Int J Clin Exp Pathol 2016;9(9):9443-9448
www.ijcep.com /ISSN:1936-2625/IJCEP0019011

Original Article

Decreased expression of *let-7f* associates with the poor prognosis of glioma patients

Jianxin Zhu[1,2], Yilei Xiao[2], Li Li[2], Baojun Fang[2], Fuhua Yu[2], Shubao Zhang[2], Ming Sun[2], Qingju Zhao[2], Gang Li[1]

[1]Department of Neurosurgery, Qilu Hospital, Shandong University, Jinan 250012, Shandong, China; [2]Department of Neurosurgery, Liaocheng People's Hospital, Liaocheng 252000, Shandong, China

Received November 2, 2015; Accepted January 5, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Objectives: *Let-7f* has been reported to play regulatory roles in several types of cancer and could act as a prognostic biomarker for cancer patients. The purpose of this study was to evaluate the prognostic value of *let-7f* in glioma patients. Methods: The relative expression levels of *let-7f* in tumor tissues and corresponding normal tissues of 108 glioma patients were detected by qRT-PCR. Chi-square test was used for analyzing the association between the expression level of *let-7f* and the clinical features. Besides, Kaplan-Meier method was performed to estimate the overall survival of glioma patients. The prognostic significance of *let-7f* was evaluated by Cox regression analysis. Results: The relative expression level of *let-7f* in tumor tissues was lower than in normal tissues (0.45 vs 1.09). Chi-square test indicated that *let-7f* expression was associated with tumor size (*P*=0.004) and WHO grade (*P*=0.000). The results of Kaplan-Meier method suggested that patients with low expression level of *let-7f* had shorter overall survival time than those with high expression level (24.1 months vs 34.5 months, log rank test, *P*=0.000). Moreover, *let-7f* could act as an independent biomarker for glioma prognosis (HR=1.971, 95% CI=1.381-2.813, *P*=0.000). Conclusion: The decreased expression of *let-7f* is detected in glioma tissues and the expression levels are associated with tumor progression. *Let-7f* may be a potential indicator for prognosis of glioma patients.

Keywords: *Let-7f*, glioma, prognosis

Introduction

Glioma is one of the most common brain tumors, accounting for about 30%-40% of all intracranial tumors [1]. The prognosis of patients with glioma is poor, with a five-year mortality over 95% despite multiple therapies [2]. The high death rate of glioma is associated with its rapid growth, angiogenesis, and invasion throughout the brain [3]. Although great progresses have been made in the advanced treatments, the results are still disappointing [4]. Recently, glioma epidemiology has focused on identifying factors that are associated with tumor progression and prognosis. The relevant factors may provide valuable information about the features of the disease and therapy targets [1, 5].

MicroRNAs (miRNAs) are short non-coding RNAs, which play important regulatory roles in organism by interfering with the functions of target mRNAs [6, 7]. The fundamental roles of miRNAs are regulating basic cell cycle including proliferation, differentiation, and death [7-9]. MiRNAs may also take part in the tumor progression by interfering crucial regulators of cell cycle [9]. Many aberrant expression of miRNAs are detected in various cancers and indicating that these miRNAs are associated with the tumor progression, such as *miRNA-221* in colorectal cancer [10], *miRNA-21* in breast cancer [11] and *miRNA-25* in gastric cancer [12]. The expression levels of miRNAs are associated with cancer type, stage, and other clinical variables, so miRNAs can be emerged as biomarkers for cancer diagnosis and prognosis [13]. *Let-7f*, one of the miRNAs, belongs to let-7 family and locates at 9q22.3 [14]. Different expression of *let-7f* has been detected in various types of cancer, such as breast cancer, gastric cancer, hepatocellular carcinoma and papillary thyroid carcinoma [15-18]. In addition, Yan et al. have found that *let-7f* could inhibit the proliferation, migration and invasion of glioma cells through perisotin signal [19]. However, the function of *let-7f* in glioma prognosis remains unclear.

