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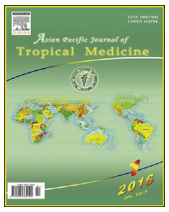

Original research http://dx.doi.org/10.1016/j.apjtm.2015.12.014

Expression of TRAP1 in gastric cancer tissue and its correlation with malignant biology

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ARTICLE INFO

Article history:
Received 15 Oct 2015
Received in revised form 20 Nov 2015
Accepted 3 Dec 2015
Available online 19 Dec 2015

Keywords:
Gastric cancer
TNF receptor-associated protein 1
Malignant biology
Proliferation
Invasion

ABSTRACT

Objective: To study the expression of tumor necrosis factor receptor-associated protein 1 (TRAP1) in gastric cancer tissue and its correlation with malignant biology.
Methods: Gastric cancer tissue and adjacent normal tissue were collected, and mRNA content and protein content of TRAP1 were detected; gastric cancer cell lines SGC7901, BGC823, AGS and MGC803 were cultured, and mRNA contents and protein contents of TRAP1, CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF were detected.
Results: mRNA and protein expression levels of TRAP1 in gastric cancer tissue were significantly higher than those in adjacent normal tissue, and mRNA and protein expression levels of TRAP1 in gastric cancer tissue with muscularis and serosa infiltration, lymph node metastasis, distant organ metastasis and TNM Ⅲ/Ⅳ stage were significantly higher than those in gastric cancer tissue with mucosa and submucosa infiltration, non-lymph node metastasis, non-distant organ metastasis and TNM Ⅰ/Ⅱ stage. mRNA and protein expression levels of TRAP1, CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in MGC803 were the highest, and mRNA and protein expression levels of TRAP1, CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in SGC7901 were the lowest. mRNA and protein expression levels of TRAP1 in gastric cancer cell lines were positively correlated with mRNA and protein expression of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF.
Conclusions: The expression of TRAP1 significantly increases in gastric cancer tissue; TRAP1 may regulate the malignant biology of cells by increasing the expression of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF, thereby resulting in the occurrence and development of gastric cancer.

1. Introduction

Gastric cancer is the malignant tumor of digestive tract system with the highest incidence in our country. Surgical resection is the main method of treating gastric cancer, and auxiliary postoperative chemotherapy and targeted therapy can effectively clear the lesions of gastric cancer and prolong the survival time of patients [1]. Nonetheless, the condition of patients with gastric cancer will inevitably develop into local recurrence and distant metastasis, and 5-year survival rate is not satisfactory [2]. At present, there are still no specific targeted therapy drugs for gastric cancer, which is related to that fact that pathogenesis-related genes of gastric caner have not been fully elucidated. Tumor necrosis factor receptor-associated protein 1 (TRAP1), also known as HSP75, is a molecular chaperone closely related to the regulation of cell proliferation and apoptosis. Studies have reported that TRAP1 is related to the occurrence of malignant tumors of digestive tract such as colon cancer, esophageal cancer [3,4]. Thus, a hypothesis was put forward that TRAP1 might regulate malignant biological behaviors of gastric cancer and participate in the development of gastric cancer. In order to verify the hypothesis, the expression of TRAP1 in gastric cancer tissue and its correlation with malignant biology were analyzed.

2. Materials and methods

2.1. Experimental materials

Gastric cancer tissue and adjacent normal tissue were from 40 cases of patients receiving radical resection for gastric cancer in

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Peer review under responsibility of Hainan Medical College.
Foundation project: Municipal Science and Technology Development Project in 2009 (No: 090103030).

our hospital from April 2012 to May 2014; gastric cancer cell lines SGC7901, BGC823, AGS and MGC803 were purchased from Cell Bank of ATCC, cell culture media were from Gibco Company and consumable materials were from Corning Company; PCR kits of TRAF1, CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF were from TAKARA Company and monoclonal antibodies were from Santa Cruz Company.

2.2. Experimental instruments

Cell culture incubator and centrifuge were from Thermo Company, and fluorescent quantitative PCR apparatus and chemiluminescence apparatus were from Bio-Rad Company.

