Effect of paeonol on proliferation, apoptosis, migration, invasion and glutamine of gastric cancer cells via circSFMBT2/miR-665 axis

Jia Li*, Gang Zhang***, Guoqiang Wu

Endoscope Room, Dalian Port Hospital, Dalian, 116001, China

*Correspondence to: gangzhang0207@outlook.com

Received September 24, 2020; Accepted October 30, 2020; Published December 31, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.8.6

Abstract: This experiment was performed to investigate the effect of paeonol on the proliferation, apoptosis, migration, invasion and glutamine of gastric cancer HGC-27 cells. It was concluded that paeonol can inhibit the proliferation, migration, invasion and glutamine decomposition of gastric cancer HGC-27 cells via circSFMBT2/miR-665 axis, and also induce cell apoptosis.

Key words: Paeonol; Gastric cancer; Proliferation; Apoptosis; Migration; Invasion; Glutamine.

Introduction

Gastric cancer has high morbidity and mortality, which is a common malignant tumor of the digestive system. Due to the lack of screening methods in the early stage of gastric cancer, most patients with gastric cancer are already in the late stage. The 5-year prognosis survival rate is lower than 30% (1, 2). Although surgery, radiotherapy and chemotherapy have achieved remarkable results, gastric cancer is still the cause of high cancer mortality in the world (3). As a small molecular phenolic compound, paeonol is the main active monomer of traditional Chinese medicine Radix Cynanchi Paniculati and Cortex Moutan, which has pharmacological activities such as anti-inflammation, anti-tumor, improving cardiovascular and cerebrovascular diseases and enhancing immunity (4). Lyu et al found that paeonol could significantly inhibit the growth, migration and invasion of gastric cancer cells by down-regulating MMP-2 and MMP-9. Circular RNA (circRNA), as a circular closed non-coding RNA molecule, is abnormally expressed in many cancers (5). For example, circSFMBT2 (Hsa_circ_0017639) is highly expressed in gastric cancer cells. CircSFMBT2 inhibits the proliferation and metastasis of gastric cancer cells by regulating miR-224-5p/USP3 (6). In recent years, miRNA has attracted much attention in the study of gastric cancer, such as the low expression of miR-665 in gastric cancer cells and gastric cancer tissues. The abnormal expression is significantly correlated with TNM stage, late metastasis and poor differentiation. Overexpression can significantly inhibit the proliferation, migration and epithelial-mesenchymal transformation of gastric cancer cells (7). However, the studies on circSFMBT2 (Hsa_circ_0017639) and paeonol have not been reported. Therefore, the purpose of this study is to explore the effects of paeonol on proliferation, apoptosis, migration, invasion and glutamine decomposition of gastric cancer via circSFMBT2/miR-665.

Materials and Methods

Cells and reagents
Normal gastric epithelial cell GES-1 and gastric cancer cell HGC-27 were purchased from Shanghai Cell Bank of Chinese Academy of Sciences; Fetal bovine serum and RPMI 1640 culture medium were purchased from Gibco company of USA; Paeonol was purchased from Zhejiang Haizheng Company; Lipoectomate TM 2000 transfection kit was purchased from Invitrogen company of USA; Vector, circSFMBT2, miRNA, miR-665, circSFMBT2 probe and Oligo probe were purchased from Shanghai Jima Company; MTT kit was purchased from Shanghai Tongren Institute of Chemistry. Transwell and Matrigel glue were purchased from Shanghai Yisheng Biological Company; MMP2
and MMP9 antibodies were purchased from CST Company of the United States; Glutamine Kit and glutamate Kit were purchased from Sigma-Aldrich Company of Germany; α-KG Kit was purchased from Abcam Company of the United States; TRIZol Kit and reverse transcription Kit were purchased from TaKaRa Company of Japan; RNA enzyme R was purchased from Epicenter Company of the United States; Actinomycin D was purchased from Shanghai Chunyou Biology Company. Apoptosis kit and luciferase kit were purchased from Beijing Solebo Co., Ltd.

Sample collection
Twenty-one patients with gastric cancer and para-cancerous tissues who underwent gastric cancer surgery were obtained. All patients did not receive preoperative chemotherapy and radiotherapy for 3 months and were immediately transferred to-80 ℃ refrigerator storage after surgical resection. All patients signed the informed consent form before the operation, and the study was approved by the Ethics Committee.

