blood 3 ml in the 3-4 days before operation. In a test tube without anticoagulant, standing for 30 minutes at room temperature. 4°C 3000 r/min rotating centrifugal for 15 minutes. Serum placed -80°C refrigerator. The intraoperative and cut a part of ovarian tissue into 8 degrees refrigerator rapidly within 0.5 hours, for the extraction of mRNA.

Detect the content of NGAL and MMP-9 in serum by ELISA. NGAL and MMP-9 kit was purchased from Wuhan Boster Company. 450 nm determination of A value with the DG5031 enzyme immunoassay instrument. Draw standard curve, and calculate the content of NGAL and MMP-9 in the sample.

Reverse transcriptase-polymerase chain reaction

Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to detect NGAL and MMP-9 expression. Total RNA was extracted from frozen tissue using Trizol reagent (KeyGEN). The total mRNA was reverse transcribed into cDNA by reverse transcriptase 8 mL. β-action as a quantitative control. The cDNA products were amplified by PCR. Taking the 5 mL PCR amplification product, the experiment was carried out in 2% agarose gel electrophoresis. Gel imaging system will gel bands imaged, and semi quantitative analysis was performed by software of quantity one. RT-PCR kit was purchased from Beijing Zhong Shan Jinqiao Company. NGAL and MMP-9 primers were purchased from Nanjing ginster. NGAL, MMP-9 and control primer sequences see **Table 1**.

Statistical methods

SPSS13.0 statistical software was used for statistical analysis. The χ^2 test was used to compare distribution of NGAL and MMP-9 between normal and cancer tissues, and spearman correlation was used to analyze the relationship of distribution among NGAL and MMP-9. P<0.05 was considered to be statistically significant.

Results

Cell nucleus dyeing distribution of NGAL and MMP-9 in ovary tissue and normal tissue

The positive rate of NGAL staining in ovary tissues 86.7% (130/150) is significantly higher than normal skin tissue and benign ovarian

Table 3. The staining positive result of EnVision immunohistochemistry for MMP-9 in ovary, benign ovarian and normal tissue

Group	n	MMP-9 positive			Negative	Positive rate (%)	Strong positive Rate (%)
		+	2+	3+	(-)		
Normal	30	0	0	0	30	0	0
Benign ovarian	42	3	2	1	36	14.3	2.3
Ovary	150	25	35	40	50	66.7	26.7
*P** value					0.219		
P▲ value					0.198		

Note: *The χ^2 test was used to compare staining positive result between normal and ovary tissues, P<0.05; ▲The χ^2 test was used to compare staining positive result between benign ovarian and ovary tissues, P<0.05.

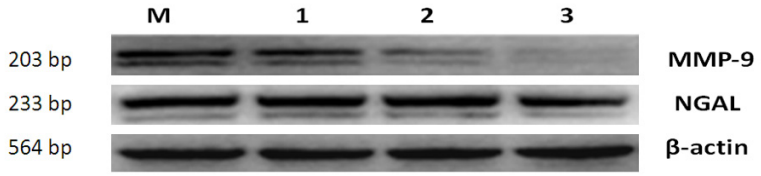

Figure 3. Electrophoresis results of expression of NGAL mRNA and MMP-9 mRNA in different ovarian tissues. M: Marker; 1: Normal group; 2: Ovarian benign tumor group; 3: Epithelial ovarian cancer group.

tumors (P<0.05; **Figure 1** and **Table 2**). And positive rate MMP-9 in ovary tissues was 66.7% (100/150), and there was significant difference in statistics than normal skin tissue and benign ovarian tumors (P<0.05; **Figure 2** and **Table 3**).

The expression of NGAL mRNA and MMP-9 mRNA in ovary tissue

NGAL and MMP-9 mRNA expression in epithelial ovarian cancer group were significantly higher than that in the normal group and ovarian benign tumor group (both P<0.05; **Figure 3**; **Table 4**). In ovarian cancer tissues, the expression of NGAL and MMP-9 mRNA increased with elevated clinical staging (both P<0.05). Lymph node metastasis group was significantly higher in no lymph node metastasis group (both P<0.05). The expression of NGAL mRNA in high, medium, low differentiation cancer reduce falls in turn (both P<0.05), while the expression of NGAL mRNA in high, medium, low differentiation cancer were enhanced in turn (both P<0.05; **Table 5**).

