Saudi Journal of Medicine (SJM)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: www.saudijournals.com

ISSN 2518-3389 (Print)
ISSN 2518-3397 (Online)

Emerging Role of microRNAs in Osteosarcoma- A Diagnostic, Prognostic, Therapeutic Prospecutive

Dr. Smita Sharma*, MD (Biochemistry), DTCD, Mr. Rami Abdulla Ali Al Dagrer, Dr. Sumant Sharma, MD
Clinical Biochemist, Maternity and Children Hospital, Nejran, KSA

***Corresponding author**
Dr. Smita Sharma

Article History
Received: 09.01.2018
Accepted: 20.01.2018
Published: 30.03.2018

DOI:
10.36348/sjm.2018.v03i03.002

Abstract: Osteosarcoma (OS), the most common primary malignant bone tumor in both children and adults, is characterized by the development of bone or osteoid substance by the tumor cells [1]. Despite of Surgical treatments and combinational chemotherapy, long term survival remains unsatisfactory because of delay in diagnosis, distant metastasis and chemoresistence. Thus, in recent years, it has become one of the most promising fields to investigate molecular mechanisms contributing to osteosarcoma carcinogenesis and progression. Several biomarkers have been proposed to predict the evolution of osteosarcoma including survivin, ErbB2, Ki67 antigen, alpha V integrins, Tumor Endothelial Marker (TEM7), IGF1, Leptin, micro RNA. Recent advances in expression biology have shifted in identifying and developing specific and sensitive biomarkers such as micro RNAs. Circulating micro RNA is emerging as promising noninvasive biomarkers for human cancers. In this review,we provide a perspective on emerging concepts and potential usefulness of microRNA as diagnostic, prognostic markers in OS and involvement of specific microRNA in OS metastasis. We tried to discuss the genetic mechanisms and molecular pathways involved by aberrant microRNA in OS. MiRNA-directed gene regulation will pave the way for improving traditional gene therapy approaches in many cancers. Moreover, since therapeutic targeting of miRNAs promises to improve the clinical management of patients with OS.
Keywords: micro RNA, OS, Metastasis.

INTRODUCTION

Osteosarcoma is aggressive disease that occurs predominantly in adolescent and young adults. Osteosarcoma is an aggressive sarcoma of the bone characterized by a high level of genetic instability and recurrent DNA deletions and amplifications. MiRNAs play an important role in cancer cell growth and migration. Detection of miRNA in serum and tissue samples may be helpful in early diagnosis, prognosis, and staging and even in chemotherapeutic resistence. microRNA (abbreviated miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) that functions in RNA silencing and post-transcriptional regulation of gene expression by translational repression or target messenger RNA degradation.

Current status of microRNA in Osteosarcoma and Molecular mechanisms involved in Pathogenesis

MiRNAs are known to function as both oncogenes as well as tumor suppressors in OS and influence the phenotypic characteristic of OS cells via regulation of their target genes. The most critical pathways in Osteosarcoma pathogenesis are the Notch, Wnt, NF-κB, p53, PI3K/Akt, and MAPK pathways. The balance between cell survival and apoptosis is determined by the Wnt and NF-κB pathways, as well as by the ratio between the activities of the MAPK and PI3K/Akt pathways. Several miRNAs (miRNA-21, -34a, -143, -148a, -195a, -199a-3p, -382) regulate multiple target genes, pathways, and processes essential for osteosarcoma pathogenesis. Tao Luo *et al*. showed that hsa-miRNA-27a-3p,hsa-miRNA-9-5p,hsa–miRNA-182-5p,FRS2,COROIC,FOXP1and CPEB4 were identified as node genes with high degrees of association in the miRNA-gene regulatory network and may serve a role in pathogenesis and development of osteosarcoma[2].

MiRNA 138 act as tumour suppressor by inhibiting Differentiated embryogenic chondrogenic gene 2(DEC2.MiR-138/DEC2 may be a novel therapeutic target in osteosarcoma [3]. MiRNA 21 act as oncomir,its high levels are associated with initiation and progression of cancer.miR-21 targets code for tumor suppressors, with a role in inhibiting cell signaling, cell proliferation and migration, e.g. phosphatase and tensin homolog (PTEN) tumor suppressor[4](Fig.1).

Vanita *et al*. showed that the miRNA-21 regulatory network plays a role in tumorigenesis of osteosarcoma. Its expression facilitates cell proliferation and decreases cellular sensitivity towards cisplatin. Both effects can be rescued by Spry2, a target protein downregulated by increased miR-21 levels This shows

that miRNA can function as novel target for Cancer treatment [5] Yuan *et al*. showed that high serum level of miRNA-21 is correlated to the advanced Enneking stage of tumors and also as a predictive marker for chemotherapeutic resistance and unfavorable prognostic factor for overall survival [6].