Cell culture and grouping
Normal gastric epithelial cells GES-1 and human gastric cancer cells HGC-27 were cultured in RPMI 1640 medium containing 10% fetal bovine serum, respectively, and incubated at 37 ℃ and 5% CO2 in the incubator. HGC-27 cells in the logarithmic growth phase were treated with different concentrations of paeonol at 0.0, 0.1, 0.2, 0.4 mg/ml, which were recorded as pae- nol groups. HGC-27 cells were divided into six groups. Control group: DMSO solution was added, paeonol concentration was 0 mg/ml; Pae group: paeonol concentration was 0.4 mg/ml; Pae+vector group: Vector was transfected and then treated with paeonol concentration of 0.4 mg/ml; Pae+circSFMBT2 group: CircSFMBT2 was transfected, and then HGC-27 cells were treated with paeonol 0.4 mg/ml; Pae+circSFMBT2+miR-NC group: circSFMBT2 and miR-NC were co-transfected, and then HGC-27 cells were treated with paeonol 0.4 mg/ml; Pae+circSFMBT2+miR-665 group: circSFMBT2 and miR-665 were co-transfected, and then HGC-27 cells were treated with paeonol 0.4 mg/ml. The procedure of the Lipofectamine TM 2000 transfection kit was strictly followed in cell transfection.

MTT method
HGC-27 cells were collected and inoculated into 96-well plates (3 × 10³ cells / well). After 48 hours, 20 μL MTT was added to each well for 4 h and 150 μL DMSO was added to mix gently. The OD value was detected by enzyme labeling instrument at 490 nm, and the cell viability was calculated.

Flow cytometry
After the HGC-27 cells were digested, we mixed the cells with a 1X binding buffer. Then 5 μL Annexin V-FITC and PI reagents were also added, followed by incubation at room temperature in the dark for 20 min. The cell apoptosis rate was detected by flow cytometry.

Transwell
Cell migration experiment: After HGC-27 cells were digested, a total of 200 μL of cell suspension was ad-

Sample collection
Twenty-one patients with gastric cancer and para-cancerous tissues who underwent gastric cancer surgery were obtained. All patients did not receive preoperative chemotherapy and radiotherapy for 3 months and were immediately transferred to-80 ℃ refrigerator storage after surgical resection. All patients signed the informed consent form before the operation, and the study was approved by the Ethics Committee.

Cell culture and grouping
Normal gastric epithelial cells GES-1 and human gastric cancer cells HGC-27 were cultured in RPMI 1640 medium containing 10% fetal bovine serum, respectively, and incubated at 37 ℃ and 5% CO2 in the incubator. HGC-27 cells in the logarithmic growth phase were treated with different concentrations of paeonol at 0.0, 0.1, 0.2, 0.4 mg/ml, which were recorded as paeonol groups. HGC-27 cells were divided into six groups. Control group: DMSO solution was added, paeonol concentration was 0 mg/ml; Pae group: paeonol concentration was 0.4 mg/ml; Pae+vector group: Vector was transfected and then treated with paeonol concentration of 0.4 mg/ml; Pae+circSFMBT2 group: CircSFMBT2 was transfected, and then HGC-27 cells were treated with paeonol 0.4 mg/ml; Pae+circSFMBT2+miR-NC group: circSFMBT2 and miR-NC were co-transfected, and then HGC-27 cells were treated with paeonol 0.4 mg/ml; Pae+circSFMBT2+miR-665 group: circSFMBT2 and miR-665 were co-transfected, and then HGC-27 cells were treated with paeonol 0.4 mg/ml. The procedure of the Lipofectamine TM 2000 transfection kit was strictly followed in cell transfection.

MTT method
HGC-27 cells were collected and inoculated into 96-well plates (3 × 10³ cells / well). After 48 hours, 20 μL MTT was added to each well for 4 h and 150 μL DMSO was added to mix gently. The OD value was detected by enzyme labeling instrument at 490 nm, and the cell viability was calculated.