The expression of NGAL and MMP-9 in serum

NGAL and MMP-9 in the serum of patients with ovarian cancer were higher than those of normal group and benign ovarian tumors (both P<0.05; **Table 6**). In ovarian cancer patient serum, the NGAL and MMP-9 levels in stage III--IV were significantly higher than in stage I--II (both P<0.05). NGAL and MMP-9 in lymph node metastasis group was significantly higher than that in the group without lymph node metastasis (both P<0.05). The degree of differentiation with ovarian cancer. Tissue differentiation was positively correlated with the level of NGAL. While the degree of differentiation with ovarian cancer. Tissue differentiation was negatively correlated with the level of MMP-9. Both difference have statistical significance (both P<0.05; **Table 7**).

Analysis between the expression of NGAL mRNA in ovarian cancer tissues and the levels of NGAL in serum

The Pearson Correlation Analysis showed that the level of serum NGAL was positively correlated with the expression of NGAL mRNA in epithelial ovarian cancer tissues (r=0.762, P<0.05). The level of serum MMP-9 was positively correlated with the expression of MMP-9 mRNA in epithelial ovarian cancer tissues (r=0.601, P<0.05). Expression of NGAL mRNA correlated positively with the expression of MMP-9 mRNA in epithelial ovarian cancer (r=0.740, P<0.05). Level of serum NGAL correlated positively with the level of serum MMP-9 in epithelial ovarian cancer (r=0.676, P<0.05).

Discussion

NGAL is the one member of lipocalin family [7, 8]. Also the molecular weight of 25 KD. NGAL contains 178 amino acid residues and is located in human chromosome of 9q34. With the length for 5869 bp, NGAL 135 KD NGAL can not only through the two disulfide bonds and neutrophil MMP-9 is formed by the combination of molecular weight 135 KD heterologous dimerization of two, but also exist as monomers, dimers and other forms of homologous two. The high expression of NGAL is in a variety of tumors, such as breast cancer, rectal cancer, colon cancer, gastric cancer [9-11]. It was suggested that NGAL may be a novel human oncogene.

Table 4. The expression of NGAL and MMP-9 mRNA in different ovarian tissue

Group	n	NGAL	P	MMP-9	P
Normal group	30	0.121±0.037	0.017[a]	0.111±0.042	0.021[a]
Ovarian benign tumor group	42	0.442±0.155	0.019[b]	0.312±0.064	0.015[b]
Epithelial ovarian cancer group	150	0.747±0.146	0.008[c]	0.625±0.144	0.002[c]

a: Normal group vs ovarian benign tumor group; b: Ovarian benign tumor group vs epithelial ovarian cancer group; c: Normal group vs epithelial ovarian cancer group.

Table 5. The relationship between NGAL and MMP-9 mRNA expression and clinical pathological characteristics of ovarian cancer

Clinical features	n	NGAL	P	MMP-9	P
The degree of differentiation					
High differentiation (G1)	39	0.824±0.065	0.012[a]	0.505±0.142	0.014[a]
Middle differentiation (G2)	66	0.770±0.064	0.007[b]	0.635±0.111	0.022[b]
Low differentiation (G3)	45	0.620±0.221	0.004[c]	0.715±0.120	0.000[c]
Staging					
I–II	51	0.684±0.179	0.009	0.528±0.136	0.000
III–IV	99	0.779±0.116		0.676±0.124	
Lymph node metastasis					
NO	69	0.678±0.179	0.032	0.539±0.132	0.002
YES	81	0.806±0.073		0.699±0.109	

a: High differentiation vs Middle differentiation; b: Middle differentiation vs low differentiation; c: High differentiation vs low differentiation.

mRNA is closely related to ovarian cancer differentiation degree. The higher the degree of differentiation, the expression of NGAL mRNA is high. From this we can infer that, high expression of NGAL cells in high differentiation of ovarian cancer related to abnormal differentiation characteristics. The abnormal expression of NGAL mRNA may be the molecular basis of differentiation form in ovarian carcinoma tissue.

