Genetic Markers Indicate that 1,25-dihydroxyvitamin D Treatment may not Protect Against Hepatocellular Carcinoma

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Abstract

Objectives: The impact of 1,25-dihydroxyvitamin D on hepatocellular carcinoma (HCC) cells is a complicated area. In this study, we aimed to clarify the effect of 1,25-dihydroxyvitamin D on HCC cells according to genetic markers.

Methods: The optimal concentration of 1,25-dihydroxyvitamin D is treated to HepG2 cells (250 nM at the 48th hour). From treated HepG2 cells, total Ribonucleic Acid (RNA) was isolated, and Ki-67, MMP-2, MMP-9, HIF-1α, hTERT, and piR-823 gene expressions were determined by SYBR Green-based real-time polymerase chain reaction.

Results: Increased expressions of Ki-67, hTERT, and piR-823 were determined compared with the control group at the 48th hour after treatment (p<0.001), while decreased gene expressions of MMP-2, MMP-9, and HIF-1α were observed compared with the control group (p<0.001).

Conclusion: Currently, there are several different opinions about the usage of vitamin D, especially in HCC. In addition to researchers who argue that vitamin D has anticarcinogenic and protective properties, an increasing number of researchers argue that tumor cells can become aggressive after HCC occurs. According to our results, it was determined that vitamin D causes HepG2 HCC cells to become aggressive in terms of gene expression in the parameters used as a marker for proliferation, adhesion, and differentiation.

Keywords: 1.25-Dihydroxyvitamin D, hepatocellular carcinoma, motility, PIWI interacting RNA, proliferation

Hepatocellular carcinoma (HCC) is the fourth lethal cancer type worldwide. It can be treated easily if it is diagnosed at early stages. Nowadays, some natural compounds, synthetic essential molecules, and vitamins are used to protect against cancer. Vitamin D is a lipophilic molecule, and some forms can be used directly in the liver and kidney. 1,25-Dihydroxyvitamin D is the active form of vitamin D and acts on the molecular mechanisms of cells. Some previous studies suggested that 1,25-dihydroxyvitamin D is used to protect against cancer, especially HCC. However, recent studies indicated that 1,25-dihydroxyvitamin D may have negative effects on cells and suggested that 1,25-dihydroxyvitamin D usage cannot be effective for treatment during carcinogenesis.

Ki-67 is a transcription factor, and its expression increases in cancer cases. Ki-67 expression has been known as a biomarker of proliferation. High Ki-67 expression, as well as hypoxia-inducible factor-1 alpha (HIF-1α) expression, results in low differentiation and poor prognosis in HCC. HIF-1α is another transcription factor and prognostic marker that is correlated with a higher rate in lymph node metastasis for HCC. Matrix metalloproteinases (MMPs)
are special enzymes that degrade the extracellular matrix and promote angiogenesis and tumor invasion and migration. When the extracellular matrix is disrupted, cancer cells begin to invade adjacent tissues, and metastasis occurs.\[^{[14]}\] \( \text{MMP-2} \) and \( \text{MMP-9} \) are functional MMP members that have high gene expression patterns during metastasis and invasion of cancer cells.\[^{[15]}\] The human telomerase reverse transcriptase (\( \text{hTERT} \)) gene region is another proliferation marker of cancer cells. By determining the expression changes of \( \text{hTERT} \), researchers can have an idea of cancer cell proliferation and survival. \( \text{hTERT} \) is a catalytic enzyme that regulates telomerase activity. In cancer cells, approximately 90% of tumors have high \( \text{hTERT} \) expression. \( \text{hTERT} \) indicates not only telomerase activity but also proliferation rates of cancer cells.\[^{[16]}\] PIWI-interacting RNAs (piRNAs; piRs) are small noncoding RNAs that are 24-31 nucleotides in length. piRNAs act to regulate target genes by transposon silencing and reduce proliferation and proangiogenic activity of cancer cells or promote apoptosis. They can be classified as tumor-suppressive or oncogenic piRNAs according to their functions. piR-823 is a member of the piRNA family and has an oncogenic activity in cancer cells.\[^{[17]}\]

In this study, we aimed to determine the effect of 1,25-dihydroxyvitamin D on HepG2 HCC cells according to gene expressions of various markers. We observed the proliferation changes by detecting \( \text{Ki-67} \), \( \text{hTERT} \), and \( \text{HIF-1a} \) gene expressions, while the adhesion of cells was detected by \( \text{MMP-2} \) and \( \text{MMP-9} \) gene expressions. Moreover, we identified that 1,25-dihydroxyvitamin D causes the upregulation of an oncogenic piRNA expression, piR-823. These data are significant for HCC researchers to understand what 1,25-dihydroxyvitamin D treatment causes cellular behaviors in a genetic perspective.

