miR-203 Acts as a Tumor Suppressor Gene in Osteosarcoma by Regulating RAB22A

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Abstract
microRNAs (miRNAs), small noncoding RNAs of 19–25 nt, play an important roles in the pathological processes of tumorigenesis. The object of this study was to study the expression and function of miR-203 and to found its target gene in osteosarcoma. In our study, we found the expression level of miR-203 was significantly downregulated in osteosarcoma cell lines and tissues. In addition, overexpression of miR-203 inhibited the osteosarcoma cell proliferation and migration and inhibited Mesenchymal-to-Epithelial reversion Transition (MErT). Moreover, we identified RAB22A as a direct target of miR-203 and RAB22A overexpression blocks the roles of miR-203 in osteosarcoma cell. Furthermore, we demonstrated that RAB22A expression was upregulated in human osteosarcoma cell lines and tissues. Take together, our results demonstrated that miR-203 act as a tumor suppressor miRNA through regulating RAB22A expression and suggested its involvement in osteosarcoma progression and carcinogenesis.

Introduction
Osteosarcoma (OS) has become the most common malignant bone tumor and occurs mainly in adolescents and young adults, which accounts for approximately 60% of malignant bone tumors in the first 2 decades of life[1–4]. It mainly present in the long bones of the body, such as the knee joint, lower femur and upper tibia in about 80% patients[5–7]. Approximately 40–50% of osteosarcoma patients will develop metastases and the main sites of metastases of OS are the lungs, pleura, and the heart[8–11]. Therefore, it is urgent to develop better prognosis, new therapeutic targets and approaches for osteosarcoma treatment.

MicroRNAs (miRNAs) are a novel series of small endogenous, non-coding, single-stranded RNAs, which negatively regulate a wide variety of genes expression mainly through direct interaction with the 3’untranslated regions (3’UTR) of their corresponding mRNA targets[12–15]. Increasing evidences have demonstrated that miRNAs play important roles in pathological and physiological processes such as cell-cycle regulation, differentiation, proliferation, apoptosis, and migration[7, 16–20]. Numerous miRNAs have been implicated in different cancers
including bladder cancer, renal cell carcinoma, breast cancer, gastric cancer, playing important roles in tumorigenesis[21–26]. Therefore, miRNAs have been suggested as potential and novel targets for the diagnosis, prognosis and treatment of osteosarcoma[27, 28].

Previous studies demonstrated that miR-203 acts as an important role in various cancers[29–31]. For example, Liao et al[32] reported that miR-203 expression was lower in imatinib-resistant glioblastoma (GBM) cells (U251AR, U87AR) that underwent epithelial-mesenchymal transition (EMT) than in their parental cells (U251, U87). Xu et al[33] showed that miR-203 could be a potential prognostic marker and functions as a tumor suppressor in human renal cancer by post-transcriptionally targeting FGF2. Moreover, Lee et al[34] demonstrated that miR-203 induces the cells apoptosis by directly targeting Yes-1 in oral cancer cells. However, the role of miR-203 in osteosarcoma is still unknown. In this study, we confirmed that the miR-203 expression is significantly decreased in osteosarcoma tissues and revealed that miR-203 could inhibit cell proliferation through directly targeting RAB22A in osteosarcoma.

Materials and Methods

Ethics Statement

Our experiments involving tissues were approved by the ethical board of The Second Affiliated Hospital of Xi’an Jiaotong University and complied with the Declaration of Helsinki. All of these patients gave written informed consent.

Human tissue samples and cell lines

Osteosarcoma tissues and adjacent nontumor tissues (located 3 cm away from the tumor) were collected during surgery in our hospital. Human osteosarcoma cell lines (MG-63, SOSP-9607, SAOS-2, and U2OS) and one normal osteoblast cell line (hFOB) were purchased from the Cell Bank of Chinese Academy of Medical Sciences (Beijing, China). These cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) (Gibco).

Oligonucleotides transfection

miR-203 mimic and negative control (scramble) were obtained from RIBOBIO (Guangzhou, China). Cells were transfected with miR-203 mimic or scramble by Lipofectamine 2000 (Invitrogen) following to the manufacturer’s instructions.

Cell proliferation and migration assay

Cell proliferation was performed using CCKK-8 according manufacturer’s protocol. For cell migration, a sterile white pipette tip was used to perform similar-sized wounds. Wounded cells were washed by PBS to remove cell debris and then photographed. The wound closure was measured and photographed at 0h and 48 h.