Proved by experiments, using NGAL gene as a target, the design of small interfering RNA, downregulation of NGAL gene expression by gene silencing, observed cell apoptosis significantly increased tumor cell proliferation significantly reduce. Suggesting that NGAL could be blocked by specific apoptosis inducing tumor cell apoptosis factor induced, thus protecting the survival of tumor cells [12, 13]. In vitro, NGAL can activate ERalpha/Slug pathway, induced by mesenchymal-epithelial transition. You can also recombine cancer cell skeleton by ectopic E-cadherin, Rac1 and catenins, so that the adhesion decrease between cells and matrix, so as to improve the invasive ability of tumor cells [14, 15].

In this study, the expression of NGAL mRNA in ovarian cancer tissue was significantly higher than that in normal ovarian tissue and ovarian benign tumors. NGAL mRNA expression in ovarian cancer tissue increased with elevated clinical staging. Lymph node metastasis group was significantly higher than that in the group without lymph node metastasis. These differences indicate that NGAL play some role in the formation, invasion and metastasis of the ovarian cancer. In addition, the expression of NGAL

MMP-9 is the Zn2+ dependent activation of proteolytic enzyme catalysis. MMP-9 main degradation of type IV collagen and gelatin, and thus promote the malignant tumor growth and angiogenesis. The study found that the expression of MMP-9 mRNA in ovarian cancer tissues is increased, which suggesting that MMP-9 may play a certain role in the carcinogenesis of ovarian tissue. Expression of MMP-9 mRNA was markedly increased in ovarian carcinoma. The expression levels during III-IV period in epithelial ovarian carcinoma was significantly higher than that I-II period, which suggesting that MMP-9 may play a certain role in the invasion, metastasis and evolution of ovarian tissue. The level of serum MMP-9 in ovarian cancer has higher degree with clinical staging, pathological grading and lymph node metastasis in the data organization. Therefore, MMP-9 has important significance in judging the malignant degree of ovarian cancer invasion and metastasis, and prognosis estimation.

The study by Nuntago [16] et al. found that NGAL through the formation of complexes with MMP-9, stable MMP-9 activity, reduce the degradation, and promote bile duct cancer cell invasion. NGAL/MMP-9 complex in the serum

Table 6. The level of NGAL and MMP-9 in different serum of the patients

Group	n	NGAL	P	MMP-9	P
Normal group	30	17.787±2.655	0.024[a]	24.176±3.405	0.017[a]
Ovarian benign tumor group	42	31.944±4.190	0.031[b]	35.321±8.759	0.019[b]
Epithelial ovarian cancer group	150	44.799±8.723	0.003[c]	39.925±7.455	0.008[c]

a: Normal group vs ovarian benign tumor group; b: Ovarian benign tumor group vs epithelial ovarian cancer group; c: Normal group vs epithelial ovarian cancer group.

Table 7. The relationship between NGAL and MMP-9 level and clinical pathological characteristics of ovarian cancer (ng/ml)

Clinical features	n	NGAL	P	MMP-9	P
The degree of differentiation					
High differentiation (G1)	39	48.535±8.520	0.010[a]	37.625±7.197	0.017[a]
Middle differentiation (G2)	66	44.907±9.611	0.035[b]	38.639±7.748	0.019[b]
Low differentiation (G3)	45	40.319±5.125	0.004[c]	43.808±6.033	0.013[c]
Staging					
I–II	51	40.434±7.642	0.010	36.834±6.988	0.034
III–IV	99	47.048±8.484		41.519±7.279	
Lymph node metastasis					
NO	69	39.985±7.110	0.004	36.799±6.9761	0.032
YES	81	47.280±8.523		41.537±7.273	

a: High differentiation vs Middle differentiation; b: Middle differentiation vs low differentiation; c: High differentiation vs low differentiation.