**Methods**

**Cell Culture and 1,25-Dihydroxyvitamin D Treatment:** HepG2 HCC cell line (ATCC, Washington D.C., USA) was maintained in a humidified incubator with 5% CO\(_2\) at 37°C. Dulbecco’s Modified Eagle’s Medium with phenol red (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS, Capricorn, Germany) and 1% penicillin/streptomycin (Capricorn, Germany) was used to culture HepG2 cells.

Before treatment of 4-hydroxycoumarin (Sigma, USA) to HepG2 cells, 7\(\times\)10\(^3\) cells were plated into each well of 96-well plate (SPL, Korea). According to our previous study, the detected IC\(_{50}\) concentration of 1,25-dihydroxyvitamin D was treated to HepG2 HCC cells.\[^{[18]}\] IC\(_{50}\) concentration of 1,25-dihydroxyvitamin D was 250 nM at the 48th hour.

**Total RNA Isolation and Real-Time Polymerase Chain Reaction:** Total RNA was isolated according to the manufacturer’s instructions of NucleoSpin RNA Kit (Macchery-Nagel, Germany). Total RNA was converted to cDNA using a reverse transcription kit (Bioneer AccuPower, Korea). In the obtained cDNA, \( \text{piR-823, Ki-67, MMP-2, MMP-9, HIF-1a, and hTERT} \) expressions were determined using real-time polymerase chain reaction (RT-PCR) (LightCycler96, Roche, USA). Glyceraldehyde-3-phosphate was used as an internal control. SYBR Green-based primers were designed and supplied by Oligomer (Ankara, Turkey). RT-PCR was conducted at the following conditions: pre-denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 10 s, and annealing/extension at 60°C for 30 s. Gene expressions were calculated using the delta-delta CT (\( \Delta\Delta\text{CT} \)) formula. The primer sequences are shown in Table 1.

**Statistical Analysis**

Normal distribution of continuous variables was determined using the Shapiro-Wilk suitability test. Comparisons between groups of normally distributed variables were evaluated using one-way analysis of variance. Multiple comparisons of gene expressions were performed using Student’s t-test. All statistical analysis was performed using IBM SPSS Statistics 21.0 software package at Eskisehir Ozmangazi University, Department of Statistics, Turkey. Data are presented as mean±standard deviation. In the figures, only the mean values have been shown.

| Gene name | Forward sequence | Reverse sequence |
|-----------|------------------|------------------|
| piR-823   | 5′-AGCGTTTGGTTGTATAGTGGT-3′ | 5′-CTTATGCGCGCTGGGACCCTGACC-3′ |
| Ki-67     | 5′-TCTTGGGGGACCTAAGGCGTC-3′ | 5′-AGTTGTTGAGCGTCCTGTTAGTG-3′ |
| MMP-2     | 5′-TCTCTGACATGGCACTTGG-3′ | 5′-CAAGTGCTGGCTGAGTATAGCATC-3′ |
| MMP-9     | 5′-TTGACACGGACAAGAAGTGG-3′ | 5′-GACATTACGCTGCTTCCTAT-3′ |
| HIF-1a    | 5′-GGGCCGGAACGCAAAGAAAG-3′ | 5′-CTTATCAAGATCGGAACCTCACA-3′ |
| hTERT     | 5′-TCTGACACCTACCTACCCAC-3′ | 5′-CACTGCTCTCCAGCAAATTCAC-3′ |
| GAPDH     | 5′-CGAGGCGGCGACGAAAAAGG-3′ | 5′-GGCCAGCCCGACGCTCAAAG-3′ |