Flow cytometry
After the HGC-27 cells were digested, we mixed the cells with a 1X binding buffer. Then 5 μL Annexin V-FITC and PI reagents were also added, followed by incubation at room temperature in the dark for 20 min. The cell apoptosis rate was detected by flow cytometry.

Transwell
Cell migration experiment: After HGC-27 cells were digested, a total of 200 μL of cell suspension was ad-
control, then HGC-27 cells were further incubated with Mmur280 streptavidin magnetic beads, and then the biotin-coupled RNA complex was pulled down. After 4 h, the magnetic beads were cleaned, and the binding RNA complex on the magnetic beads was extracted by TRIzol reagent and analyzed by RT-PCR.

Statistical analysis

All the experiments were statistically analyzed by SPSS 22.0 and GraphPad Prism 7.0. T-test was used for comparison between the two groups, and single-factor analysis of variance was used for comparison between multiple groups. Pearson correlation analysis was used to evaluate the correlations between circSFMBT2 and miR-665, with P < 0.05 indicating a statistically significant difference between the two groups.

Results

Effects of paeonol on viability, apoptosis, migration, invasion and glutamine decomposition of human gastric cancer cells

After gastric cancer cells were treated with different concentrations of paeonol (0.0, 0.1, 0.2, 0.4 mg/ml), the results showed that with the increase of paeonol concentration, the cell viability, the number of migrating cells, the number of invasive cells and the expression of MMP2 and MMP9 protein decreased gradually, while the apoptosis rate increased gradually, as shown in Figure 1A-E. The decomposition of glutamine was evaluated by observing the expression levels of glutamine, glutamate and α-KG. The results showed that the expression levels of glutamine, glutamate and α-KG decreased gradually with the increase of paeonol concentration, as shown in Figure 1F-G.

Up-regulation of circSFMBT2 in gastric cancer tissues and gastric cancer cell lines

As shown in Figure 2A, circSFMBT2 was a circular structure derived from exon Exon5, 6, 7, 8. Compared with paracancerous tissues and normal gastric epithelial cell line GES-1, the expression of circSFMBT2 in gastric cancer tissue and gastric cancer cell line HGC-27 was significantly increased (Figure 2B-C). The circular stability of circSFMBT2 was verified by RNase R + restriction enzyme digestion test and the actinomycin D experiment. The results showed that the expression of circSFMBT2 did not change significantly, but the expression of linear SFMBT2 decreased significantly after the treatment of RNase R +. After the treatment of actinomycin D, the expression of circSFMBT2 was significantly higher than that of linear SFMBT2, indicating that circSFMBT2 has good ring stability, as shown in Figure 2D-E. In addition, we also detected the location of circSFMBT2 by RT-PCR, and the results showed that circSFMBT2 mainly existed in the cytoplasm, as shown in Figure 2F.

Effect of paeonol partially restored by circSFMBT2 on the biological function of gastric cancer cells

As shown in Figure 3A, after treated with different concentrations of paeonol (0.0, 0.1, 0.2, 0.4 mg/ml), the expression of circSFMBT2 decreased gradually in gastric cancer cells. According to the experimental results of Figure 1, this study carried out the follow-up experiment by selecting the concentration of paeo-
Effect of paeonol on gastric cancer cells via circSFMBT2/miR-665 axis.

At 0.4 mg/ml. Compared with the control group, circSFMBT2 expression, cell viability, number of migrating cells, number of invasive cells, expression of MMP2 and MMP9 protein, expression of glutamine,
glutamate and α-KG decreased significantly in the Pae group, while cell apoptosis rate increased significantly in the Pae+circSFMBT2 group. Compared with the Pae+vector group, circSFMBT2 expression, cell viability, the number of migratory cells, the number of invasive cells, expression of MMP2 and MMP9 protein increased significantly in the Pae+circSFMBT2 group. The expression of glutamine, glutamate and α-KG increased significantly, while the apoptosis rate decreased significantly, as shown in Figure 3B-J.

Targeted binding sites between circSFMBT2 and miR-665.