2.3. Experimental methods

2.3.1. Tissue sample collecting

At the end of operation, gastric cancer tissue and adjacent normal tissue were collected, immunohistochemistry staining was used to confirm the nature of tissue, then normal saline was used to wash away residual blood and clot from the tissue, and after absorbing the moisture, tissue was put in freezing tube and then preserved in ultralow temperature freezer in the long term.

2.3.2. Cell culturing and collecting

Media containing 10% fetal bovine serum were used for cell culture, and cell growth was observed under microscope. When the density of cells in culture bottles grew to about 80%, trypsin digestion and passage were carried out with 0.125% concentration of trypsin, and after passage, cells were seeded in Petri dishes with diameter of 3.5 mm; when the density of cells in Petri dishes grew to 90%, media were discarded and Petri dishes were collected and preserved in ultralow temperature freezer.

2.3.3. Detection of mRNA expression

Tissue and cell samples were taken, and RNA extraction kits and reverse transcription kits were used for experiment. After cDNA samples were obtained, primers of target genes to be tested were designed and PCR kits were used to amplify *TRAF1*, *CyclinB1*, *CyclinD1*, *CyclinE*, *MMP-2* and *VEGF*. *β-actin* was used as standardized internal reference among groups and mRNA expression levels of above target genes were calculated. PCR amplification conditions were as follows: 95 °C for 15 s, specific annealing temperature for 20 s, 72 °C for 25 s, and repeating 40 cycles.

2.3.4. Detection of protein expression

Tissue and cell samples were taken and added to protein lysate, and obtained protein samples were treated according to process and then added to polyacrylamide gel for electrophoresis, transmembrane, antigen site closure, antibody incubation and ECL development in turn to get corresponding bands of proteins, and grey values of the bands were scanned. β-actin was used as standardized internal reference among groups and protein expression levels of above target genes were calculated.

2.3.5. Statistical methods

SPSS21.0 software was used for statistical processing. After experimental data was input, comparison between two groups was by t test, comparison among groups was by variance analysis and correlation analysis was by Pearson test. $P < 0.05$ was the standard of statistical significance in difference.

3. Results

3.1. TRAP1 expression in gastric cancer tissue

mRNA and protein expression levels of TRAP1 in gastric cancer tissue were significantly higher than those in adjacent normal tissue; analysis of TRAP1 expression in gastric cancer tissue with different clinical pathological features was as follows: mRNA and protein expression levels of TRAP1 in gastric cancer tissue with muscularis and serosa infiltration, lymph node metastasis, distant organ metastasis and TNM Ⅲ/Ⅳ stage were significantly higher than those in gastric cancer tissue with mucosa and submucosa infiltration, non-lymph node metastasis, non-distant organ metastasis and TNM Ⅰ/Ⅱ stage (Table 1).

3.2. TRAP1 expression in gastric cancer cell lines

mRNA expression levels of TRAP1 in gastric cancer cell lines SGC7901, BGC823, AGS and MGC803 were 62.35 ± 7.84, 163.45 ± 19.33, 100 ± 12.85 and 253.35 ± 28.69 respectively; protein expression levels were 57.68 ± 6.34, 168.68 ± 22.12, 100 ± 12.59 and 278.38 ± 31.45 respectively. Variance analysis showed: mRNA expression levels and protein expression levels of TRAP1 in gastric cancer cell lines SGC7901,

Table 1
mRNA and protein expression levels of TRAP1 in gastric cancer tissue.