Ming Xu *et al*. showed that 181 b served as onco-miRNA via direct targeting and suppressing RASSF8 expression may be a promising therapeutic target of osteosarcoma.RASSF8 is a member of the Ras-association domain family, several of which are believed to Tumour suppressor genes[7]. Both vivo and vitro studies conducted by Wang *et al*. [8]showed that overexpression of RASSF8 inhibited melanoma cells growth,migration and inhibition as a result of downregulating P65 which lead to G1-S arrest and apoptosis induction through increasing p53 and p21 expression.In addition,following RASSF8 depletion can increase its accumulation in the cytoplasm in the cytoplasm and then delocalized to the nucleus to activate the canonical Wnt signaling pathway[9,10].The detailed molecular mechanisms of RASSF8 and whether miRNA- 181b involved in these pathways in OS should be investigated in future.

Shi-hong Xu *et al*. showed that increased miR-9 expression could be a valuable marker of tumor progression and for prognosis of osteosarcoma [11]. Cao *et al*. reported upregulation of miR-802 in OS tumor tissue and induced proliferation by regulating p27 expression, thereby classifying miR-802 as an onco-miRNA. At the molecular level, their results further revealed that expression of p27, a negative cell-cycle regulator, was negatively regulated by miR-802 [12].

Lie-Dao Yu *et al*. showed that higher expression of miR-130b is correlated with adverse clinic pathological features and poor prognosis in Osteosarcoma.miR-130b may regulate proliferation and invasion of osteosarcoma cells by targeting PPARϒ, suggesting miR-130b may play a key role in progression of osteosarcoma.[13]. While the mechanisms underlying PPARγaction are not fully established, it has been shown that PPARγ can inhibit the cell cycle, which is accomplished at least in part through downregulating the protein phosphatase PP2Aupon PPARγactivation [14]. The PPARγligands can also inhibit the G1/S transition by inhibiting Rb phosphorylation [15]. Furthermore, PPARγupregulates the CDK inhibitors p18 and p21 [16]. PPARγligand PGJ2 induces both CDKp21 and the proapoptotic Bax but downregulates the anti-apoptotic Bcl-xL [17]. Synthetic PPARγagonist treatment in human pancreatic cancer and bladder cancer cell lines resulted in G1 cell cycle arrest secondary to p21 induction[18, 19]. Further insight into the cross-talk between these different mechanisms will guide future antitumortherapies.

Yang H showed that, miR-148a act as a tumour suppressor in OS tissues and cell lines by targetting Rho-associated coiled-coil kinase 1 (ROCK1) . Therefore, the miR-148a/ROCK1 axis may become a potential therapeutic target for OS[20].

Four plasma miRNA including miR 195-5p-199a-3p,miRNA 320 a and miR-374a-5p were significantly increased in Osteosarcoma patients.these 4 mi-RNA were significantly decreased after surgery.These findings imply that these miRNA may reflect tumour dynamics and are available as new biomarkers to evaluate tumour removal.Also plasma miRNA including miRNA-195-5p,miRNA-199a-3p were significantly increased in metastatic patients. While miRNA-199a-3p and miRNA 320 a were correlated with histological (osteoblasic) subtype [21] ..MiRNA-195–5p could inhibit osteosarcoma cell migration and invasion through targeting FASN (fatty acid synthase) [22]. MiR-199a-3p could inhibit osteosarcoma cell growth, migration, and induce the apoptosis via p53 signaling pathway [23, 24]. Jian *et al*. showed that miRNA-195 plays a key role in inhibiting osteosarcoma cell migration and invasion through targeting.Fatty acid synthase(FASN)and strongly suggest that exogenous miRNA-195 may have therapeutic role in treating osteosarcoma.Fatty acid synthase is an enzyme crucial to endogenous lipogenesis in mammals and is responsible for catalyzing the synthesis of long chain fatty acids. FASN is critical in sustaining biological features of cancer cells. FASN is highly expressed in variety of tumour cells but is low in normal cells. Jian *et al*. showed that expression of oncogene FASN is negatively regulated by mi-RNA -195 through a special binding site in FASN3ʾ-UTR [25].

MiRNA-503 is tumour suppressor by repressing LICAM expression during the development of osteosarcoma. LICAM is the prototype member of the LI-family of closely related neural adhesion molecule. LICAM was discovered as cell adhesion molecule in the nervous system. Subsequent work in tumour biology has showed that LICAM is overexpressed in many cancers including Osteosarcoma. LICAM expression is generally associated with poor prognosis,an aggressive phenotype and advanced tumour stages. Yang Chong *et al*. Showed that LICAM expression was upregulated in osteosarcoma cells. The expression of LICAM was negatively correlated with miRNA 503 levels and this leads to enhanced metastasis of cancer [26].