We detected the expression of circSFMBT2 after biotin transfection by RIP and RT-PCR assays. The results showed that the circSFMBT2 probe was enriched in the vector group and circSFMBT2 group, as shown in Figure 4A. In addition, as shown in the Venn diagram, there were four miRNA combined with circSFMBT2 predicted by circBank and Starbase, namely miR-107, miR-665, miR-4644 and miR-103a-3p. The expression of miRNA in si-circSFMBT2 transfected gastric cancer cell line HGC-27 was detected by RT-PCR assay. The results showed that miR-107, miR-665, miR-4644 and miR-103a-3p in the si-circSFMBT2 group were significantly higher than those in the si-NC group, and there was a significant difference in miR-665 (Figure 4B-C). The results of Figure 4D show that there were complementary nucleotide sequences between circSFMBT2 and miR-665. Compared with the miR-NC group, circSFMBT2 WT luciferase activity in the miR-665 group decreased significantly, while circSFMBT2 MUT had no significant change, as shown in Figure 4E.

Compared with paracancerous tissues and normal gastric epithelial cell line GES-1, the expression of miR-665 in gastric cancer tissue and gastric cancer cell line HGC-27 were significantly decreased, and there was a negative correlation between circSFMBT2 and miR-665, as shown in Figure 4F-H. As shown in Figure 4I, the expression of miR-665 increased gradually after treated with different concentrations of paeonol (0.0, 0.1, 0.2, 0.4 mg/ml) in gastric cancer cells.

Overexpression of miR-665 could partially restore the effects of paeonol and circSFMBT2 on the biological function of gastric cancer cells.

Compared with the control group, miR-665 and apoptosis rate were significantly increased, cell viability, the number of migrating cells, the number of invasive cells, expression of MMP2 and MMP9 protein, expression of glutamine, glutamate and α-KG were significantly decreased in the Pae group. Compared with Pae+vector, miR-665 and cell apoptosis rate decreased significantly, cell viability, the number of migrating cells, the number of invasive cells, the expression of MMP2 and MMP9 protein, and the expression of glutamine, glutamate and α-KG increased significantly in the Pae+circSFMBT2 group. Compared with Pae+circSFMBT2+miR-NC, miR-665 and apoptosis rate were significantly increased, cell viability, the number of migrating cells, the number of invasive cells, the expression of MMP2 and MMP9 protein, the expression of glutamine, glutamate and α-KG were significantly decreased in the Pae+circSFMBT2+miR-665 group (Figure 5A-J).

Discussion

Paeonol, also known as 2-hydroxy-4-methoxycacetophenone, is a class of small molecular phenolic compounds with a variety of pharmacological activities. In recent years, paeonol has achieved obvious results in anti-tumor, such as liver cancer, cervical cancer, breast cancer and gastric cancer (8). Lei et al. found that paeonol could enhance the radiosensitivity of lung adeno-

Figure 4. There were targeted binding sites between circSFMBT2 and miR-665. A. The expression of circSFMBT2 was detected after biotin transfection; B. Venn Diagram Wien graphic analysis software circBank and Starbase jointly predicted the expression level of miRNA in si-circSFMBT2 transfected with miRNA; C. Expression level of miRNA transfected with si-circSFMBT2; D. Starbase predicted the binding site between circSFMBT2 and miR-665; E. circSFMBT2 and miR-665 could bind to each other; F. The expression of miR-665 in gastric cancer; G. There was a negative correlation between circSFMBT2 and miR-665; H. Down-regulation of miR-665 expression in gastric cancer cell line HGC-27; I. Effects of different concentrations of paeonol (0.0, 0.1, 0.2, 0.4 mg/ml) on the expression of miR-665.
carcinoma by promoting radiation-induced apoptosis and inhibiting the PI3K/Akt pathway (9). Studies have shown that paeonol had an obvious inhibitory effect on the growth of breast cancer cells, and its mechanism may be related to its induction of apoptosis. CXCL4/CXCR3-B may promote apoptosis by regulating the expression of BACH1 and Nrf2 and down-regulating HO-1 (10). Paeonol has attracted wide attention in the treatment of gastric cancer. The results showed that paeonol could significantly inhibit the proliferation, migration and invasion of BGC823 cells, reduce the expression of MMP-2 and MMP-9 protein, and promote the apoptosis of SGC-7901 cells (11,12), which was similar to this study. The results showed that paeonol decreased the viability, invasion, migration, glutamine, glutamate and α-KG expression of HGC-27 cells in a dose-dependent manner, increased the apoptosis rate, and down-regulated the expression of MMP-2 and MMP-9 proteins, indicating that paeonol could significantly inhibit the proliferation, migration, invasion and glutamine decomposition of gastric cancer cells, and promote apoptosis.