Clinical pathological features	Groups	Case (n)	mRNA content of *TRAF1*	Protein content of TRAF1
Tissue origin	Adjacent normal tissue	40	100.00 ± 14.52	100.00 ± 12.96
	Gastric cancer tissue	40	237.28 ± 28.75[a]	262.39 ± 31.46[a]
Infiltration depth	Mucosa and submucosa	22	165.63 ± 18.95	178.65 ± 21.47
	Muscularis and serosa	18	289.52 ± 31.34[b]	314.67 ± 32.68[b]
Lymph node metastasis	Without	25	149.59 ± 16.34	159.65 ± 16.84
	With	15	313.58 ± 36.95[c]	338.91 ± 37.52[c]
Distant organ metastasis	Without	28	157.49 ± 17.14	166.79 ± 18.44
	With	12	304.59 ± 34.27[d]	329.42 ± 35.67[d]
TNM stage	Ⅰ/Ⅱ stage	24	170.34 ± 21.42	189.43 ± 21.34
	Ⅲ/Ⅳ stage	16	276.75 ± 29.58[e]	301.38 ± 36.41[e]

[a] Compared with adjacent normal tissue, $P < 0.05$. [b] Compared with gastric cancer tissue with mucosa and submucosa infiltration, $P < 0.05$. [c] Compared with gastric cancer tissue without lymph node metastasis, $P < 0.05$. [d] Compared with gastric cancer tissue without distant organ metastasis, $P < 0.05$. [e] Compared with gastric cancer tissue with TNM Ⅰ/Ⅱ stage, $P < 0.05$.

Table 2
mRNA contents of malignant biological behavior-related molecules in gastric cancer cell lines.

Cell lines	Proliferation-related molecules			Invasion molecules	
	CyclinB1	*CyclinD1*	*CyclinE*	*MMP-2*	*VEGF*
SGC7901	59.97 ± 7.85	66.72 ± 7.54	47.23 ± 5.02	51.34 ± 7.39	62.21 ± 7.23
BGC823	162.32 ± 23.12	146.79 ± 15.96	152.46 ± 16.75	144.58 ± 17.38	149.04 ± 16.12
AGS	100.00 ± 12.46	100.00 ± 13.04	100.00 ± 14.14	100.00 ± 12.71	100.00 ± 11.59
MGC803	294.58 ± 32.42	328.39 ± 36.82	309.39 ± 34.68	336.82 ± 37.38	278.72 ± 29.42

Table 3
Protein contents of malignant biological behavior-related molecules in gastric cancer cell lines.

Cell lines	Proliferation-related molecules			Invasion molecules	
	CyclinB1	CyclinD1	CyclinE	MMP-2	VEGF
SGC7901	63.34 ± 7.97	54.53 ± 6.51	71.47 ± 8.87	68.12 ± 7.24	59.18 ± 5.67
BGC823	177.59 ± 19.54	152.37 ± 17.86	166.45 ± 18.14	170.23 ± 19.24	161.23 ± 18.52
AGS	100.00 ± 14.81	100.00 ± 11.94	100.00 ± 12.52	100.00 ± 13.42	100.00 ± 14.12
MGC803	287.43 ± 29.28	312.59 ± 36.58	298.46 ± 34.13	305.32 ± 35.67	273.31 ± 31.52

BGC823, AGS and MGC803 were different; LSD-t pair wise comparison showed: mRNA expression levels and protein expression levels of TRAP1 in MGC803 were the highest, and mRNA expression levels and protein expression levels of TRAP1 in SGC7901 were the lowest.

3.3. Expression of malignant biological behavior-related molecules in gastric cancer cell lines

Variance of analysis showed that mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in gastric cancer cell lines SGC7901, BGC823, AGS and MGC803 had differences; pair wise comparison by LSD-*t* test showed that mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in MGC803 were the highest, and mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in SGC7901 were the lowest (Tables 2 and 3).

3.4. Correlation between TRAP1 expression level and expression levels of malignant biological behavior-related molecules

Pearson correlation analysis showed that mRNA and protein expression levels of TRAP1 in gastric cancer cell lines were positively correlated with mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF ($P < 0.05$) (Table 4).

Table 4
Correlation between TRAP1 expression level and expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF.