As a potential tumour suppressor gene miRNA-375 downregulated in Osteosarcoma and unfavorable progonostic factor low levels are associated with chemoresistence. As chemoresistence remains a substantial problem in OS.miRNA-375 might suppress osteosarcoma growth through mediation of PI3K/Akt pathway [27].So its potential value as noninvasive

biomarker for osteosarcoma diagnosis, prognosis and chemosensitivity prediction. Junbo Dong etal showed that MiRNA -223 is downregulated in osteosarcoma patients and lower levels are associated with distant metastasis. Xu *et al*. demonstrated that miRNA-223 was a tumour suppressor in Osteosarcoma and showed that miR-223/Ect2/p21 signalling played a role in osteosarcoma cell cycle progression and proliferation [28]. MiRNA-34 is suppressed in Osteosarcoma, miR-34 inhibited the p53-mediated cell cycle arrest and apoptosis in OS cells [29]. The Notch signaling pathway is involved in maintaining the balance between cell proliferation and differentiation and altered Notch signaling has been associated with various disorders, including cancer[30][31]. MiRNA-34c inhibits osteoblast differentiation and increases osteoclast genesis through suppression of Notch signaling (resulting in inhibition of osteoprotegerin expression) in mouse model [32]. Additionally, p53 also induced the upregulation of miRNA-192, miRNA-194, and miRNA-215 in U2OS cells carrying wild-type p53 [33]. MiRNA-192 and miRNA-215 induce the expression of p21, and U2OS cells transfected with an expression vector for miRNA-192 formed significantly fewer colonies than those transfected with that for a control miRNA or miRNA-34a [33]. The loss of miRNA-31 was associated with defects in the p53 pathway, and overexpression of miRNA-31 significantly inhibited the proliferation of OS cell lines [34].

Duan Z *et al*. found that miR-199a-3p, miR-127-3p, and miR-376 were significantly decreased in the OS cell lines as compared to osteoblasts, while the expression of miR-151-3p and miR-191 was increased. Among these miRNAs, overexpression of miR-199a-3p in OS cell lines was associated with a significant decrease in cell growth with G1 arrest. Furthermore, miR-199a-3p suppressed the expression of oncogenic and ant apoptotic proteins, MET, motor, STAT3, MCL-1, and BCL-X. This suggests that miR-199a-3p plays a significant functional role in regulating the proliferation of OS cells Taken together; these findings indicate that miR-199a-3p can prove to be a promising candidate for gene therapy [35].

MiRNA-133a was downregulated in osteosarcoma cell lines and primary human osteosarcoma tissues, and its decrease was significantly correlated with tumor progression and prognosis of the patients. that the anti-tumor effect of miR-133a was probably due to targeting and repressing of Bcl-xL and Mcl-1 expression. This showed that roles of miR-133a in osteosarcoma pathogenesis and implicate its potential in cancer therapy [36](Fig-2).

microRNA cluster (including *miR-127-3p, miR-154, miR-299-5p, miR-329, miR-337-3p, miR-376a, miR-376c, miR-377, miR-382, miR-409-3p, miR-409-5p, miR-410, miR-432, miR-493, miR-495, miR-453, miR-654-5p,* and *miR-758*) at the chromosome 14q32 locus is significantly downregulated in osteosarcoma compared to normal bone tissue[37].The cMyc oncogene is inhibited by this miRNA cluster, thus miRNA downregulation at this locus results in inhibition of apoptosis through increased cMyc activity[37, 38].An inverse correlation has been demonstrated between aggressive tumor behavior (such as increased metastatic potential and accelerated time to death) and the residual expression of representative 14q32 miRNAs (*miR-134, miR-382,* and *miR-544*) in samples from human osteosarcoma patients[39].

Yan zhou *et al.* that miR-124 was down-regulated in OS cell lines and tissues. Furthermore, the low level of miR-124 was associated with increased expression of Sphingosine kinase 1 (SPHK1) in OS cells and tissues. Up-regulation of miR-124 significantly inhibited cell proliferation, invasion, and MMP-2 and -9 expressions of OS cells [40].

MiR-127-3p is suggested to act mainly via the suppression of SETD8 expression. Overall, the results revealed that miR-127-3p acts as a tumor suppressor and that its down-regulation in cancer may contribute to OS progression and metastasis, suggesting that miR-127-3p could be a potential therapeutic target in the treatment of OS [41].

Current status of microRNA in osteosarcoma Metastasis

MicroRNAs (miRNAs) are thought to have an important role in tumor metastasis by regulating diverse cellular pathways pulmonary metastasis is the predominant site of osteosarcoma recurrence and the most common cause of death.