Table 1. The sequences of the primers used in this study

Name	Sequences	
Let-7f	Forward	5'-GCCGTGAGGTAGTAGATTGTAT-3'
	Reverse	5'-GTGCAGGGTCCGAGGT-3'
U6	Forward	5'-CGCTTCGGCAGCACATATAC-3'
	Reverse	5'-TTCACGAATTTGCGTGTCAT-3'

Table 2. The association between *let-7f* expression and clinical features of glioma patients

Characteristics	Total number (n)	*Let-7f* expression		x^2	*P* value
		High (n)	Low (n)		
Gender				0.160	0.689
Male	56	28	28		
Female	52	24	28		
Age				0.060	0.807
≥ 55	61	30	31		
< 55	47	22	25		
Tumor location				0.252	0.616
Supratentorial	68	34	34		
Subtentorial	40	18	22		
Tumor size				8.306	0.004
> 3 cm	55	19	36		
≤ 3 cm	53	33	20		
Karnofsy performance status				0.572	0.449
≥ 80	56	25	31		
< 80	52	27	25		
WHO grade				13.588	0.000
I+II	57	37	20		
III+IV	51	15	36		
Relapse				0.250	0.617
Yes	67	31	36		
No	41	21	20		

The purpose of this study was to evaluate the clinical significance of *let-7f* in glioma. We detected the expression levels of *let-7f* in tumor tissues and corresponding normal tissues of 108 glioma patients. The associations between the gene expression and clinical features of glioma patients were analyzed and the prognostic value of *let-7f* was also estimated in the study.

Materials and methods

Patients and tissue specimens

108 glioma patients confirmed by pathological and clinical diagnoses in Liaocheng People's Hospital from December 2011 to February 2014 were enrolled in this study. The pathological tissue specimens and corresponding normal tissue samples were collected from the patients and the samples were put in liquid nitrogen immediately then stored at -80°C for use. None of the patients had received chemotherapy or radiotherapy before the surgery. The study was approved by the Ethic Committee of the hospital and all the participators had signed written informed consents in advance.

The follow-up investigation was about 5 years and all of the patients were participated in. The investigation information and the clinical features of the glioma patients were collected to estimate the clinical significance of *let-7f* in glioma patients.

RNA extraction and qRT-PCR

Total RNA of the pathological tissues and corresponding normal tissues of glioma patients were extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's introduction. The residuary DNA in the total RNA was dealt with DNase. Concentration of the total RNA was detected by UV absorbance (A260/A280) and 1% agarose gel electrophoresis was used for testing the quality of the total RNA.

PrimeScript RT reagent kit (Takara, China) was used to obtain the complementary DNA (cDNA) from the total RNA. Moreover, fluorescence quantitative real-time PCR (qRT-PCR) was performed using SYBR Green assay (Takara, China). *U6* was emerged as the internal control. The data analysis was calculated by $2^{-\Delta\Delta Ct}$ method. The sequences of the primers used in this study were listed in **Table 1**. Each sample was in triplicate.

Statistical analysis

The relative expression levels of *let-7f* in the pathological tissues and corresponding normal

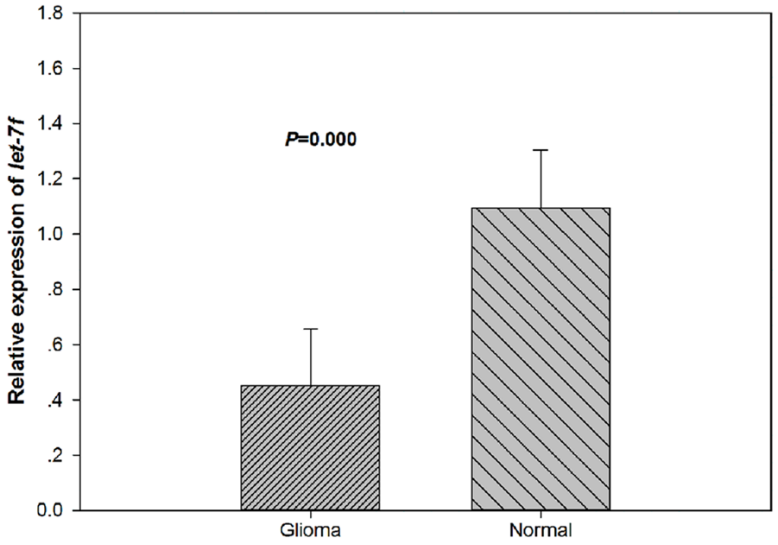

Figure 1. Relative expression of *let-7f* in tumor tissues and corresponding normal tissues of glioma patients (U6 as normalized control).