of clear cell renal cell carcinoma has high expression, and also the patients has poor prognosis. Compared with low expression group, the total survival rate was decreased obviously [17]. In this experiment, the expression of NGAL and MMP-9 in ovarian cancer tissue and serum has the positive correlation. It can be inferred that both of them may have synergistic effects in the occurrence and development of ovarian cancer. However, NGAL in serum and tissue has positive correlation to the degree of differentiation in ovarian cancer, while MMP-9 was negatively correlated with the extent of tissue differentiation. Therefore, both the mechanism of action are still needing further research [18, 19].

Levels of NGAL and MMP-9 in the ovarian cancer tissues and serum were significantly higher than that of other research group. Between the NGAL and MMP-9 relationship with clinical pathological features and RT-PCR findings are consistent. The level of NGAL in serum is related to NGAL mRNA in ovarian cancer tissue, and the level of MMP-9 in serum is related to MMP-9 mRNA in ovarian cancer tissue. It can be inferred that, MMP-9 and NGAL elevated in serum may partly derived from cancer tissue. Therefore, monitoring of NGAL and MMP-9 can serve as an important index to judge the prognosis of patients with ovarian cancer before operation [20-22]. To explore the combined detection of NGAL and MMP-9 serum levels, the aim is to make it clear to be used as a diagnostic index used for play a certain clinical value in early diagnosis of ovarian cancer, which is needed to further research.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wen-Ying Lu, Pathological Science Laboratory, The Sixth People's Hospital of Yancheng, Yancheng 224500, Jiangsu, China. Tel: +86-515-88508709; E-mail: piaoxue1982717@sina.com

References

[1] Mezzanzanica D. Ovarian cancer: a molecularly insidious disease. Chin J Cancer 2015; 34: 1-3.

[2] Kim Y, Guntupalli SR, Lee SJ, Behbakht K, Theodorescu D, Lee JK, Diamond JR. Retrospec-