MitsuhikoOsaki *et al*. showed that miRNA-143 act as tumour suppressor and its downregulation of miR-143 correlates with the lung metastasis of human osteosarcoma cells by promoting cellular invasion, probably via MMP-13 upregulation, suggesting that miRNA could be used to develop new molecular targets for osteosarcoma metastasis [42]. Chen B showed that miR-145 functions as a tumor metastasis suppressor gene by down-regulating MMP16 and may be a potential target in osteosarcoma treatment [43].

Guoqing Duan *et al.* showed that downregulation of miR-26b in osteosarcoma tissues, negatively correlated with the expression of connective tissue growth factor (CTGF) and Smad1. Their data indicated that downregulation of miR-26b in osteosarcoma elevated the levels of CTGF and Smad1, facilitating osteosarcoma metastasis [44].

Qifei Liu *et al*. showed that MiR-489-3p act as tumour suppressor in OS cells especially in high metastatic potential cells and was also significantly decreased in metastatic lesions compared with their corresponding primary tumor samples paired box gene

3 (PAX3) was identified as a functional target of miR-489-3p in OS cells. Mechanistic investigations indicated that prometastasis function of PAX3 was mediated by upregulating downstream target MET tyrosine kinase receptor. Their results revealed that miR-489-3p-PAX3-MET signaling is critical to OS metastasis. Targeting this pathway may open new therapeutic prospects to restrict the metastatic potential of OS[45].

Kang Han *et al*. showed that miRNA195 act as tumour metastasis suppressor by down-regulating CCND1 and can be used as a potential target in the treatment of osteosarcoma [46]. CCND1 encodes Cyclin D1 protein which is essential for G1 to S transition. it has been reported that cyclin D1 involve in tumor progression and metastasis[47].

Wei Wang *et al*. showed that Lowlevel expression of miRNA-144 was significantly associated with distant metastasis and poor prognosis. MiRNA-144 suppresses OS progression by directly downregulating ROCK1 and ROCK2 expression, and may be a promising therapeutic target for OS [48]. Rho-associated kinase (ROCK) is an essential downstream effector of the Rho small GTPase, which acts as a molecular switch that binds GTP (active) and GDP (inactive) to regulate cell survival, proliferation and cytoskeleton organization, inducing alterations in cell shape/morphology, adhesion and movement [49]. Two existing isoforms, ROCK1 and ROCK2, are known. Increased expression of ROCK is well documented in tumors, and related to cancer progression, metastasis and poor prognosis [50].

Guangfeng Niu, *et al*. revealed that transforming growth factor beta 2 (TGF-β2) was negatively regulated by miR-153. Furthermore, overexpression of miRNA-153 decreased p-SMAD2, p-SMAD3, epidermal growth factor receptor (EGFR) and insulin-like growth factor binding protein-3 (IGFBP-3) expressions, which were the downstream signaling molecules of TGF-β. Furthermore, miRNA-153 suppressed TGF-β-mediated MG-63 proliferation and migration. Therefore, their results suggested that miRNA-153 may act as a tumor suppressor in osteosarcoma through targeting TGF-β2 [51]. Lei Shen *et al*. demonstrated that miR-217 functions as a tumor-suppressive miRNA and inhibits the osteosarcoma tumorigenesis through targeting WASF3 [52]. The WASF3 gene was a member of the Wiskott Aldrich syndrome family of proteins (WASP), which contained verprolincofilin-acidic domains at their C-terminal ends [53, 54]. These domains were thought to coordinate the recruitment of monomeric actin and the ARP2/3 complex of proteins to facilitate actin polymerization, which was essential for cell movement and invasion [54, 55]. Tang Y confirmed that WASF3 was up-regulated in osteosarcoma tissues and overexpression of WASF3 induced osteosarcoma cells proliferation and invasion. However, the relevant mechanisms were still unclear. The ability of miRNA-217 to target WASF3 might provide one possible mechanism of post-transcriptional control of WASF3.Consequently; their findings provided a molecular basis for the role of miRNA-217/WASF3 in the progression of human osteosarcoma and suggested that this miRNA could be a potential target for the treatment of osteosarcoma in future (Fig-3).

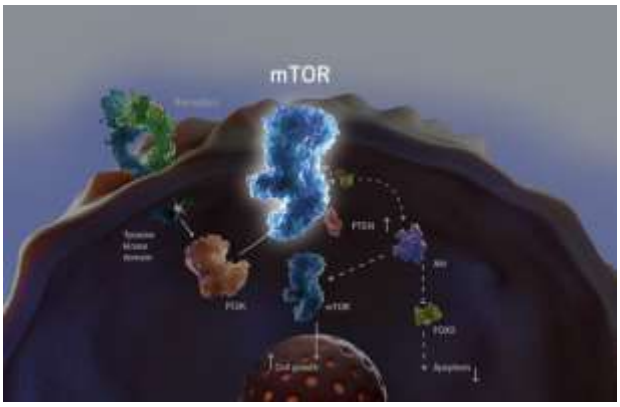

Fig-1

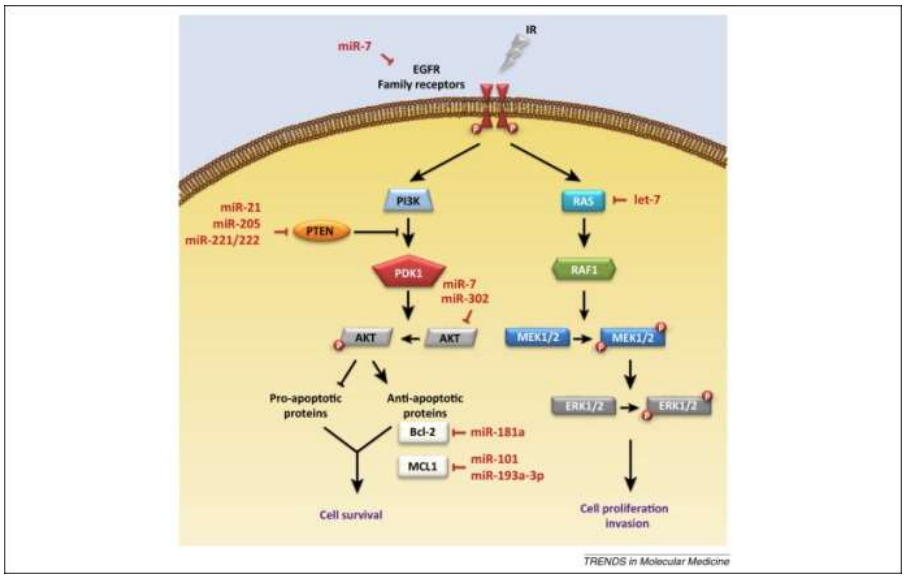

Fig-2

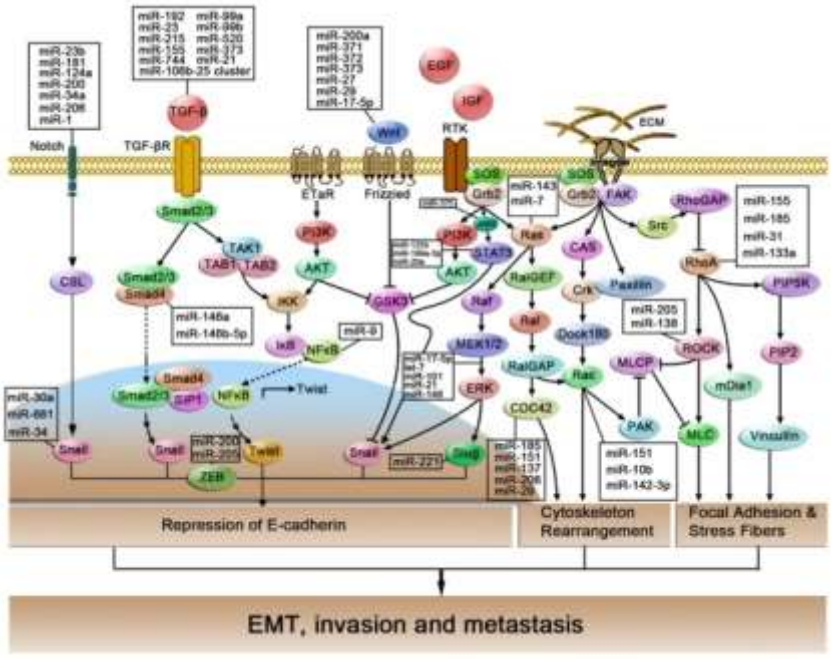

Fig-3

CONCLUSION

It is evident miRNAs play a role in the progression of OS by regulating proliferation, invasion, adhesion, metastasis, and apoptosis. Therefore, they can be helpful in early diagnosis, prognosis and staging of Osteosarcoma. Since therapeutic targeting of miRNA promises to improve the clinical management of patients with OS, future studies should be able design miRNA-based treatments efficiently with high quality of delivery, therapeutic effects and better safety profiles in OS.

REFERENCES

1. Lamoureux, F., Trichet, V., Chipoy, C., Blanchard, F., Gouin, F., & Redini, F. (2007). Recent advances in the management of osteosarcoma and forthcoming therapeutic strategies. *Expert review of anticancer therapy*, *7*(2), 169-181.
2. Luo, T., Yi, X., & Si, W. (2017). Identification of miRNA and genes involving in osteosarcoma by comprehensive analysis of microRNA and copy number variation data. *Oncology letters*, *14*(5), 5427-5433.
3. Jiang, B., Mu, W., Wang, J., Lu, J., Jiang, S., Li, L., ... & Tian, H. (2016). MicroRNA-138 functions as a tumor suppressor in osteosarcoma by targeting differentiated embryonic chondrocyte gene 2. *Journal of Experimental & Clinical Cancer Research*, *35*(1), 69.

4. Meng, F., Henson, R., Wehbe–Janek, H., Ghoshal, K., Jacob, S. T., & Patel, T. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*, *133*(2), 647-658.
5. Vanas, V., Haigl, B., Stockhammer, V., & Sutterlüty-Fall, H. (2016). MicroRNA-21 increases proliferation and cisplatin sensitivity of osteosarcoma-derived cells. *PloS one*, *11*(8), e0161023.
6. Yuan, J., Chen, L., Chen, X., Sun, W., & Zhou, X. (2012). Identification of serum microRNA-21 as a biomarker for chemosensitivity and prognosis in human osteosarcoma. *Journal of International Medical Research*, *40*(6), 2090-2097.
7. Xu, M., & Li, J. M. (2016). MicroRNA-181b promotes osteosarcoma cell proliferation, invasion and migration in vitro via targeting RASSF8. *INTERNATIONAL JOURNAL OF CLINICAL AND EXPERIMENTAL PATHOLOGY*, *9*(6), 6145-6153.
8. Wang, J., Hua, W., Huang, S. K., Fan, K., Takeshima, L., Mao, Y., & Hoon, D. S. (2015). RASSF8 regulates progression of cutaneous melanoma through nuclear factor-κb. *Oncotarget*, *6*(30), 30165.
9. Brembeck, F. H., Rosário, M., & Birchmeier, W. (2006). Balancing cell adhesion and Wnt signaling, the key role of β-catenin. *Current opinion in genetics & development*, *16*(1), 51-59.
10. MacDonald, B. T., Tamai, K., & He, X. (2009). Wnt/β-catenin signaling: components, mechanisms, and diseases. *Developmental cell*, *17*(1), 9-26.
11. Xu, S. H., Yang, Y. L., Han, S. M., & Wu, Z. H. (2014). MicroRNA-9 expression is a prognostic biomarker in patients with osteosarcoma. *World journal of surgical oncology*, *12*(1), 195.
12. Cao, Z. Q., Shen, Z., & Huang, W. Y. (2013). MicroRNA-802 promotes osteosarcoma cell proliferation by targeting p27. *Asian Pacific Journal of Cancer Prevention*, *14*(12), 7081-7084.
13. Yu, L. D., Jin, R. L., Gu, P. C., Ling, Z. H., Lin, X. J., & Du, J. Y. (2015). Clinical significance of microRNA-130b in osteosarcoma and in cell growth and invasion. *Asian Pacific journal of tropical medicine*, *8*(9), 752-756.
14. Altiok, S., Xu, M., & Spiegelman, B. M. (1997). PPARγ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. *Genes & development*, *11*(15), 1987-1998.
15. Barrera, G., Toaldo, C., Pizzimenti, S., Cerbone, A., Pettazzoni, P., Dianzani, M. U., & Ferretti, C. (2008). The role of PPAR ligands in controlling growth-related gene expression and their interaction with lipoperoxidation products. *PPAR research*, *2008*.
16. Morrison, R. F., & Farmer, S. R. (1999). Role of PPARγ in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18 (INK4c) and p21 (Waf1/Cip1), during adipogenesis. *Journal of Biological Chemistry*, *274*(24), 17088-17097.
17. Shen, Z. N., Nishida, K., Doi, H., Oohashi, T., Hirohata, S., Ozaki, T., ... & Inoue, H. (2005). Suppression of chondrosarcoma cells by 15-deoxy-Δ12, 14-prostaglandin J2 is associated with altered expression of Bax/Bcl-xL and p21. *Biochemical and biophysical research communications*, *328*(2), 375-382.
18. Eibl, G., Wente, M. N., Reber, H. A., & Hines, O. J. (2001). Peroxisome proliferator-activated receptor γ induces pancreatic cancer cell apoptosis. *Biochemical and Biophysical Research Communications*, *287*(2), 522-529.
19. Yin, F., Wakino, S., Liu, Z., Kim, S., Hsueh, W. A., Collins, A. R., ... & Law, R. E. (2001). Troglitazone inhibits growth of MCF-7 breast carcinoma cells by targeting G1 cell cycle regulators. *Biochemical and biophysical research communications*, *286*(5), 916-922.
20. Wei, L., Surma, M., Shi, S., Lambert-Cheatham, N., & Shi, J. (2016). Novel insights into the roles of Rho kinase in cancer. *Archivum immunologiae et therapiae experimentalis*, *64*(4), 259-278.
21. Lian, F., Cui, Y., Zhou, C., Gao, K., & Wu, L. (2015). Identification of a plasma four-microRNA panel as potential noninvasive biomarker for osteosarcoma. *PloS one*, *10*(3), e0121499.
22. Mao, J. H., Zhou, R. P., Peng, A. F., Liu, Z. L., Huang, S. H., Long, X. H., & Shu, Y. (2012). microRNA-195 suppresses osteosarcoma cell invasion and migration in vitro by targeting FASN. *Oncology letters*, *4*(5), 1125-1129.
23. Tian, Y., Zhang, Y. Z., & Chen, W. (2014). MicroRNA-199a-3p and microRNA-34a regulate apoptosis in human osteosarcoma cells. *Bioscience reports*, *34*(4), e00132.
24. Duan, Z., Choy, E., Harmon, D., Liu, X., Susa, M., Mankin, H., & Hornicek, F. (2011). MicroRNA-199a-3p is downregulated in human osteosarcoma and regulates cell proliferation and migration. *Molecular cancer therapeutics*, *10*(8), 1337-1345.
25. Mao, J. H., Zhou, R. P., Peng, A. F., Liu, Z. L., Huang, S. H., Long, X. H., & Shu, Y. (2012). microRNA-195 suppresses osteosarcoma cell invasion and migration in vitro by targeting FASN. *Oncology letters*, *4*(5), 1125-1129.
26. Chong, Y., Zhang, J., Guo, X., Li, G., Zhang, S., Li, C., ... & Shao, M. (2014). MicroRNA-503 acts as a tumor suppressor in osteosarcoma by targeting L1CAM. *PloS one*, *9*(12), e114585.
27. Liu, W., Zhao, X., Zhang, Y. J., Fang, G. W., & Xue, Y. (2017). MicroRNA-375 as a potential serum biomarker for the diagnosis, prognosis, and chemosensitivity prediction of osteosarcoma. *Journal of International Medical Research*, 0300060517734114.
28. Dong, J., Liu, Y., Liao, W., Liu, R., Shi, P., & Wang, L. (2016). miRNA-223 is a potential

diagnostic and prognostic marker for osteosarcoma. *Journal of bone oncology*, *5*(2), 74-79.

29. He, C., Xiong, J., Xu, X., Lu, W., Liu, L., Xiao, D., & Wang, D. (2009). Functional elucidation of MiR-34 in osteosarcoma cells and primary tumor samples. *Biochemical and biophysical research communications*, *388*(1), 35-40.
30. Wang, Z., Li, Y., Banerjee, S., & Sarkar, F. H. (2009). Emerging role of Notch in stem cells and cancer. *Cancer letters*, *279*(1), 8-12.
31. Wang, Z., Li, Y., Kong, D., Ahmad, A., Banerjee, S., & Sarkar, F. H. (2010). Cross-talk between miRNA and Notch signaling pathways in tumor development and progression. *Cancer letters*, *292*(2), 141-148.
32. Bae, Y., Yang, T., Zeng, H. C., Campeau, P. M., Chen, Y., Bertin, T., ... & Lee, B. H. (2012). miRNA-34c regulates Notch signaling during bone development. *Human molecular genetics*, *21*(13), 2991-3000.
33. Braun, C. J., Zhang, X., Savelyeva, I., Wolff, S., Moll, U. M., Schepeler, T., ... & Dobbelstein, M. (2008). p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. *Cancer research*, *68*(24), 10094-10104.
34. Creighton, C. J., Fountain, M. D., Yu, Z., Nagaraja, A. K., Zhu, H., Khan, M., ... & Anderson, M. L. (2010). Molecular profiling uncovers a p53-associated role for microRNA-31 in inhibiting the proliferation of serous ovarian carcinomas and other cancers. *Cancer research*, *70*(5), 1906-1915.
35. Duan, Z., Choy, E., Harmon, D., Liu, X., Susa, M., Mankin, H., & Hornicek, F. (2011). MicroRNA-199a-3p is downregulated in human osteosarcoma and regulates cell proliferation and migration. *Molecular cancer therapeutics*, *10*(8), 1337-1345.
36. Ji, F., Zhang, H., Wang, Y., Li, M., Xu, W., Kang, Y., ... & Li, C. (2013). MicroRNA-133a, downregulated in osteosarcoma, suppresses proliferation and promotes apoptosis by targeting Bcl-xL and Mcl-1. *Bone*, *56*(1), 220-226.
37. Maire, G., Martin, J. W., Yoshimoto, M., Chilton-MacNeill, S., Zielenska, M., & Squire, J. A. (2011). Analysis of miRNA-gene expression-genomic profiles reveals complex mechanisms of microRNA deregulation in osteosarcoma. *Cancer genetics*, *204*(3), 138-146.
38. Thayanithy, V., Sarver, A. L., Kartha, R. V., Li, L., Angstadt, A. Y., Breen, M., ... & Subramanian, S. (2012). Perturbation of 14q32 miRNAs-cMYC gene network in osteosarcoma. *Bone*, *50*(1), 171-181.
39. Sarver, A. L., Thayanithy, V., Scott, M. C., Cleton-Jansen, A. M., Hogendoorn, P. C., Modiano, J. F., & Subramanian, S. (2013). MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. *Orphanet journal of rare diseases*, *8*(1), 7.
40. Zhou, Y., Han, Y., Zhang, Z., Shi, Z., Zhou, L., Liu, X., & Jia, X. (2017). MicroRNA-124 upregulation inhibits proliferation and invasion of osteosarcoma cells by targeting sphingosine kinase 1. *Human cell*, *30*(1), 30-40.
41. Zhang, J., Hou, W., Chai, M., Zhao, H., Jia, J., Sun, X., ... & Wang, R. (2016). MicroRNA-127-3p inhibits proliferation and invasion by targeting SETD8 in human osteosarcoma cells. *Biochemical and biophysical research communications*, *469*(4), 1006-1011.
42. Osaki, M., Takeshita, F., Sugimoto, Y., Kosaka, N., Yamamoto, Y., Yoshioka, Y., ... & Ito, H. (2011). MicroRNA-143 regulates human osteosarcoma metastasis by regulating matrix metalloprotease-13 expression. *Molecular therapy*, *19*(6), 1123-1130.
43. Chen, B., Huang, Z., Zhang, Y., Chen, Y., & Li, Z. (2015). MicroRNA-145 suppresses osteosarcoma metastasis via targeting MMP16. *Cellular Physiology and Biochemistry*, *37*(6), 2183-2193.
44. Duan, G., Ren, C., Zhang, Y., & Feng, S. (2015). MicroRNA-26b inhibits metastasis of osteosarcoma via targeting CTGF and Smad1. *Tumor Biology*, *36*(8), 6201-6209.
45. Liu, Q., Yang, G., & Qian, Y. (2017). Loss of MicroRNA-489-3p promotes osteosarcoma metastasis by activating PAX3-MET pathway. *Molecular carcinogenesis*, *56*(4), 1312-1321.
46. Han, K., Chen, X., Bian, N., Ma, B., Yang, T., Cai, C., ... & Zhao, T. (2015). MicroRNA profiling identifies MiR-195 suppresses osteosarcoma cell metastasis by targeting CCND1. *Oncotarget*, *6*(11), 8875.
47. Li, Z., Wang, C., Prendergast, G., & Pestell, R. G. (2006). Cyclin D1 functions in cell migration. *Cell cycle*, *5*(21), 2440-2442.
48. Wang, W., Zhou, X., & Wei, M. (2015). MicroRNA-144 suppresses osteosarcoma growth and metastasis by targeting ROCK1 and ROCK2. *Oncotarget*, *6*(12), 10297.
49. Patel, R. A., Forinash, K. D., Pireddu, R., Sun, Y., Sun, N., Martin, M. P., ... & Sebti, S. M. (2012). RKI-1447 is a potent inhibitor of the Rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer. *Cancer research*, *72*(19), 5025-5034.
50. Oellers, P., Schröer, U., Senner, V., Paulus, W., & Thanos, S. (2009). Rocks are expressed in brain tumors and are required for glioma-cell migration on myelinated axons. *Glia*, *57*(5), 499-509.
51. Niu, G., Li, B., Sun, L., & An, C. (2015). MicroRNA-153 inhibits osteosarcoma cells proliferation and invasion by targeting TGF-β2. *PloS one*, *10*(3), e0119225.
52. Shen, L., Wang, P., Yang, J., & Li, X. (2014). MicroRNA-217 regulates WASF3 expression and suppresses tumor growth and metastasis in osteosarcoma. *PloS one*, *9*(10), e109138.

53. Teng, Y., Mei, Y., Hawthorn, L., & Cowell, J. K. (2014). WASF3 regulates miR-200 inactivation by ZEB1 through suppression of KISS1 leading to increased invasiveness in breast cancer cells. *Oncogene*, *33*(2), 203.
54. Teng, Y., Ghoshal, P., Ngoka, L., Mei, Y., & Cowell, J. K. (2013). Critical role of the WASF3 gene in JAK2/STAT3 regulation of cancer cell motility. *Carcinogenesis*, *34*(9), 1994-1999.
55. Teng, Y., Ren, M., Cheney, R., Sharma, S., & Cowell, J. K. (2010). Inactivation of the WASF3 gene in prostate cancer cells leads to suppression of tumorigenicity and metastases. *British journal of cancer*, *103*(7), 1066.