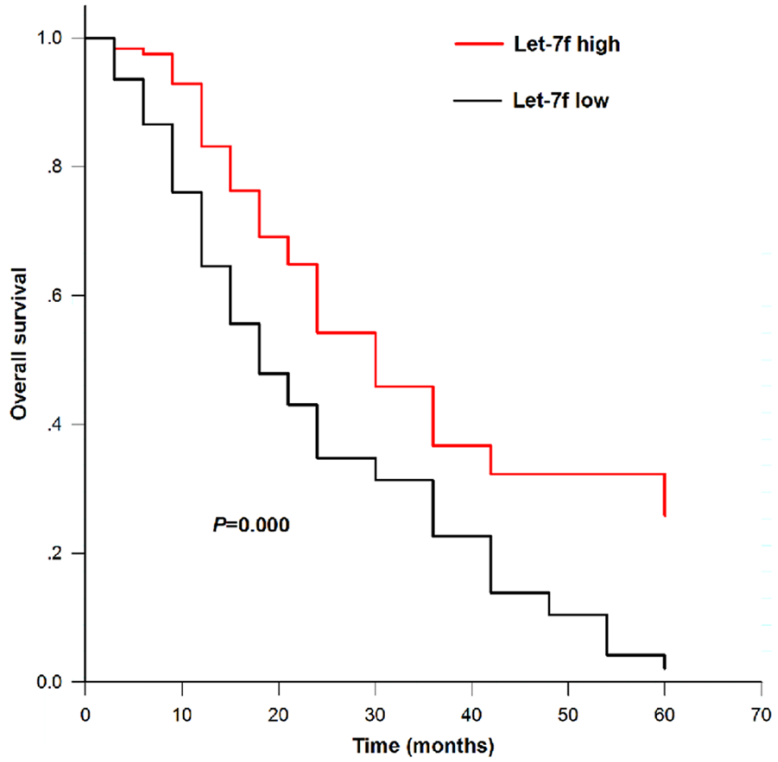

Figure 2. Overall survival of glioma patients with *let-7f* high expression (red line) and low expression (black line) (Log rank test, *P*=0.000).

tissues were analyzed by T test and the results were shown as mean ± SD. The correlation between expression level of the gene and clinical features of glioma patients was estimated by Chi-square test. And the overall survival analysis was assessed using Kaplan-Meier method. In addition, Cox regression analysis was used to evaluate the prognostic significance of *let-7f*. All the data analyses were performed in SPSS 18.0 software and Sigma Plot software was used for drawing. $P < 0.05$ was considered as statistical significance.

Results

Relative expression of let-7f in glioma patients

The 108 glioma patients collected in this study included 56 males and 52 females and their average age was 56.3 years. The clinical information of the glioma patients was summarized in **Table 2**.

QRT-PCR was used to test the relative expression of *let-7f* in pathological tissues and corresponding normal tissues of glioma patients. The relative expression level in tumor tissues was 0.45± 0.203, while that in the corresponding normal tissues was 1.09±0.211. There was a significant difference between them (**Figure 1**, *P*= 0.000).

The relationship between let-7f expression and clinical features

In order to analyze the connection between *let-7f* expression and the clinical features, the glioma patients were divided into two groups according to their average expression level. The results of Chi-square test indicated that the expression level of *let-7f* was associated with tumor size (*P*=0.004) and WHO grade (*P*=

Table 3. Univariate and multivariate analyses for prognostic factors in glioma patients