tive analysis of survival improvement by molecular biomarker-based personalized chemotherapy for recurrent ovarian cancer. PLoS One 2014; 9: e86532.
[3] Bolignano D, Donato V, Lacquaniti A, Fazio MR, Bono C, Coppolino G, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL) in human neoplasias: a new protein enters the scene. Cancer Lett 2010; 288: 10-6.
[4] Chae H, Ryu H, Cha K, Kim M, Kim Y, Min CK. Neutrophil gelatinase-associated lipocalin as a biomarker of renal impairment in patients with multiple myeloma. Clin Lymphoma Myeloma Leuk 2015; 15: 35-40.
[5] Akter H, Park M, Kwon OS, Song EJ, Park WS, Kang MJ. Activation of matrix metalloproteinase-9 (MMP-9) by neurotensin promotes cell invasion and migration through ERK pathway in gastric cancer. Tumour Biol 2015; 36: 6053-62.
[6] Tang D, Piao Y, Zhao S, Mu X, Li S, Ma W, Song Y, Wang J, Zhao W, Zhang Q. Expression and correlation of matrix metalloproteinase-9 and heparanase in patients with breast cancer. Med Oncol 2014; 31: 26.
[7] Wu BL, Li CQ, Du ZP. Functional analysis of the mRNA profile of neutrophil gelatinase-associated lipocalin overexpression in esophageal squamous cell carcinoma using multiple bioinformatic tools. Mol Med Rep 2014; 10: 1800-12.
[8] Kos FT, Sendur MA, Aksoy S. Evaluation of renal function using the level of neutrophil gelatinase-associated lipocalin is not predictive of nephrotoxicity associated with cisplatin-based chemotherapy. Asian Pac J Cancer Prev 2013; 14: 1111-4.
[9] Shimura T, Dagher A, Sachdev M, Ebi M, Yamada T, Yamada T, Joh T, Moses MA. Urinary ADAM12 and MMP-9/NGAL Complex Detect the Presence of Gastric Cancer. Cancer Prev Res (Phila) 2015; 8: 240-8.
[10] Peres LA, da Cunha AD Jr, Assumpção RA. Evaluation of the cisplatin nephrotoxicity using the urinary neutrophil gelatinase-associated lipocalin (NGAL) in patients with head and neck cancer. J Bras Nefrol 2014; 36: 280-8.
[11] Duvillard L, Ortega-Deballon P, Bourredjem A. A case-control study of pre-operative levels of serum neutrophil gelatinase-associated lipocalin and other potential inflammatory markers in colorectal cancer. BMC Cancer 2014; 14: 912.
[12] Ding L, Zhang X, Zhang Y. Clinical significance of the detection of serum neutrophil gelatinase-associated lipocalin in human colorectal cancer. Zhonghua Wei Chang Wai Ke Za Zhi 2014; 17: 589-93.
[13] Lippi G, Meschi T, Nouvenne A, Mattiuzzi C, Borghi L. Neutrophil gelatinase-associated lipocalin in cancer. Adv Clin Chem 2014; 64: 179-219.
[14] Volpe V, Raia Z, Sanguigno L, Somma D, Mastrovito P, Moscato F, Mellone S, Leonardi A, Pacifico F. NGAL controls the metastatic potential of anaplastic thyroid carcinoma cells. J Clin Endocrinol Metab 2013; 98: 228-35.
[15] Barresi V, Leni A, Tuccari G. Neutrophil gelatinase-associated lipocalin (NGAL) immunohistochemical expression in follicular cell-derived thyroid tumors: a novel diagnostic tool? Histol Histopathol 2012; 27: 329-36.
[16] Nuntagowat C, Leelawat K, Tohtong R. NGAL knockdown by siRNA in humancholangiocarcinoma cells suppressed invasion by reducing NGAL/MMP-9 complex formation. Clin Exp Metastasis 2010; 27: 295-305.
[17] Perrin C, Patard JJ, Jouan F, Collet N, Théoleyre S, Edeline J, Zerrouki S, Laguerre B, Bellaud-Roturaud MA, Rioux-Leclercq N, Vigneau C. The neutrophil gelatinase-associated lipocalin, or LCN 2, marker of aggressiveness in clear cell renal cell carcinoma. Prog Urol 2011; 21: 851-858.
[18] Shimura T, Dagher A, Sachdev M, Ebi M, Yamada T, Yamada T, Joh T, Moses MA. Urinary ADAM12 and MMP-9/NGAL Complex Detect the Presence of Gastric Cancer. Cancer Prev Res (Phila) 2015; 8: 240-8.
[19] Ruiz-Morales JM, Dorantes-Heredia R, Arrieta O, Chávez-Tapia NC, Motola-Kuba D. Neutrophil gelatinase-associated lipocalin (NGAL) and matrix metalloproteinase-9 (MMP-9) prognostic value in lung adenocarcinoma. Tumour Biol 2015; 36: 3601-10.
[20] Odabasi M, Yesil A, Ozkara S, Paker N, Ozkan S, Eris C, Yildiz MK, Abuoglu HH, Gunay E, Tekeşin K. Role of human neutrophil gelatinase associated lipocalin (NGAL) and Matrix Metalloproteinase-9 (MMP-9) overexpression in neoplastic colon polyps. Int J Clin Exp Med 2014; 7: 2804-11.
[21] Gilet A, Zou F, Boumenir M, Frippiat JP, Thornton SN, Lacolley P, Ropars A. Aldosterone upregulates MMP-9 and MMP-9/NGAL expression in human neutrophils through p38, ERK1/2 and PI3K pathways. Exp Cell Res 2015; 331: 152-63.
[22] Surlin P, Silosi I, Rauten AM, Cojocaru M, Foia L. Involvement of TSP1 and MMP9/NGAL in angiogenesis during orthodontic periodontal remodeling. ScientificWorldJournal 2014; 2014: 421029.