\( \text{piR-823: PIWI Interacting RNA-823; MMP-2: Matrix Metalloproteinase-2; MMP-9: Matrix Metalloproteinase-9; HIF-1a: Hypoxia-inducible factor-1 alpha; hTERT: The human telomerase reverse transcriptase; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.} \)
Results

According to our data, Ki-67 gene expression was up-regulated in the 1,25-dihydroxyvitamin D-treated group (0.73±0.013) compared with the control (-2.6±0.089; Fig. 1a; p<0.001). Furthermore, the upregulation of hTERT gene expression was observed in the 1,25-dihydroxyvitamin D-treated group (0.547±0.006) compared with the control (-0.203±0.01; Fig. 1b; p<0.001). High piR-823 expression was detected after 1,25-dihydroxyvitamin D treatment (1.169±0.003) compared with the control (0.797±0.003; Fig. 1c; p<0.001).

MMP-2 and MMP-9 gene expressions were downregulated in the 1,25-dihydroxyvitamin D-treated group (0.447±0.014 and -0.35±0.007) compared with the control statistically (0.68±0.017 and 0.18±0.008; Fig. 2a and 2b; p<0.001). Moreover, the downregulation of HIF-1α gene expression was detected in the 1,25-dihydroxyvitamin D-treated HepG2 cells (0.06±0.005) compared with the control (0.207±0.008; Fig. 2c; p<0.001).

Discussion

Vitamin D is a lipophilic vitamin, and its 1,25-dihydroxyvitamin D form is the active form that can be used in essential molecular mechanisms of cells. Vitamin D has a significant role in immune system regulation, proliferation, differentiation, and apoptosis of cells under physiological and pathological conditions.[19] 1,25-Dihydroxyvitamin D enforces molecular mechanisms through vitamin D receptor (VDR) activation.[20] 1,25-Dihydroxyvitamin D binding to VDR makes conformational changes that facilitate 25(OH)D3 with several transcriptional factors in the nucleus. The interaction of 25(OH)D3 with transcriptional factors activates gene transcription.[21] This gene region may be a region that is related to apoptosis, proliferation, differentiation, or adhesion characteristics of cells. In recent studies, it has been argued that vitamin D cannot have any antiproliferative therapeutic effect in liver cancer according to its characteristic features and cellular mechanism.[22,23] Lappe et al.[24] indicated that vitamin D and Ca supplements cause cancer in older women. Pivonello et al.[25] (2016) reported that vitamin D administration may cause increased proliferation of JHH6 HCC cells and suggested that vitamin D is a mitogen in HCC cells. 1,25-Dihydroxyvitamin D have an impact on different gene regions. In a study, HIF-1α induced colon cancer cells, and 1,25-dihydroxyvitamin D treatment reduced cellular proliferation through HIF-1α and VEGF.[23] 1,25-Dihydroxyvitamin D modulated cytokine-induced MMP synthesis and collagen degradation by human lung fibroblasts.[26] Moreover, 1,25-dihydroxyvitamin D promoted cell differentiation and decreased telomerase activity in different healthy cell lines.[27,28]

Generally, Ki-67, a cell proliferation antigen, is used in cancer histopathology.[29] In a study, Ki-67 expression varies due to cell-cycle regulation via CDK4/CDK6 on cellular proliferation.[29] Antibodies raised against the Ki-67 protein which is important for evaluating cell proliferation immunohistologically, particularly useful on the prognostic value of cell growth in clinical specimens of human neoplasms.[30] To identify the proliferation of HepG2 cells, especially, the upregulation of Ki-67 gene expression showed that 1,25-di-
hydroxyvitamin D treatment increases proliferation. hTERT induced the activity of telomerase. In cancer cells, high telomerase activity and hTERT expression were observed.\cite{31,32} 1,25-Dihydroxyvitamin D treatment caused HepG2 cells to increase hTERT expression. High hTERT gene expression supported the increase in the proliferation of HepG2 cells. Generally, high hTERT expression caused HepG2 cells to survive and proliferate. To determine the adhesion changes of cells, MMP-2 and MMP-9 gene expressions were observed. As a result of 1,25-dihydroxyvitamin D treatment, the adhesion functions were increased due to low MMP-2 and MMP-9 expressions. HIF-1α gene expression was used to determine the differentiation of cells after 1,25-dihydroxyvitamin D treatment. Low HIF-1α expression supplied high survival of HepG2 cells. HIF-1α is widely used to identify the transition of epithelial cells to mesenchymal cells.\cite{13,25} Decreased HIF-1α expressions suggested that 1,25-dihydroxyvitamin D treatment caused HepG2 cells to increase their adhesive properties. This high adhesive property was also supported by observing low MMP-2 and MMP-9 gene expressions.