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Let-7f (low vs high)	1.927	1.362-2.728	0.000	1.971	1.381-2.813	0.000
Gender (male vs female)	0.900	0.635-1.276	0.554	0.847	0.589-1.219	0.372
Age (≥ 55 vs < 55)	1.052	0.742-1.494	0.775	1.077	0.752-1.544	0.685
Tumor location (supratentorial vs subtentoria)	0.994	0.700-1.412	0.975	0.979	0.674-1.423	0.913
Tumor size (≥ 3 cm vs < 3 cm)	1.297	0.908-1.853	0.153	1.371	0.947-1.985	0.095
Karnofsy performance status (≥ 80 vs < 80)	0.885	0.625-1.254	0.493	0.895	0.627-1.278	0.542
WHO grade (I+II vs III+IV)	1.164	0.821-1.649	0.393	1.112	0.768-1.610	0.573
Relapse (yes vs no)	1.027	0.722-1.461	0.881	0.910	0.625-1.325	0.621

0.000). However, it had nothing to do with age, gender, tumor location, Karnofsy performance status or relapse ($P > 0.05$).

Association between let-7f expression and the overall survival of glioma patients

Kaplan-Meier method was used for overall survival analysis. The patients with low expression level of the *let-7f* had shorter overall survival time than those with high expression level (24.1 months vs 34.5 months, **Figure 2**). There was a remarkable difference between the two groups (Log rank test, P=0.000).

Univariate and multivariate Cox regression analyses were used for analyzing prognostic factors in glioma patients. The results indicated that *let-7f* was associated the glioma prognosis and could act as an independent biomarker for prognosis of glioma patients (**Table 3**, HR=1.971, 95% CI=1.381-2.813, P=0.000).

Discussion

Recent years, glioma as a typical type of brain tumor has attracted more and more attentions but the therapeutic efficacy for glioma patients are not satisfactory. The prognosis of glioma is poor despite multiple advance treatments, due to excessive proliferation, relentless invasion, and angiogenesis [20]. The identification of new biomarkers for glioma therapy target and prognosis may improve the clinical outcomes of the patients. Recently, with the development of the molecular biological techniques, the genes which could alter during the tumor progression are proved to improve the outcomes of the cancer patients. Guo et al. had reported that *FRAT1* was an important factor in the tumorigenesis and progression of glioma and could be explored as an indicator for pathological diagnosis, therapy target and prognosis [21]. *CDKN2A* (*p16*) mRNA was proved to be a prognostic marker for malignant high-grade glioma [22]. Wang et al. indicated that transmembrane-4-L-six-family-1 was a potential predictor for prognosis in human glioma [23]. These studies suggested that the endogenous biomarkers might improve the clinical outcomes of patients with glioma.

The *let-7* family is a high conserved family of miRNAs, including fourteen members [24]. Currently, many members of *let-7* family were reported to be associated with cancer tumorigenesis and progression. *Let-7a* had been reported to inhibit growth and migration of breast cancer cells [25]. Han et al. demonstrated that *let-7b* could suppress invasion and migration of gastric cancer cells through directly targeting the tumor metastasis-associated gene *ING1* [26]. And *let-7f* was also proved to play regulatory roles in several types of cancer, such as gastric cancer, breast cancer, hepatocellular carcinoma and so on [15-17]. Then weather *let-7f* participates in the development of glioma or affects the clinical outcome requires further investigation.

In this study, we detected the expression level of *let-7f* in pathological tissues and corresponding normal tissues of patients with glioma. The results suggested that the expression level of *let-7f* was decreased in tumor tissues and a further analysis indicated that it was associated with tumor size and WHO grade. These results might suggest that the abnormal expression of *let-7f* in tumor tissues was associated with the cancer development. The findings were accordance with previous studies, such as Yan et al., had reported that *let-7f* overexpression inhibited glioma cell proliferation, migration, and invasion via targeting periostin [19]. Nahid et al.

had proved that *let-7f* expression was decreased during hepatic differentiation [27]. Decreased expression of *let-7f* was also detected in metastasis gastric cancer tissues and the over-expression of the gene could inhibit invasion and migration of gastric cancer cells through directly targeting the tumor metastasis-associated gene *MYH9* [28]. In a word, all of the results indicated that *let-7f* might be a potential tumor suppressor.