CircRNA is a kind of non-coding RNA molecules with single-strand circular closure, which is highly conservative and stable. It can act as miRNAs sponges that regulate downstream targets, interact with genes and participate in the occurrence and development of a variety of cancers, including gastric cancer (13, 14). The results of Shen et al. (15) showed that the expression of circRNA_001569 was up-regulated and the expression of miR-145 was down-regulated in gastric cancer tissues and cells. Overexpression of circRNA_001569 could significantly inhibit the proliferation of gastric cancer cells and induce apoptosis. CircRNA_001569 plays a role in regulating the expression of miR-145 in gastric cancer, which is similar to the results of this study. The results of Liang et al. (16) showed that the expression of hsa_circ_006100 was up-regulated and the expression of miR-195 was down-regulated in gastric cancer, which was significantly related to tumor stage, cell differentiation and lymph node metastasis. Hsa_circ_006100 inhibits the proliferation, migration and invasion of extracellular cells and promotes apoptosis by regulating the miR-195/GPRC5A axis. The expression of circ_0000144 was up-regulated and the expression of miR-623 was down-regulated in gastric cancer tissues and cells. Interfering with circ_0000144 could inhibit the proliferation, migration, invasion and glutamine decomposition of gastric cancer cells by up-regulating miR-623, and promote cell apoptosis (17). MiR-665 is expressed in a variety of cancers and can play a role in cell proliferation, apoptosis, migration and invasion (18, 19). For example, miR-665 was down-regulated in gastric cancer cells, and miR-665 inhibited gastric cancer cell proliferation, invasion and epithelial-mesenchymal transformation by down-regulating PPP2R2A (20).
This study showed that the expression of circSFMBT2 was up-regulated and the expression of miR-665 was down-regulated in gastric cancer tissues and gastric cancer cells. After treated with paeonol, the expression of circSFMBT2 was down-regulated and the expression of miR-665 was up-regulated in HGC-27 cells. Overexpression of circSFMBT2 could partially restore the effects of paeonol on proliferation, apoptosis, migration, invasion and glutamine decomposition of gastric cancer cells. Further results showed that circSFMBT2 targeted negative regulation of miR-665 expression, and overexpression of miR-665 could partially restore the effects of paeonol and circSFMBT2 on gastric proliferation, apoptosis, migration, invasion and glutamine decomposition, indicating that paeonol plays a role in gastric cancer cells by regulating circSFMBT2/miR-665 axis. In general gastric cancer has many factors and components that need to be carefully evaluated (21-24).

Gastric cancer is the fourth most common cancer in the world and the second leading cause of death. Environmental factors as well as genetic factors play an important role in the development and progression of this disease. The most important of these is Helicobacter pylori, which is present in most cancerous tissues (25-30). With the advent of microRNAs in the field of genetic findings, these powerful molecules have opened their place in the field of genetic diseases. miRNAs are small, non-coding molecules involved in various cellular processes, such as cell differentiation and death (31-36).

To sum up, paeonol can inhibit the proliferation, migration, invasion and glutamine decomposition of gastric cancer, and induce apoptosis, and its mechanism may be related to the regulation of the circSFMBT2/miR-665 axis.

Acknowledgement
This work was supported by the Heilongjiang Youth Science Foundation (QC.2018113). Project name: Study on the anti-tumor mechanism of paeonol and metastasis of gastric cancer.