Indexes	mRNA expression level			Protein expression level		
	Correlation coefficient *r*	Statistics	*P* value	Correlation coefficient *r*	Statistics	*P* value
CyclinB1	0.781	14.123	<0.05	0.691	15.284	<0.05
CyclinD1	0.669	9.994	<0.05	0.745	12.145	<0.05
CyclinE	0.727	12.478	<0.05	0.772	10.955	<0.05
VEGF	0.718	10.949	<0.05	0.663	8.494	<0.05
MMP-2	0.678	15.502	<0.05	0.714	13.176	<0.05

4. Discussion

TRAP1 is a member of heat shock proteins (HSP) and also known as HSP75 [5]. The protein locates in the mitochondria and can inhibit the survival of reactive oxygen species ROS, thereby protecting cells against the effect of mitochondrial apoptosis mechanism and guaranteeing the continuing proliferation state of cells [6,7]. TRAP1 is widely distributed in multiple organs and tissues in the body, and mRNA of *TRAP1* is detected in parts including liver, heart, digestive tract, skeletal muscle, kidney and breast [8–11]. In recent years, the relationship between TRAP1 and the occurrence and development of malignant tumors has received attention from more and more scholars, and TRAP1 positive expression is regarded as an independent predictor of the survival and recurrent time of patients with malignant tumors [12,13]. Studies of Condelli [14] report that TRAP1 can participate in the occurrence and development of colorectal cancer through BRAF pathway, and studies of Ou [12] believe that TRAP1 can activate STAT3/MMP2 pathway and cause the occurrence of esophageal cancer.

Based on above studies and reports, TRAP1 is related to the occurrence of a variety of digestive tract tumors, therefore it was speculated that TRAP1 might be involved in the occurrence of gastric cancer. In order to verify whether TRAP1 was related to the occurrence of gastric cancer, TRAP1 expression levels in gastric cancer tissue and adjacent normal tissue were analyzed at first, and results showed that mRNA and protein expression levels of TRAP1 in gastric cancer tissue were significantly higher than those in adjacent normal tissue, which could preliminarily indicate that TRAP1 was related to the occurrence of gastric cancer. In order to further verify whether TRAP1 was related to the clinical progress and pathological process of gastric cancer, the expression levels of TRAP1 in gastric cancer tissue with different clinical pathological features were analyzed, and results showed that mRNA and protein expression levels of TRAP1 in gastric cancer tissue with muscularis and serosa infiltration, lymph node metastasis, distant organ metastasis and TNM Ⅲ/Ⅳ stage significantly increased, which indicated that TRAP1 was involved in the development of gastric cancer and closely related to the local infiltration and distant metastasis of gastric cancer.

Progress processes of gastric cancer mainly include tumor size increase as well as local infiltration and distant metastasis of cancer cells, and above changes are closely related to malignant biological behaviors such as cancer cell proliferation, migration and invasion [15,16]. According to the above analysis, TRAP1 participates in the occurrence of gastric cancer and disease progress, and occurrence and development of gastric cancer involve the changes of malignant biological behaviors of cancer cells. Therefore, it was considered that TRAP1 might be involved in the occurrence and development of gastric cancer by regulating malignant biological behaviors such as gastric cancer cell proliferation, migration and invasion. In order to clarify whether TRAP1 had regulatory effect on malignant biological behaviors of gastric cancer cells, four different gastric cancer cell lines were selected as research subjects, expression of TRAP1 in cells was analyzed, and results showed that mRNA and protein expression levels of TRAP1 in MGC803 were the highest, and mRNA and protein expression levels of TRAP1 in SGC7901 were the lowest. It indicated that expression levels of TRAP1 in different gastric cancer cell lines had differences, which might cause changes of biological behaviors of cancer cells.

Existing basic research suggests that malignant biological behaviors related to the occurrence and development of gastric cancer include cancer cell proliferation, migration and invasion. Completion of these biological behaviors requires the participation of a variety of molecules. Cyclins are a group of proteins closely related to cell cycle progress and cell proliferation, include CyclinB1, CyclinD1 and CyclinE, *etc* [17,18], and can form Cyclins–CDKs complex with corresponding cyclin-dependent kinases (CDKs), thus regulating the start and running of cell cycle and conducive to cell proliferation [19,20]. VEGF and MMP2 are two molecules closely related to cell invasion and metastasis. MMP2 can degrade extracellular matrix and make cancer cells break through the limit of local basement membrane and infiltrate to local parts [21,22]; VEGF can increase the number of local angiogenesis, which provides the energy for cellular infiltration as well as the path for cellular transfer through blood [23,24]. Analysis of the expression of proliferation and invasion-related molecules in four cell lines showed that mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in MGC803 were the highest, and mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF in SGC7901 were the lowest.