In the previous studies, many miRNAs were proved to act as prognostic biomarkers and as non-invasive biomarkers, they could provide more credible outcomes for patients [29, 30]. For example, *miRNA-124* had been reported to be associated with prognosis of lung cancer and could act as a potential biomarker for further risk stratification in the treatment of lung cancer [31]. Yuan et al. reported that *miRNA-940* could inhibit the growth of hepatocellular carcinoma and might be a indicator for prognosis [32]. Over-expression of *miRNA-155* was proved to correlate with a poor outcome for patients with bladder cancer [33]. In this study, we evaluated the prognostic value of miRNA *let-7f* in glioma. The results suggested that patients with low expression levels of the gene had lower survival rate and the expression levels of *let-7f* was associated with glioma prognosis. *Let-7f* could act as an independent predictor in patients with glioma.

In conclusion, the decreased expression of *let-7f* is found in tumor tissues of glioma patients. And the gene is correlated with tumor size and WHO grade. Besides, the expression levels of *let-7f* are associated with survival rate of patients and *let-7f* may be a potential biomarker for prognosis and therapy target for glioma.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Gang Li, Department of Neurosurgery, Qilu Hospital of Shandong University, Jinan 250012, Shandong, China. E-mail: zhaone373h@sina.com

References

[1] Zeng T, Cui D and Gao L. Glioma: an overview of current classifications, characteristics, molecular biology and target therapies. Front Biosci (Landmark Ed) 2015; 20: 1104-1115.

[2] McLendon RE and Halperin EC. Is the long-term survival of patients with intracranial glioblastoma multiforme overstated? Cancer 2003; 98: 1745-1748.

[3] Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA and Cavenee WK. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 2007; 21: 2683-2710.

[4] Shen J, Li G, Liu Q, He Q, Gu J, Shi Y and Lou H. Marchantin C: a potential anti-invasion agent in glioma cells. Cancer Biol Ther 2010; 9: 33-39.

[5] Schwartzbaum JA, Fisher JL, Aldape KD and Wrensch M. Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol 2006; 2: 494-503; quiz 491 p following 516.

[6] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[7] Hwang HW and Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2007; 96 Suppl: R40-44.

[8] Bueno MJ and Malumbres M. MicroRNAs and the cell cycle. Biochim Biophys Acta 2011; 1812: 592-601.

[9] Carleton M, Cleary MA and Linsley PS. MicroRNAs and cell cycle regulation. Cell Cycle 2007; 6: 2127-2132.

[10] Qin J and Luo M. MicroRNA-221 promotes colorectal cancer cell invasion and metastasis by targeting RECK. FEBS Lett 2014; 588: 99-104.

[11] Petrovic N, Mandusic V, Stanojevic B, Lukic S, Todorovic L, Roganovic J and Dimitrijevic B. The difference in miR-21 expression levels between invasive and non-invasive breast cancers emphasizes its role in breast cancer invasion. Med Oncol 2014; 31: 867.

[12] Gong J, Cui Z, Li L, Ma Q, Wang Q, Gao Y and Sun H. MicroRNA-25 promotes gastric cancer proliferation, invasion, and migration by directly targeting F-box and WD-40 Domain Protein 7, FBXW7. Tumour Biol 2015; 36: 7831-40.

[13] Lee YS and Dutta A. MicroRNAs in cancer. Annu Rev Pathol 2009; 4: 199-227.

[14] Kuehbacher A, Urbich C, Zeiher AM and Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res 2007; 101: 59-68.

[15] Tao WY, Liang XS, Liu Y, Wang CY and Pang D. Decrease of let-7f in low-dose metronomic Paclitaxel chemotherapy contributed to upregulation of thrombospondin-1 in breast cancer. Int J Biol Sci 2015; 11: 48-58.