Epigenetic mechanisms can be classified as DNA methylation, histone modifications, and small noncoding RNAs, which cannot change the nucleotide sequences but can change the expression patterns of genes. piRNAs are the novel members of small noncoding RNAs and are members of epigenetic mechanisms. piRNAs especially target for expressing or repressing methylated or acetylated regions of DNA.\cite{34} In vitamin D intracellular signaling, VDR and CYP genes have large CpG islands where suitable places for DNA methylation are in their promoter regions. Furthermore, histone modifications (acetylation, phosphorylation, etc.) are altered by VDR and its target genes. This is evidence that increased levels of methylation in the VDR gene can lead to altered transcription and disruption of vitamin D synthesis associated with various diseases.\cite{19,35} The relationship between VDR genes, DNA methylation, and histone modifications is also evidence that these epigenetically variable gene regions may effect piRNA expressions. According to this perspective, we wanted to observe piR-823 expression changes after 1,25-dihydroxyvitamin D treatment. piR-823 is one of the first investigated piRNA region that has an oncogenic activity in various cancer cells. piR-823 is effective on the expression of HSP27, HSP60, and HSP70 simultaneously to promote proliferation and inhibit apoptosis by binding to common transcription factors HSF1.\cite{36} piR-823 is a predetermined type of piRNA in gastric cancer. Studies on piR-823 reported that the level of expression of piR-823 was positively associated with lymph node metastasis and distant metastasis.\cite{37,38} piR-823 inhibition could effectively inhibit tumor growth in therapeutic xenograft models and offer an encouraging strategy for the treatment of multiple myeloma.\cite{39} Moreover, piR-823 expression is a significant point of our research. piR-823 acted as an oncogene after 1,25-dihydroxyvitamin D treatment. 1,25-Dihydroxyvitamin D treatment resulted in detecting high piR-823 expression. These findings are preliminary in vitro results of active vitamin D application for HCC. According to our results, 1,25-dihydroxyvitamin D form may not be beneficial for...
anticancer usage after being HCC. Moreover, increase in proliferation, adhesion, and telomerase activity indicated that this treatment made HCC cells to become more adhesive instead of healing. These results are significant for understanding the impact of the active form of vitamin D on genetic biomarkers of cellular carcinogenesis mechanisms as proliferation, adhesion, differentiation, and telomerase activity. Furthermore, it is indicated that the active form of vitamin D may affect the epigenetic mechanisms of cells via piR-823 expression, which is oncogenic in several cancers. This result is preliminary, which presents the relationship between 1,25-dihydroxyvitamin D and piRNA in HCC cells. We suggest that vitamin D usage after being HCC is harmful for individuals in the treatment of cancer according to in vitro results.

Disclosures

Ethics Committee Approval: It was a cell culture study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – O.C., E.C.; Design – O.C.; Supervision – O.C.; Materials – O.C.; Data collection &/or processing – O.C., E.C.; Analysis and/or interpretation – O.C., E.C.; Literature search – O.C.; Writing – O.C., E.C.; Critical review – O.C., E.C.

References

1. Manson JE, Mayne ST, Clinton SK. Vitamin D and prevention of cancer--ready for prime time? N Eng J Med 2011;364:1385–7.
2. Farhan M, Rizvi A, Naseem I, Hadi SM, Ahmad A. Targeting increased copper levels in diethylnitrosamine induced hepatocellular carcinoma cells in rats by epigallocatechin-3-gallate. Tumour Biol 2015;36:8861–7.
3. Chiang KC, Yeh CN, Chen MF, Chen TC. Hepatocellular carcinoma and vitamin D: a review. J Gastroenterol Hepatol 2011;26:1597–603.
4. McGlynn KA, London WT. The global epidemiology of hepatocellular carcinoma: present and future. Clin Liver Dis 2011;15:223–43, vii–x.
5. Mahmoud AM, El-Shemy HA. Cytotoxic profiling of some compounds of natural origin against HepG2 liver cancer cell line in-vitro. J Arid Land 2012;22:191–4.
6. Glowka E, Stasiak J, Lulek J. Drug delivery systems for vitamin D supplementation and therapy. Pharmaceutics 2019;11:347.
7. Pivonello C, Provvisiero DP, Negri M, Di GG, De AC, Galdiero G. Potential role of vitamin D in restoring sensitivity to mTOR inhibitors in hepatocellular carcinoma (HCC): 1,25(OH)vitamin D (VITD) reverts everolimus (EVE) resistance in a HCC cell line. Endocrine Abstracts 2016;41.
8. Pourgholami MH, Akhter J, Lu Y, Morris DL. In vitro and in vivo inhibition of liver cancer cells by 1,25-dihydroxyvitamin D3. Cancer Lett 2000;151:97–102.
9. Fingas CD, Altinbas A, Schlattjan M, Beifuss A, Sowa JP, Sydor S, et al. Expression of apoptosis- and vitamin D pathway-related genes in hepatocellular carcinoma. Digestion 2013;87:176–81.
10. Huang J, Yang G, Huang Y, Kong W, Zhang S. 1,25(OH)2D3 inhibits the progression of hepatocellular carcinoma via down-regulating HDAC2 and upregulating P21(WAF1/CIP1). Mol Med Rep 2016;13:1373–80.
11. Huang J, Yang G, Huang Y, Zhang S. 1,25(OH)2D3 induced apoptosis of human hepatocellular carcinoma cells in vitro and inhibited their growth in a nude mouse xenograft model by regulating histone deacetylase 2. Biochimie 2018;146:28–34.
12. Schmitt-Graff A, Ertelt V, Allgaier HP, Koelble K, Olschewski M, Nitschke R, et al. Cellular retinol-binding protein-1 in hepatocellular carcinoma correlates with beta-catenin, Ki-67 index, and patient survival. Hepatology 2003;38:470–80.
13. Zhang JG, Zhou HM, Zhang X, Mu W, Hu JN, Liu GL, et al. Hypoxic induction of vasculogenic mimicry in hepatocellular carcinoma: role of HIF-1 alpha, RhoA/ROCK and Rac1/PAK signaling. BMC cancer 2020;20:32.
14. Lin XL, Li K, Yang Z, Chen B, Zhang T. Dulcitol suppresses proliferation and migration of hepatocellular carcinoma via regulating SIRT1/p53 pathway. Phytomedicine 2020;66:153112.
15. Huang S, Van Arsdall M, Tedjarati S, McCarty M, Wu W, Langley R, et al. Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice. JNCI 2002;94:1134–42.
16. Choi SH, Cho KJ, Yun SH, Jin B, Lee HY, Ro SW, et al. HKR3 regulates cell cycle through the inhibition of hTERT in hepatocellular carcinoma cell lines. J Cancer 2020;11:2442–52.
17. Öner Ç, Two different mechanisms of two different non-coding RNAs—MicroRNAs and PIWI-interacting RNAs: From origin to cancer. In: Mallick B, editor. AGO-Driven Non-Coding RNAs. 1st ed. Cambridge, Massachusetts: Elsevier; 2019. p. 3–34.
18. Öner Ç, Isan H, Aktaş RG, Çolak E. The effect of Vitamin D on hepatocellular carcinoma. Osmangazi Journal of Medicine 2020;42:301–10.
19. Mankgopo MK, Hairwadzi H. Role of vitamin D levels in viral infections and possible epigenetic alterations. Cellular Immunology & Immunotherapeutics 2016;1:1–6.
20. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res 1998;13:325–49.
21. Umesono K, Murakami KK, Thompson CC, Evans RM. Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. Cell 1991;65:1255–66.
22. Jenkins K. Vitamin D does not prevent cancer: Study. Medscape Medical News; 2018. Available at: https://www.med-
scape.com/viewarticle/899716#:~:text=Conflicting%20Results,lung%20cancer%2C%20even%20in%20nonsmokers. Accessed May 26, 2021.