References
1. Choi YJ, Kim N. Gastric cancer and family history. Korean J Intern Med 2016; 31(6): 1042-1053.
2. Coccolini F, Nardi M, Montori G, et al. Neoadjuvant chemotherapies in advanced gastric and esophago-gastric cancer. Meta-analysis of randomized trials. Int J Surg 2018; 51: 120-127.
3. Tan Z. Recent advances in the surgical treatment of advanced gastric cancer: A review. Med Sci Monit 2019; 25: 3537-3541.
4. Zhang L, Li DC, Liu LF. Paeonol: pharmacological effects and mechanisms of action. Int Immunopharmacol 2019; 72: 413-421.
5. Lyu ZK, Li CL, Jin Y, Liu YZ, Zhang X, Zhang F, Ning LN, Liang ES, Ma M, Gao W, Zhang MX, Liu DS. Paeonol exerts potential activities to inhibit the growth, migration and invasion of human gastric cancer BGC-823 cells via downregulating MMP-2 and MMP-9. Mol Med Rep 2017; 16(5):7513-7519.
6. Li BJ, Jin MM, Cao FF, et al. Hsa_circ_0017639 expression promotes gastric cancer proliferation and metastasis by sponging miR-224-5p and upregulating USP3. Gene 2020; 750: 144753.
7. Wu KZ, Zhang CD, Zhang C, et al. miR-665 suppresses the epithelial-mesenchymal transition and progression of gastric cancer by targeting CRM1. Cancer Manag Res 2020; 12: 3489-3501.
8. Adki KM, Kulkarni YA. Chemistry, pharmacokinetics, pharmacology and recent novel drug delivery systems of paeonol. Life Sci 2020; 250:117544. doi: 10.1016/j.lfs.2020.117544. Epub 2020 Mar 13. PMID: 32179072.
9. Lei Y, Li HX, Jin WS, Peng WR, Zhang CJ, Bu LJ, Du YY, Ma T, Sun GP. The radiosensitizing effect of Paeonol on lung adenocarcinoma by augmentation of radiation-induced apoptosis and inhibition of the PI3K/Akt pathway. Int J Radiat Biol 2013; 89(12):1079-86.
10. Gao L, Wang Z, Lu D, Huang J, Liu J, Hong L. Paeonol induces cytoprotective autophagy via blocking the Akt/mTOR pathway in ovarian cancer cells. Cell Death Dis 2019; 10(8): 609.
11. Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. Mod Genet J 2020; 15(2): 75-92.
12. Fu J, Yu LH, Luo J, et al. Paeonol induces the apoptosis of the SGC-7901 gastric cancer cell line by downregulating ERBB2 and inhibiting the NF-κB signaling pathway. Int J Mol Med 2018; 42(3): 1473-1483.
13. Fang X, Wen J, Sun M, Yuan Y, Xu Q. CircRNAs and its relationship with gastric cancer. J Cancer. 2019; 10(24): 6105-6113.
14. Li R, Jiang JI, Shi H, et al. CircRNA: a rising star in gastric cancer. Cell Mol Life Sci 2020; 77(9): 1661-1680.
15. Shen FQ, Liu PJ, Xu ZQ, et al. CircRNA_001569 promotes cell proliferation through absorbing miR-145 in gastric cancer. J Biochem, 2019; 165(1): 27-36.
16. Liang M, Huang GQ, Liu ZY, et al. Elevated levels of hsa_circ_006100 in gastric cancer promote cell growth and metastasis via miR-195/GPRC5A signalling. Cell ProliF 2019; 52(5): e12661.
17. Mi LL, Lei LH, Yin XL, et al. CircRNA_0000144 functions as a miR-623 sponge to enhance gastric cancer progression via up-regulating GPRC5A. Biosci Rep 2020; 40(8): BSR20201313.
18. Fan JH, Li HP, Nie X, et al. MiR-665 aggravates heart failure via suppressing CD34-mediated coronary microvessel angiogenesis. Aging (Albany NY) 2018; 10(9): 2459-2479.
19. Dong CH, Du QY, Wang ZM, et al. MicroRNA-665 suppressed the invasion and metastasis of osteosarcoma by directly inhibiting RAB2B. Am J Transl Res 2016; 8(11): 4975-4981.
20. Zhang MJ, Wang S, Yi AW, et al. microRNA-665 is down-regulated in gastric cancer and inhibits proliferation, invasion, and EMT by targeting PPP2R2A. Cell Biochem Funct 2020; 38(4): 409-418.
21. association between gastric cancer and hopQ alleles in Helicobacter pylori. Genetika 2016; 48(3): 893-902 Kazemi E, Kahrizi D. Lack of
22. Kazemi E, Kahrizi D, Moradi MT, Sohrabi M, Yari K. Gastric cancer and Helicobacter pylori: impact of hopQ1 gene. Cell Mol Biol 2016; 62(2): 107-110.
23. Kazemi E, Kahrizi D, Moradi MT, Sohrabi M, Amini A, Moussavi SAR, Yari K. Association between Helicobacter pylori hopQ1 genotyping and human gastric cancer. Cell Mol Biol 2016; 62(1): 6-9.
24. Kazemi E, Kahrizi D, Moradi MT, Sohrabi, M, Amini S, Moussavi S.A.R., Yari K. Association between Manganese Superoxide Dismutase (MnSOD Val-9Ala) genotypes with the risk of generalized aggressiveness of periodontitis disease. Cell Mol Biol 2016; 61 (8): 49-52.
25. Zhang J, Liu B. A review on the recent developments of sequence-based protein feature extraction methods. Curr Bioinform 2019;14(3):190-9. DOI: 10.2174/1574893614666181212102749.
26. Xu L, Jiang S, Zou Q. An in silico approach to identification, categorization and prediction of nucleic acid binding proteins. bioRxiv. 2020. DOI: 10.1093/bib/bbaa171.
27. Zhu S, Wang X, Zheng Z, Zhao XE, Bai Y, Liu H. Synchronous measuring of triiodoide changes in rat brain and blood and its application to a comparative pharmacokinetic study in normal and Alzheimer’s disease rats. Journal of Pharmaceutical and Biomedical
Analysis. 2020; 113263. 10.1016/j.jpba.2020.113263.
28. Chen G, Li Y, Ren Z, Gu Y, Tang F, Mao J, Zhu J, Wang L, Li Y. Clinical Significance of MicroRNA-155-Regulated Autophagy and Apoptosis by Targeting Rictor/Fos in Gastric Cancer Progression. Nanosci Nanotechnol Lett 2020; 12(4):525-35.
29. Alkhudhayri AA, Wahab R, Siddiqui MA, Ahmad J. Selenium Nanoparticles Induce Cytotoxicity and Apoptosis in Human Breast Cancer (MCF-7) and Liver (HepG2) Cell Lines. Nanosci Nanotechnol Lett 2020;12(3):324-30.
30. Jin Y, Zhu H, Zhu H, Jin F, Shi C, Yang L, Qian J, Zhang S. Fluorouracil Nanoliposomes Promote Apoptosis of Human Gastric Cancer Xenografts in Nude Mice. Nanosci. Nanotechnol. Lett 2020; 12: 690–695.
31. Lin J, Wang Y, Wei X, Kong S, Liu Z, Liu J, Zhang F, Lin S, Ji B, Zhou Z, Guo Z. Controllable antibacterial and bacterially anti-adhesive surface fabricated by a bio-inspired beetle-like macromolecule. Int J Biol Macromol 2020. Doi: 10.1016/j.ijbiomac.2020.04.207.
32. Zhang D, Lu Z, Sun B. Highly Sensitive Gold Nanoparticle-Polymerase Chain Reaction in the Detection of Anaplastic Lymphoma Kinase-Positive Gastric Cancer from a Biopsy Specimen. Nanosci Nanotechnol Lett 2020; 12(4):498-505.
33. Wu Y, Liu C, Gao M, Liang Q, Jiang Y. Effect of Titanium Nanoparticles on Osteoblast Proliferation. Nanosci Nanotechnol Lett 2020;12(4):455-60.
34. Bai J, Guo T, Dong W, Song Y, Guo T, Cui M. Reduction of Breast Cancer Lymph Node Metastasis by Nano-Carbon Absorption of 5-Fluorouracil. Nanoscience and Nanotechnology Letters. 2020 Mar 1;12(3):407-12.
35. Zhang T, Su H, Xing Y, Zhang J, Xu D. Protective Mechanism of Lipid-Lowering Ketone and Self-Assembled OA Chitosan Nanoparticles on Insulin Oxidative Stress. Induced by High Fat in BRL-3A Cells. Nanosci Nanotechnol Lett 2020; 12: 715–719.
36. Kazemi E, Zargooshi J Kaboudi M, Heidari P, Kahrizi D, Mahaki B, Mohammadian Y, Khazaeei H, Ahmed K. A genome-wide association study to identify candidate genes for erectile dysfunction. Brief Bioinform 2020; bbaa338, https://doi.org/10.1093/bib/bbaa338.