[16] Liu WJ, Xu Q, Sun LP, Dong QG, He CY and Yuan Y. Expression of serum let-7c, let-7i, and let-7f microRNA with its target gene, pepsinogen C,

in gastric cancer and precancerous disease. Tumour Biol 2015; 36: 3337-3343.
[17] Ge W, Yu DC, Li QG, Chen X, Zhang CY and Ding YT. Expression of serum miR-16, let-7f, and miR-21 in patients with hepatocellular carcinoma and their clinical significances. Clin Lab 2014; 60: 427-434.
[18] Geraldo MV, Fuziwara CS, Friguglieti CU, Costa RB, Kulcsar MA, Yamashita AS and Kimura ET. MicroRNAs miR-146-5p and let-7f as prognostic tools for aggressive papillary thyroid carcinoma: a case report. Arq Bras Endocrinol Metabol 2012; 56: 552-557.
[19] Yan S, Han X, Xue H, Zhang P, Guo X, Li T, Yuan G, Deng L and Li G. Let-7f Inhibits Glioma Cell Proliferation, Migration, and Invasion by Targeting Periostin. J Cell Biochem 2015; 116: 1680-92.
[20] Moller HG, Rasmussen AP, Andersen HH, Johnsen KB, Henriksen M and Duroux M. A systematic review of microRNA in glioblastoma multiforme: micro-modulators in the mesenchymal mode of migration and invasion. Mol Neurobiol 2013; 47: 131-144.
[21] Guo G, Zhong CL, Liu Y, Mao XG, Zhang Z, Jin J, Liu J, Yang L, Mao JM, Guo YH and Zhao YL. Overexpression of FRAT1 is associated with malignant phenotype and poor prognosis in human gliomas. Dis Markers 2015; 2015: 289750.
[22] Sibin MK, Bhat DI, Narasingarao KV, Lavanya C and Chetan GK. CDKN2A (p16) mRNA decreased expression is a marker of poor prognosis in malignant high-grade glioma. Tumour Biol 2015; 36: 7607-14.
[23] Wang P, Bao W, Zhang G, Cui H and Shi G. Transmembrane-4-L-six-family-1, a potential predictor for poor prognosis, overexpressed in human glioma. Neuroreport 2015; 26: 455-461.
[24] Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE, Degnan B, Muller P, Spring J, Srinivasan A, Fishman M, Finnerty J, Corbo J, Levine M, Leahy P, Davidson E and Ruvkun G. Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 2000; 408: 86-89.
[25] Liu K, Zhang C, Li T, Ding Y, Tu T, Zhou F, Qi W, Chen H and Sun X. Let-7a inhibits growth and migration of breast cancer cells by targeting HMGA1. Int J Oncol 2015; 46: 2526-2534.
[26] Han X, Chen Y, Yao N, Liu H and Wang Z. MicroRNA let-7b suppresses human gastric cancer malignancy by targeting ING1. Cancer Gene Ther 2015; 22: 122-129.
[27] Davoodian N, Lotfi AS, Soleimani M, Mola SJ and Arjmand S. Let-7f microRNA negatively regulates hepatic differentiation of human adipose tissue-derived stem cells. J Physiol Biochem 2014; 70: 781-789.
[28] Liang S, He L, Zhao X, Miao Y, Gu Y, Guo C, Xue Z, Dou W, Hu F, Wu K, Nie Y and Fan D. MicroRNA let-7f inhibits tumor invasion and metastasis by targeting MYH9 in human gastric cancer. PLoS One 2011; 6: e18409.
[29] Fabbri M. miRNAs as molecular biomarkers of cancer. Expert Rev Mol Diagn 2010; 10: 435-444.
[30] Wang F, Lou JF, Cao Y, Shi XH, Wang P, Xu J, Xie EF, Xu T, Sun RH, Rao JY, Huang PW, Pan SY and Wang H. miR-638 is a new biomarker for outcome prediction of non-small cell lung cancer patients receiving chemotherapy. Exp Mol Med 2015; 47: e162.
[31] Zhang Y, Li H and Han J. Down-regulation of microRNA-124 is correlated with tumor metastasis and poor prognosis in patients with lung cancer. Int J Clin Exp Pathol 2015; 8: 1967-1972.
[32] Yuan B, Liang Y, Wang D and Luo F. MiR-940 inhibits hepatocellular carcinoma growth and correlates with prognosis of hepatocellular carcinoma patients. Cancer Sci 2015; 106: 819-24.
[33] Wang H and Men CP. Correlation of Increased Expression of MicroRNA-155 in Bladder Cancer and Prognosis. Lab Med 2015; 46: 118-122.