23. Scragg R, Khaw KT, Toop L, Slayter J, Lawes CMM, Waayer D, et al. Monthly high-dose vitamin D supplementation and cancer risk: a post hoc analysis of the vitamin D assessment randomized clinical trial. JAMA Oncol 2018;4:e182178.

24. Lappe J, Watson P, Travers-Gustafson D, Recker R, Garland C, Gorham E, et al. Effect of vitamin D and calcium supplementation on cancer incidence in older women: a randomized clinical trial. JAMA 2017;317:1234–43.

25. Ben-Shoshan M, Amir S, Dang DT, Dang LH, Weisman Y, Mabjeesh NJ. 1alpha,25-dihydroxyvitamin D3 (Calcitriol) inhibits hypoxia-inducible factor-1/vascular endothelial growth factor pathway in human cancer cells. Mol Cancer Ther 2007;6:1433–9.

26. Kim SH, Baek MS, Yoon DS, Park JS, Yoon BW, Oh BS, et al. Vitamin D inhibits expression and activity of matrix metalloproteinase in human lung fibroblasts (HFL-1) cells. Tuberc Respir Dis (Seoul) 2014;77:73–80.

27. Kang SN, Kim SH, Chung SW, Lee MH, Kim HJ, Kim TS. Enhancement of 1 alpha,25-dihydroxyvitamin D(3)-induced differentiation of human leukemia HL-60 cells into monocytes by parthenolide via inhibition of NF-kappa B activity. Br J Pharmacol 2002;135:1235–44.

28. Zarei M, Zarezadeh M, Hamedi Kalajahi F, Javanbakht MH. The relationship between vitamin D and telomere/telomerase: a comprehensive review. J Frailty Aging 2021;10:2–9.

29. Sobecki M, Mrouj K, Colinge J, Gerbe F, Jay P, Krasinska L, et al. Cell-cycle regulation accounts for variability in Ki-67 expression levels. Cancer Res 2017;77:2722–34.

30. Endl E, Gerdes J. The Ki-67 protein: fascinating forms and an unknown function. Exp Cell Res 2000;257:231–7.

31. Liu X, Wang Y, Chang G, Wang F, Wang F, Geng X. Alternative splicing of hTERT Pre-mRNA: a potential strategy for the regulation of telomerase activity. Int J Mol Sci 2017;18:567.

32. Zhang Y, Toh L, Lau P, Wang X. Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/β-catenin pathway in human cancer. J Biol Chem 2012;287:32494–511.

33. Leao R, Apolonio JD, Lee D, Figueiredo A, Tabori U, Castelo-Branco P. Mechanisms of human telomerase reverse transcriptase (hTERT) regulation: clinical impacts in cancer. J Biomed Sci 2018;25:22.

34. Xiao Z, Shen J, Zhang L, Li M, Hu W, Cho C. Therapeutic targeting of noncoding RNAs in hepatocellular carcinoma: Recent progress and future prospects. Oncol Lett 2018;15:3395–402.

35. Zhu H, Wang X, Shi H, Su S, Harshfield GA, Gutin B, et al. A genome-wide methylation study of severe vitamin D deficiency in African American adolescents. J Pediatr 2013;162:1004–9.e1.

36. Yin J, Jiang XY, Qi W, Ji CG, Xie XL, Zhang DX, et al. piR-823 contributes to colorectal tumorigenesis by enhancing the transcriptional activity of HSF1. Cancer Sci 2017;108:1746–56.

37. Cui L, Lou Y, Zhang X, Zhou H, Deng H, Song H, et al. Detection of circulating tumor cells in peripheral blood from patients with gastric cancer using piRNAs as markers. Clin Biochem 2011;44:1050–7.

38. Cheng J, Deng H, Xiao B, Zhou H, Zhou F, Shen Z, et al. piR-823, a novel non-coding small RNA, demonstrates in vitro and in vivo tumor suppressive activity in human gastric cancer cells. Cancer Lett 2012;315:12–7.

39. Yan H, Wu QL, Sun CY, Ai LS, Deng J, Zhang L, et al. piRNA-823 contributes to tumorigenesis by regulating de novo DNA methylation and angiogenesis in multiple myeloma. Leukemia 2015;29:196–206.