According to the analysis of experimental results in above cell lines, expression level of TRAP1 in MGC803 cell lines was the highest, and expression level of TRAP1 in SGC7901 cell lines was the lowest; correspondingly, expression levels of proliferation and invasion-related molecules in MGC803 cell lines were the highest, and expression levels of proliferation and invasion-related molecules in SGC7901 cell lines were the lowest. Therefore, it was analyzed that the trend of TRAP1 expression levels in different gastric cancer cell lines was consistent with that of expression levels of proliferation and invasion-related molecules, and Pearson correlation analysis showed that mRNA and protein expression levels of TRAP1 were positively correlated with mRNA and protein expression levels of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF, which could further indicate that the changes of TRAP1 expression levels in gastric cancer cell lines might affect the expression of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF. Above opinions about TRAP1 regulating the expression of proliferation and invasion-related molecules come from correlation analysis and still need next experiments to overexpress or knockdown TRAP1 to verify the expression changes of proliferation and invasion-related molecules.

Based on the above discussion, it is believed that the expression of TRAP1 significantly increases in gastric cancer tissue; TRAP1 may regulate the malignant biology of cells by increasing the expression of CyclinB1, CyclinD1, CyclinE, MMP-2 and VEGF, thereby resulting in the occurrence and development of gastric cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Ben Nasr S, Ayadi M, Bahloul R, Guesmi S, Allani B, Chrait N, et al. Perioperative chemotherapy in locally advanced gastric cancer. A retrospective study about 25 cases. *Tunis Med* 2015; **93**(4): 228-230.
[2] Maruo H, Suzuki K, Ishikawa S, Higashi Y, Shoji T, Nishiyama R, et al. Stage Ⅳ gastric cancer made resectable following a chemotherapy regimen that included trastuzumab–a case report. *Gan To Kagaku Ryoho* 2015; **42**(8): 997-1000.
[3] Chen R, Pan S, Lai K, Lai LA, Crispin DA, Bronner MP, et al. Up-regulation of mitochondrial chaperone TRAP1 in ulcerative colitis associated colorectal cancer. *World J Gastroenterol* 2014; **20**(45): 17037-17048.
[4] Tian X, Ma P, Sui CG, Meng FD, Li Y, Fu LY, et al. Suppression of tumor necrosis factor receptor-associated protein 1 expression induces inhibition of cell proliferation and tumor growth in human esophageal cancer cells. *FEBS J* 2014; **281**(12): 2805-2819.
[5] Amoroso MR, Matassa DS, Sisinni L, Lettini G, Landriscina M, Esposito F. TRAP1 revisited: novel localizations and functions of a 'next-generation' biomarker (review). *Int J Oncol* 2014; **45**(3): 969-977.
[6] Kadye R, Kramer AH, Joos-Vandewalle J, Parsons M, Njengele Z, Hoppe H, et al. Guardian of the furnace: mitochondria, TRAP1, ROS and stem cell maintenance. *IUBMB Life* 2014; **66**(1): 42-45.
[7] Zhang P, Lu Y, Yu D, Zhang D, Hu W. TRAP1 provides protection against myocardial ischemia-reperfusion injury by ameliorating mitochondrial dysfunction. *Cell Physiol Biochem* 2015; **36**(5): 2072-2082.
[8] Gao JY, Song BR, Peng JJ, Lu YM. Correlation between mitochondrial TRAP-1 expression and lymph node metastasis in colorectal cancer. *World J Gastroenterol* 2012; **18**(41): 5965-5971.
[9] Zhang Y, Jiang DS, Yan L, Cheng KJ, Bian ZY, Lin GS. HSP75 protects against cardiac hypertrophy and fibrosis. *J Cell Biochem* 2011; **112**(7): 1787-1794.
[10] Lee C, Park HK, Jeong H, Lim J, Lee AJ, Cheon KY, et al. Development of a mitochondria-targeted Hsp90 inhibitor based on the crystal structures of human TRAP1. *J Am Chem Soc* 2015; **137**(13): 4358-4367.
[11] Guzzo G, Sciacovelli M, Bernardi P, Rasola A. Inhibition of succinate dehydrogenase by the mitochondrial chaperone TRAP1 has anti-oxidant and anti-apoptotic effects on tumor cells. *Oncotarget* 2014; **5**(23): 11897-11908.
[12] Ou Y, Liu L, Xue L, Zhou W, Zhao Z, Xu B, et al. TRAP1 shows clinical significance and promotes cellular migration and invasion through STAT3/MMP2 pathway in human esophageal squamous cell cancer. *J Genet Genomics* 2014; **41**(10): 529-537.
[13] Ozgur A, Tutar L, Tutar Y. Regulation of heat shock proteins by miRNAs in human breast cancer. *Microrna* 2014; **3**(2): 118-135.
[14] Condelli V, Maddalena F, Sisinni L, Lettini G, Matassa DS, Piscazzi A, et al. Targeting TRAP1 as a downstream effector of