Real-time quantitative PCR

Total RNA was extracted from tissues or cells by using TRIZol. Real-time quantitative PCR (qRT-PCR) and TaqMan microRNA assays were done to quantify the expression of mature miR-203 using SYBR Green PCR mix (Applied Biosystems) in a 7300 Real-time PCR System (Applied Biosystems). Gene expression was measured relative to U6 or GAPDH. The following primers were used: for RAB22A, (forward) 5’-TTGTAGCCATTGCGAGGA-3’ and (reverse) 5’-AGGCTGCTTCTGAGTTTGA-3’; for GAPDH, (forward) 5’-GACTCATGACCACAGTC CATGC-3’ and (reverse) 5’-AGAGGCAGGGATGATTTCTG-3’.
Western blot analysis

Western blot analysis was measured as described previously[35]. Total proteins were prepared from tissues or cells by using the RIPA buffer (Pierce). Protein was electrophoreses in a 10% SDS–PAGE and transferred onto membrane. Proteins were measured with anti-GAPDH and anti-RAB22A antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). The signal was detected using an ECL detection system (Millipore).

Dual luciferase reporter assay

A total of miR-203 mimic (or scramble), pGL3, RAB22A-3'UTR-WT or RAB22A-3'UTR-MUT vectors were cotransfected into the MG-63 cells using Lipofectamine 2000 following the manuscript’s instruction (Invitrogen). Relative luciferase activity was measured 48 h after-transfection using a Dual-Luciferase Reporter kit (Promega, USA).

Statistical analysis

Data are showed as the mean±SD. Difference between groups was analyzed using one-way ANOVA or Student’s t test. A P-value of p<0.05 was considered statistically significant.

Result

miR-203 was downregulated in human osteosarcoma cell lines and tissues

We examined the miR-203 levels in human osteosarcoma cell lines (MG-63, SOSP-9607, SAOS-2, and U2OS) and one normal osteoblast cell line (hFOB) using qRT-PCR and found that miR-203 expression was downregulated in human osteosarcoma cell lines compared to in hFOB(Fig 1A). Furthermore, the expression of miR-203 was downregulated in osteosarcoma tissues compared with their corresponding nontumor tissues (Fig 1B and 1C).

miR-203 overexpression inhibited cell proliferation and migration

Up-regulation of miR-203 expression was discovered at 48 hours after miR-203 mimic transfection (Fig 2A). As showed in Fig 2B and 2C, miR-203 overexpression reduced cell proliferation and inhibited cell migration in the MG-63 cells.

Overexpression of miR-203 inhibited Mesenchymal-to-Epithelial reversion Transition (MERt)

We showed that overexpression of miR-203 can induce the E-cadherin mRNA expression and inhibit N-cadherin and Vimentin mRNA expression (Fig 3A). Furthermore, as determined by western blot, we also demonstrated that ectopic expression of miR-203 can enhance the protein expression of E-cadherin and inhibit the protein expression of N-cadherin and Vimentin (Fig 3B). We also found that miR-203 overexpression inhibited the mRNA expression of Twist, Snail, Slug, and Zeb1, which are the EMT transcription factors (Fig 3C).

miR-203 directly targets the RAB22A in osteosarcoma cells

To find the molecular mechanisms responsible for the effect of miR-203 in osteosarcoma, we used Target Scan databases to predict target genes of miR-203. We identified that RAB22A gene harbored a potential miR-203 binding site using a stringent bioinformatics approach (Fig 4A). Ectopic expression of miR-203 led to a reduction of luciferase activity when the reporter...
construct contained the RAB22A 3'UTR in MG-63 cells (Fig 4B). miR-203 overexpression in MG-63 cells reduced RAB22A mRNA and protein expression (Fig 4C and 4D).

RAB22A overexpression blocks the roles of miR-203

We found that RAB22A expression was upregulated in human osteosarcoma cell lines compared to in hFOB (Fig 5A). Furthermore, the expression of RAB22A was upregulated in osteosarcoma tissues compared with their corresponding nontumor tissues (Fig 5B). Western blot data confirmed that the protein expression of RAB22A was overexpressed when cells transfected with RAB22A vector (Fig 5C). We rescued the expression of RAB22A in miR-203 overexpressing MG-63 cells. CCK8 assay showed that overexpression of RAB22A increased the
miR-203 overexpressing MG-63 cells proliferation (Fig 5D). Moreover, overexpression of RAB22A increased the N-cadherin and Vimentin mRNA expression and inhibited E-cadherin mRNA expression in miR-203 overexpressing MG-63 cells (Fig 5E). Furthermore, the migration abilities of miR-203 overexpressing MG-63 cells were increased after RAB22A vector transfection (Fig 5F).

**Discussion**

Altered patterns of miRNA expression have been well demonstrated in nearly all types of human diseases and, especially in cancers[27, 36, 37]. miRNAs are involved in tumor cell proliferation and migration by regulating numerous genes such as tumor suppressor genes and oncogenes[35, 38, 39]. In our study, we found the expression level of miR-203 was downregulated in osteosarcoma cell lines and tissues. In addition, forced overexpression of miR-203 inhibited the osteosarcoma cell proliferation and migration and inhibited MEnT. Moreover, we identified RAB22A as a direct target of miR-203 and RAB22A overexpression blocks the roles of miR-203 in osteosarcoma cell. Take together, our results demonstrated that miR-203 act as a...
tumor suppressor miRNA and suggested its involvement in osteosarcoma progression and carcinogenesis.

Increasing data have shown that miR-203 is deregulated and functions as a tumor suppressor gene in various human cancers including cervical cancer, prostate cancer, breast cancer, colorectal cancer and glioblastoma[30, 40–43]. For example, enforced expression of miR-203 attenuated colorectal cancer cell proliferation, invasion and migration by regulating Zinc finger protein 217 (ZNF217) expression[44]. Xiang et al[45] demonstrated that the expression of miR-203 was significantly downregulated in prostate cancer specimens and miR-203 overexpression inhibited cell proliferation, adhesion and invasion by inhibiting Rap1A expression. However, the expression and role of miR-203 in osteosarcoma is still unknown. In this study, our results showed that expression of level of miR-203 was downregulated in osteosarcoma cell lines and tissues. In addition, forced overexpression of miR-203 inhibited the osteosarcoma cell proliferation and migration and inhibited reversion of EMT. These studies may help to explain the important role of miR-203 in development and progression in osteosarcoma.

RAB22A was found as a direct target of miR-203 in osteosarcoma cells by using bioinformatics analysis, Dual luciferase reporter assar and western blot. There is a potential miR-203 binding site of RAB22A gene. Ectopic expression of miR-203 led to a reduction of luciferase activity when the reporter construct contained the RAB22A 3’UTR in MG-63 cells. Furthermore, miR-203 overexpression in MG-63 cells reduced RAB22A mRNA and protein expression. Moreover, RAB22A overexpression blocks the roles of miR-203. Furthermore, we demonstrated that RAB22A expression was upregulated in human osteosarcoma cell lines compared to in hFOB and the expression of RAB22A was upregulated in osteosarcoma tissues compared with their corresponding nontumor tissues. RAB22A belongs to a Ras superfamily of GTPases and play important roles in development of cancers[46]. Recent study has shown that RAB22A overexpression is associated with decreased overall and metastasis-free survival in the primary tumor
and RAB22A knockdown impairs cancer metastasis in breast cancer[46]. Moreover, previous studies showed that miR-373 suppresses human epithelial ovarian cancer invasion and metastasis by directly targeting RAB22A gene[47]. Yang et al[48]. also reported that miR-193b as a novel tumor suppressor plays an important role in breast cancer progression by inhibiting RAB22A expression. Take together, our results showed that miR-203 may act as a tumor suppressor gene in osteosarcoma partly by regulating RAB22A expression.

In conclusion, we determined that miR-203 is downregulated in osteosarcoma cell lines and tissues, and overexpression of miR-203 inhibited osteosarcoma cell proliferation and migration via targeting RAB22A. These data demonstrated that restoration of miR-203 may be a potential therapeutic strategy for treatment of osteosarcoma.

Author Contributions
Conceived and designed the experiments: DY GL KW. Performed the experiments: DY GL KW. Analyzed the data: DY GL KW. Contributed reagents/materials/analysis tools: DY GL KW. Wrote the paper: DY GL KW.

References
1. Salah Z, Arafeh R, Maximov V, Galasso M, Khawaled S, Abou-Sharieha S, et al. miR-27a and miR-27a* contribute to metastatic properties of osteosarcoma cells. Oncotarget. 2015; 6(7):4920–35. Epub 2015/03/03. PMID: 25749032.

2. Han K, Chen X, Bian N, Ma B, Yang T, Cai C, et al. MicroRNA profiling identifies MiR-195 suppresses osteosarcoma cell metastasis by targeting CCND1. Oncotarget. 2015. Epub 2015/04/01. PMID: 25823925.

3. Han G, Wang Y, Bi W, C-Myc overexpression promotes osteosarcoma cell invasion via activation of MEK-ERK pathway. Oncology research. 2012; 20(4):149–56. Epub 2012/01/01. PMID: 23461061.

4. Tang J, Shen L, Yang Q, Zhang C. Overexpression of metadherin mediates metastasis of osteosarcoma by regulating epithelial-mesenchymal transition. Cell proliferation. 2014; 47(5):427–34. Epub 2014/09/02. doi: 10.1111/cpr.12129 PMID: 25174891.

5. Namlos HM, Meza-Zepeda LA, Baroy T, Ostensen IH, Kresse SH, Kuijjer ML, et al. Modulation of the osteosarcoma expression phenotype by microRNAs. PloS one. 2012; 7(10):e48086. Epub 2012/11/08. doi: 10.1371/journal.pone.0048086 PMID: 23133552; PubMed Central PMCID: PMC3445101.

6. Thayanithy V, Park C, Sarver AL, Kartha RV, Korpela DM, Graef AJ, et al. Combinatorial treatment of DNA and chromatin-modifying drugs cause cell death in human and canine osteosarcoma cell lines. PloS one. 2012; 7(9):e3720. Epub 2012/09/09. doi: 10.1371/journal.pone.0043720 PMID: 22957032; PubMed Central PMCID: PMC3434163.

7. Yan K, Gao J, Yang T, Ma Q, Qiu X, Fan Q, et al. MicroRNA-34a inhibits the proliferation and metastasis of osteosarcoma cells both in vitro and in vivo. PloS one. 2012; 7(3):e33778. Epub 2012/03/30. doi: 10.1371/journal.pone.0033778 PMID: 22457788; PubMed Central PMCID: PMC3310405.

8. Wang Y, Zhao W, Fu Q, miR-335 suppresses migration and invasion by targeting ROCK1 in osteosarcoma cells. Molecular and cellular biochemistry. 2013. Epub 2013/08/27. doi: 10.1007/s11100-013-1786-4 PMID: 23975506.

9. Zhao G, Cai C, Yang T, Qiu X, Liao B, Li W, et al. MicroRNA-221 induces cell survival and cisplatin resistance through PI3K/Akt pathway in human osteosarcoma. PloS one. 2013; 8(1):e53906. Epub 2013/02/02. doi: 10.1371/journal.pone.0053906 PMID: 23372675; PubMed Central PMCID: PMC3553141.

10. Zhou X, Wei M, Wang W. MicroRNA-340 suppresses osteosarcoma tumor growth and metastasis by directly targeting ROCK1. Biochemical and biophysical research communications. 2013; 437(4):653–8. Epub 2013/07/23. doi: 10.1016/bbrc.2013.07.033 PMID: 23872151.

11. Guo S, Bai R, Liu W, Zhao A, Zhao Z, Wang Y, et al. miR-22 inhibits osteosarcoma cell proliferation and migration by targeting HMGB1 and inhibiting HMGB1-mediated autophagy. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014; 35(7):7025–34. Epub 2014/04/23. doi: 10.1177/1013536131454884 PMID: 24752578.

12. Yu X, Li Z, Shen J, Wu WK, Liang J, Weng X, et al. MicroRNA-10b Promotes Nucleus Pulposus Cell Proliferation through PhoC-Akt Pathway by Targeting HOXD10 in Intervetebral Disc Degeneration.
13. Yu X, Li Z. MicroRNAs regulate vascular smooth muscle cell functions in atherosclerosis (review). International journal of molecular medicine. 2014; 34(4):923–33. Epub 2014/09/10. doi:10.3892/ijmm.2014.1853 PMID: 24376640; PubMed Central PMCID: PMC3869743.

14. Li Z, Lei H, Luo M, Wang Y, