MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 4738-4744
DOI: 10.12659/MSM.908812

Received: 2018.01.03
Accepted: 2018.01.19
Published: 2018.07.09

Correlation of MACC1/c-Myc Expression in Endometrial Carcinoma with Clinical/Pathological Features or Prognosis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BEG 1 Qinghua Zhang*
BDG 2 Ping Xu*
AEF 3 Yanxia Lu
ACD 1 Hongtao Dou

1 Department of Gynecology, Central Hospital of Zibo in Shandong, Zibo, Shandong, P.R. China
2 Department of Gynecology, People's Hospital of ZhangQiu in Shandong Province, Zhangqiu, Shandong, P.R. China
3 Department of Gynecology, Third Ward, People's Hospital of Linyi City, Linyi, Shandong, P.R. Chin

* These authors contributed equally to this work

Corresponding Author: Hongtao Dou, e-mail: douhongtaohtg@sina.com
Source of support: Departmental sources

Background: Endometrial carcinoma (EC) is a type of female reproductive malignant tumor, the incidence of which is generally 20~30%. Multiple factors and genes are involved in the regulation of EC occurrence and progression. This study aimed to measure the expressions of MACC1 and c-Myc in EC patients to analyze their correlation with pathological features of EC.

Material/Methods: A total of 60 EC patients were recruited in the experimental group, while another cohort of 30 people with endometrial inflammatory hyperplasia was enrolled in the control group. The levels of serum MACC1 and c-Myc were measured by ELISA, and the protein expressions in EC cancer tissues, tumor-adjacent tissues, and controlled endometrial tissues were detected by immunohistochemistry (IHC). The correlation between gene expression and clinical/pathological features was then determined.

Results: Our data indicate that the level of serum MACC1 and c-Myc in the experimental group was 1.67±0.08 ng/ml and 1.78±0.07 ng/ml, respectively, both of which were significantly higher than that of the control group ($p<0.05$). However, no significant difference was found among levels of serum MACC1 or c-Myc at different TNM stages ($p>0.05$). In cancer tissues, the positive rate of MACC1 or c-Myc was 73.3% and 78.3%, respectively, which were significantly higher than that in adjacent or control tissues ($p<0.05$). MACC1/c-Myc expression was correlated with TNM stage, primary infiltration grade, lymph node metastasis, and distal metastasis ($p<0.05$).

Conclusions: MACC1 and c-Myc are highly expressed in serum and tumor tissues of EC patients. Both are correlated with TNM stage, primary infiltration, and lymph node or distal metastasis, which provides a scientific basis for the development of new biomarkers for the diagnosis of endometrial carcinoma.

MeSH Keywords: Endometrial Neoplasms • Fractures, Spontaneous • Genes, myc

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/908812

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Endometrial carcinoma (EC) is a type of cancer that begins in the uterus. In recent years, factors such as life-style, diet, and hormone therapy have been reported to increase EC incidence, especially in younger patients [1]. The continuous development of medical science has produced major progress in screening, diagnosis, and treatment of EC. For EC patients, however, survival time after treatment and quality of life have not significantly improved. In addition, details of pathogenesis mechanism of EC have not been fully defined. Accumulating evidence shows that oncogenes and tumor-suppressor genes may disrupt normal cellular metabolism via chromosomal translocation, rearrangement, gene amplification, and gene mutation, eventually leading to malignant transformation [2–4]. Previous findings indicated the correlation between EC occurrence and oncogene activation or inactivation/deficiency of tumor-suppressor genes. As early as 2009, Stein et al. identified the MACC1 gene, which is correlated with the occurrence and metastasis of colon cancer. It locates on human chromosome 7p21.1, and encodes 1 mRNA with 2559 nucleic acids and 1 protein containing 852 amino acids. This protein consists of ZU5 structural domain, SH3 structural domain, proline proficient module, and death structural domain. Among those domains, ZU5 induces the interaction between proteins and participates in the regulation of various signal transduction pathways [5]. Expression of the c-Myc gene contributes to DNA activity in the form of dimers. After specific binding with DNA, it can regulate multiple gene expressions [6]. It has been demonstrated that the expression of oncogene c-Myc in various human malignant tumors was altered [7]. At present, understanding of the expression profile and clinical implication of MACC1 or c-Myc in EC is incomplete. This study thus assessed the levels of MACC1 and c-Myc in EC patients and their correlation with clinical or pathological features of EC.

Material and Methods

General information

A total of 60 EC patients who had surgery in the Central hospital of Zibo In Shandong from January 2015 to June 2016 were recruited. Patients were 35–70 years old (average age=49.5±4.8 years), with 12, 24, 12, and 12 cases at stage I, II, III, and IV, respectively. All patients had confirmed diagnosis. Tumor-adjacent tissues were removed during surgery. Another cohort of 30 patients age 35–68 years (average age=48.8±4.0 years) with inflammatory endometrial hyperplasia was recruited from our hospital as the control group. Patients groups were not significantly different in age or sex and were thus comparable ($p>0.05$).

The study protocol was approved by the Research Ethics Committee of Central Hospital of Zibo in Shandong, and all patients gave informed consent before study commencement.

Experimental reagents and equipment

MACC1, c-Myc ELISA kits, blocking reagent, primary antibody for MACC1 and c-Myc, rabbit anti-mouse secondary antibody, and DAB substrate kits were all purchased from Shanfeng Chem (China). The microplate reader was from TECNA UK), the dehydration chamber was from TIYODA (Japan), and the microtome was from Leica (Germany).

ELISA for serum MACC1 and c-Myc contents in patients

Fasting venous blood samples were collected from all patients, which were then centrifuged for the collection of supernatant. ELISA was used to measure serum MACC1 and c-Myc contents. The ELISA kit was pre-warmed to room temperature for 30 min. Standard samples were diluted serially. At each concentration, 5 replicated wells were set for adding samples, rising, development, and quenching. A microplate reader was used to measure absorbance value at 450 nm wave length. A linear regression function was plotted to calculate sample concentration.

Immunohistochemistry staining for tissue expression of MACC1 and c-Myc

Tissue samples were fixed in formalin and embedded in paraffin. Tissue blocks were sectioned into 3-μm slices on a microtome. Tissue slices were mounted onto a slide and dried at 60°C overnight. Tissue slices were de-waxed in xylene and rinsed in gradient ethanol. After heated antigen retrieval, tissue slices were rinsed under tap water and incubated with H_2O_2. Blocking agent was then added, followed by primary antibody (1: 100 dilution) for 1-h incubation. Secondary antibody (1: 100 dilution) was then added after rinsing, followed by DAB development and mounting on a coverslip. Positive staining was identified [8] as dark yellow granules in membrane, cytoplasm, and nucleus as: negative (–) ≤10%; weakly positive (+) 11~25%; positive (++) 26~50%; and strongly positive (+++) >50%.

Data processing

SPSS17.0 statistical software was used for data processing. Enumeration data were analyzed by chi-square test, while measurement data were analyzed by analysis of variance (ANOVA) and are presented as mean ± standard deviation (SD). Logistic regression was used for multi-factorial analysis. Statistical significance was defined as $p<0.05$.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Serum MACC1 and c-myc levels in patient serum.

Group	N	MACC1 (ng/ml)	C-myc (ng/ml)
Experimental group	60		
Stage I		1.62±0.02*	1.77±0.06*
Stage II		1.65±0.03*	1.78±0.05*
Stage III		1.67±0.03*	1.82±0.07*
Stage IV		1.73±0.01*	1.85±0.01*
Control group	30	0.03±0.01	0.05±0.01

* p<0.05 compared to control group.

Table 2. MACC1 expression in patient tissues.

Group	N	MACC1 expression level			Positive rate (%)
		–	+-++	+++	
Cancer tissue in patients	60	16	33	11	73.3*#
Tumor adjacent tissues	60	56	4	0	15
Control group	30	27	3	0	10

* p<0.05 compared to adjacent tissues; # p<0.05 compared to control group.

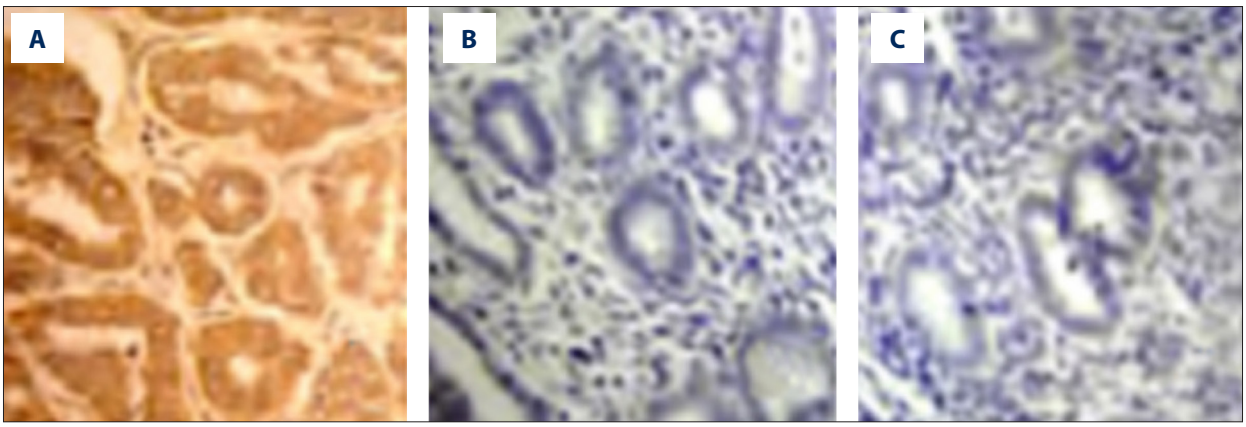

Figure 1. MACC1 expression in patient tissues (×400). (**A**) MACC1 expression (+++) in cancer tissues from experimental group; (**B**) MACC1 expression (–) in tumor-adjacent tissues; (**C**) MACC1 expression (–) in control group.

Results

Serum MACC1 and c-Myc levels in patients

Peripheral venous blood samples from EC patient were collected, centrifuged, and the sera were collected for further testing. Results showed the level of MACC1 or c-Myc reached 1.67±0.08 ng/ml and 1.78±0.07 ng/ml, respectively, in experimental group (p<0.05 compared to control group). No significant change in MACC1 or c-Myc level was observed at different TNM stages of the experimental group (p>0.05, Table 1).

MACC1 protein expression in patient tissues

The expression of MACC1 protein in EC tumor tissues, adjacent tissues, and control endometrial tissues were tested. Our data showed that in the experimental group, 11 cases presented strongly positive (+++) expression of MACC1 in cancer tissues, with 33 cases of positive expression (++), and the overall positive rate was 73.3%, which was significantly higher than that of tumor-adjacent or control tissues (p<0.05, Table 2, Figure 1).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. C-myc protein expression in patient tissues.

Group	N	C-myc expression level			Positive rate (%)
		–	+-++	+++	
Cancer tissue in patients	60	13	37	10	78.3*#
Tumor adjacent tissues	60	52	8	0	13.3
Control group	30	54	6	0	10

* p<0.05 compared to adjacent tissues; # p<0.05 compared to control group.

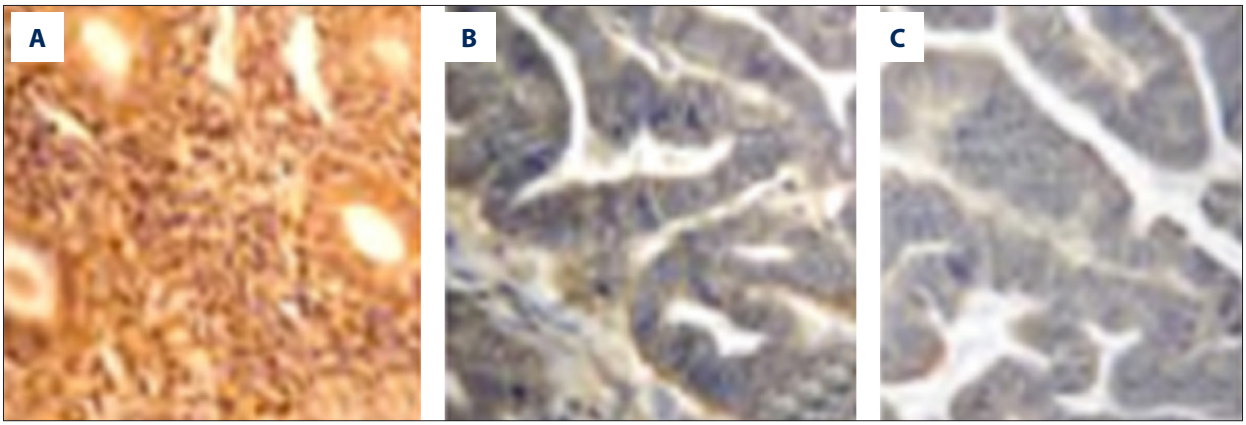

Figure 2. C-Myc expression in patient tissues (×400). (**A**) C-Myc expression (+++) in cancer tissues from experimental group; (**B**) C-Myc expression (–) in tumor-adjacent tissues; (**C**) C-Myc expression (–) in control group.

C-Myc protein expression in patient tissues

To study the difference in c-Myc protein expression in EC tumor tissues, adjacent tissues, and control endometrial tissues, we performed IHC staining and found a 78.3% positive rate of c-Myc in cancer tissues from the experimental group, which were significantly higher than that from the tumor-adjacent and control groups (p<0.05, Table 3, Figure 2).

Correlation between MACC1/c-Myc expression and clinical/pathological features

We further analyzed the correlation between MACC1/c-Myc expression and the clinical relative factors, including EC patient age, pathological grade, clinical stage, histology subtype, differentiation grade, vascular infiltration, and lymph node metastasis. Of note, results showed that MACC1/c-Myc expression level was correlated with TNM stage, primary lesion infiltration level, lymph node metastasis, and distal metastasis (p<0.05). For those EC patients with advanced TNM stage, multiple primary lesions, advanced primary lesion infiltration, lymph node, and distal metastasis, the MACC1/c-Myc level was significantly elevated (p<0.05, Table 4).

Multi-variant analysis of MACC1/c-Myc expression and clinical/pathological features of endometrium

The data of logistic multi-variant analysis showed that TNM stage, primary lesion infiltration, lymph node metastasis, and distal metastasis were all risk factors affecting MACC1/c-Myc expression and causing EC occurrence and progression (p<0.05, Table 5).

Discussion

EC, which is a type of female reproductive malignant tumor, generally occurs in post-menstrual women, and only 10% of patients are under 40 years old [9]. A clinical survey for endometrial proliferation showed about 1~3% cancer transformation rate in those with pure endometrial proliferation, while the cancer rates were 3~4% and 23% for patients with complex proliferation of endometrium and atypical endometrial proliferation, respectively [10]. C-Myc protein represents one of the most important cytokines involved in cell cycle modulation. Its over-expression may lead to abnormal mitosis, accelerated proliferation, and transition of resting phase to mitosis phase in cell cycle, which thereby facilitates cell proliferation and growth [11–13]. Previous studies also indicated MACC1 over-expression appeared in multiple types of malignant tumor

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 4. Correlation between MACC1/c-myc expression and pathological features.

Index	N	MACC1	C-myc
Age			
<45	27	21 (77.8)	23 (85.2)
≥45	33	22 (66.7)	22 (66.7)
P value		>0.05	>0.05
Pathological grade			
G1	10	5 (50)	5 (50)
G2	12	8 (66.7)	7 (58.3)
G3	38	30 (78.9)	33 (86.8)
P value		P<0.05	P<0.05
Clinical stage			
Ia	11	4 (36.4)	4 (36.4)
Ib	17	9 (52.9)	10 (58.8)
IIa	18	16 (88.9)	17 (94.4)
IIb	14	14 (100)	14 (100)
P value		P<0.05	P<0.05
Histology type			
Squamous	43	31 (72.1)	29 (67.4)
Adenoma	17	12 (70.6)	16 (94.1)
P value		>0.05	>0.05
Differentiation			
Low	23	22 (95.7)	22 (95.7)
Moderate to high	37	21 (56.8)	23 (62.2)
P value		P<0.05	P<0.05
Vascular infitratoin			
Yes	16	16 (100)	15 (93.8)
No	44	27 (61.4)	30 (68.2)
P value		P<0.05	P<0.05
Lymph node metastasis			
Yes	10	10 (100)	10 (100)
No	50	33 (66)	35 (70)
P value		P<0.05	P<0.05

tissues, including colon cancer, pancreatic carcinoma, liver cancer, breast carcinoma, lung cancer, prostate carcinoma, and brain glioma [14–16]. The present study thus assessed the levels of MACC1 and c-Myc in EC patients and analyzed their role in the clinical changes of EC.

We found over-expression of MACC1 and c-Myc in both serum and tumor tissues of EC patients, and we found significant differences in serum MACC1 and c-Myc levels of EC patients with different TNM stages. A previous study measured MACC1 protein and mRNA expression in normal endometrial tissues, pure hyperplasia endometrium, atypical hyperplasia endometrium, and EC tissues, and demonstrated the involvement of MACC1 in EC pathogenesis and progression [17]. Some reports showed that activation of c-Myc gene in malignant tumors caused over-expression of c-Myc proteins [18]. Bai et al.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 5. Correlation between MACC1/c-myc expression and clinical/pathological features of EC.

Parameter	MACC1			C-myc		
	Regression coefficient	P value	Relative risk	Regression coefficient	P value	Relative risk
TNM stage	0.724	0.003	2.125	0.697	0.003	2.015
Primary infiltration	1.132	0.002	2.027	1.233	0.002	2.325
Lymph node metastasis	1.113	0.002	2.892	1.015	0.001	2.457
Distal metastasis	0.748	0.002	2.156	0.878	0.002	2.054

measured P62 protein, which is the product of c-Myc, in proliferating endometrium, endometrial hyperplasia, and EC tissues, and found the highest level of P62 protein expression in EC tissues [19], consistent with our results.

In our study of the correlation between MACC1/c-Myc expression and clinical/pathological features, including age, pathological grade, clinical stage, histology type, differentiation grade, vascular infiltration, and lymph node metastasis, we found that MACC1/c-Myc expression level was correlated with TNM stage, primary lesion infiltration grade, lymph node metastasis, and distal metastasis. TNM stage, primary lesion infiltration, lymph node metastasis, and distal stasis were all risk factors for changed MACC1/c-Myc expression and EC pathogenesis/occurrence. Regarding the MACC1 functional mechanism, a basic study indicated that the major mechanism of MACC1 may involve abnormal activation of the HGF/c-Met signal transduction pathway. In the cell nucleus, MACC1 binds with c-Met promoter to regulate its activity, leading to the over-expression of c-Met protein. The over-reaction of cells towards HGF facilitates MACC1 translocation from cytoplasm to nucleus. Such a repeated cycle eventually leads to EC occurrence and progression [20]. For c-Myc, its functional mechanism mainly depends on the induction of mitosis and cell cycle growth response. The co-activation of c-Myc and cyclin E can regulate cell cycle, potentiate hyperplasia of atypically proliferated endometrium, and elevate estrogen receptor level in endometrium as well as the c-Myc transcription, eventually leading to gene amplification and over-proliferation of endometrial cell proliferation and EC occurrence [21]. Further research using targeted animal models based on clinical evidence is required for the validation of these results. Moreover, a large-scale study of MACC1 and c-Myc in EC patients is needed to assess the dynamic expressions of MACC1 and c-Myc should be monitored and compared before and after treatment, especially for those patients receiving surgery or chemo-/radio-therapy, in order to quickly evaluate the impact of their dynamic change on clinical symptoms and pathological features, treatment efficiency, and prognosis.

Conclusions

In EC patient serum and cancer tissues, both MACC1 and c-Myc are highly expressed, and their levels are correlated with TNM stage, primary lesion infiltration grade, lymph node metastasis, and distal metastasis. Our results may provide a novel strategy for EC diagnosis and treatment, although details of the mechanism require further investigation.

Conflict of interest

None.

References:

1. Amadio G, Masciullo V, Ferrandina MG, Scambia G: Emerging drugs for endometrial cancer. Expert Opin Emerg Drugs, 2014; 19: 497–509
2. Galimi F, Torti D, Sassi F et al: Genetic and expression analysis of MET, MACC1, and HGF in metastatic colorectal cancer: Response to met inhibition in patient xenografts and pathologic correlations. Clin Cancer Res, 2011; 17: 3146–56
3. Li M, Xin X, Wu T et al: Stromal cells of endometrial carcinoma promotes proliferation of epithelial cells through the HGF/c-Met/Akt signaling pathway. Tumour Biol, 2015; 36: 6239–48
4. Harpaz N, Taboada S, Ko HM et al: Expression of MACC1 and MET in inflammatory bowel disease-associated colonic neoplasia. Inflamm Bowel Dis, 2014; 20: 703–11
5. Stein U, Dahlmann M, Walther W: MACC1 – more than metastasis? Facts and predictions about a novel gene. J Mol Med (Berl), 2010; 88: 11–18
6. Santoni-Rugiu E, Falck J, Mailand N et al: Involvement of Myc activity in a G(1)/S-promoting mechanism parallel to the pRb/E2F pathway. Mol Cell Biol, 2000; 20: 3497–509
7. Yang W, Shen J, Wu M et al: Repression of transcription of the p27(Kip1) cyclin-dependent kinase inhibitor gene by c-Myc. Oncogene, 2001; 20: 1688–702
8. Yang YP, Qu JH, Chang XJ et al: High intratumoral metastasis-associated in colon cancer-1 expression predicts poor outcomes of cryoablation therapy for advanced hepatocellular carcinoma. J Transl Med, 2013; 11: 41
9. de Sanjose S, Bruni L, Alemany L: HPV in genital cancers (at the exception of cervical cancer) and anal cancers. Presse Med, 2014; 43: e423–28

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

10. Nelson EJ, Hughes J, Kulasingam SL: Spatial patterns of human papillomavirus-associated cancers within the state of Minnesota, 1998–2007. Spat Spatiotemporal Epidemiol, 2014; 9: 13–21
11. Stein U, Walther W, Arlt F et al: MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. Nat Med, 2009; 15: 59–67
12. Teri A, Richard L: Endomet rial hyperplasia, metaplasia and carcinoma. In: Fox H (ed.), Obstetrical and Gynaecological Pathology. Churchill Livingst, New York, 1995
13. Guo P, Dong XY, Zhao KW et al: Estrogen-induced interaction between KLF5 and estrogen receptor (ER) suppresses the function of ER in ER-positive breast cancer cells. Int J Cancer, 2010; 126: 81–89
14. Sirma H, Kumar M, Meena JK et al: The promoter of human telomerase reverse transcriptase is activated during liver regeneration and hepatocyte proliferation. Gastroenterology, 2011; 141: 326–37, 337.e1–3
15. Zhang R, Shi H, Chen Z et al: Effects of metastasis-associated in colon cancer 1 inhibition by small hairpin RNA on ovarian carcinoma OVCAR-3 cells. J Exp Clin Cancer Res, 2011; 30: 83
16. Ma J, Ma J, Meng Q et al: Prognostic value and clinical pathology of MACC-1 and c-MET expression in gastric carcinoma. Pathol Oncol Res, 2013; 19: 821–32
17. Ren SM, Shi HR, Zhang RT: MACC1, HGF and Cmet expression and its significance in endometrial carcinoma. Chinese Journal of Practical Gynecology and Obstetrics, 2013; 29: 274–78
18. Zhang RT, Shi HR, Huang HL: MACC1, HGF and Cmet protein expression and significance in epithelial ovarian cancer. Journal of Southern Medical University, 2011; 31: 1551–55
19. Bai MK, Costopoulos JS, Christoforidou BP, Papadimitriou CS: Immunohistochemical detection of the c-Myc oncogene product in normal, hyperplastic and carcinomatous endometrium. Oncology, 1994; 51: 314–19
20. Rolle CE, Kanteti R, Surati M et al: Combined MET inhibition and topoisomerase I inhibition block cell growth of small cell lung cancer. Mol Cancer Ther, 2014; 13: 576–84
21. Cheng PC, Chang HK, Chen SH: Quantitative nanoproteomics for protein complexes (QNanoPX) related to estrogen transcriptional action. Mol Cell Proteomics, 2010; 9: 209–24

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]