BRAF cytoprotective pathway: a novel strategy for human BRAF-driven colorectal carcinoma. *Oncotarget* 2015; **6**(26): 22298-22309.

[15] Guo XL, Wang YJ, Cui PL, Wang YB, Liang PX, Zhang YN, et al. Effect of BRMS1 expression on proliferation, migration and adhesion of mouse forestomach carcinoma. *Asian Pac J Trop Med* 2015; **8**(9): 724-730.

[16] Zhang JF, Qu LS, Qian XF, Xia BL, Mao ZB, Chen WC. Nuclear transcription factor CDX2 inhibits gastric cancer-cell growth and reverses epithelial-to-mesenchymal transition *in vitro* and *in vivo*. *Mol Med Rep* 2015; **12**(4): 5231-5238.

[17] Yao Y, Feng S, Xiao M, Li Y, Yang L, Gong J. MTA1 promotes proliferation and invasion in human gastric cancer cells. *Onco Targets Ther* 2015; **20**(8): 1785-1794.

[18] Li WH, Wu JH, Gao C, Gong JP. Types of primary cyclins expression in gastric carcinoma and their relationship with clinicopathological features. *Chin J Oncol* 2013; **35**(2): 114-118.

[19] Alsina M, Landolfi S, Aura C, Caci K, Jimenez J, Prudkin L, et al. Cyclin E amplification/overexpression is associated with poor prognosis in gastric cancer. *Ann Oncol* 2015; **26**(2): 438-439.

[20] Yang Q, Wang B, Gao W, Huang S, Liu Z, Li W, et al. SIRT1 is downregulated in gastric cancer and leads to G_1-phase arrest via NF-κB/Cyclin D1 signaling. *Mol Cancer Res* 2013; **11**(12): 1497-1507.

[21] Huang LF, Lu YJ, Pan S, Luo H. Correlation between the expression levels of vascular endothelial growth factor and matrix metalloproteinase-2 and gastric cancer invasion. *Chin J Exp Surg* 2014; **31**(8): 1816-1818.

[22] Avcı N, Ture M, Deligonul A, Cubukcu E, Olmez OF, Sahinturk S, et al. Association and prognostic significance of the functional -1562C/T polymorphism in the promoter region of MMP-9 in Turkish patients with gastric cancer. *Pathol Oncol Res* 2015; **21**(4): 1243-1247.

[23] Jun KH, Lee JE, Kim SH, Jung JH, Choi HJ, Kim YI, et al. Clinicopathological significance of N-cadherin and VEGF in advanced gastric cancer brain metastasis and the effects of metformin in preclinical models. *Oncol Rep* 2015; **34**(4): 2047-2053.

[24] Bilgiç CI, Tez M. Serum VEGF levels in gastric cancer patients: correlation with clinicopathological parameters. *Turk J Med Sci* 2015; **45**(1): 112-117.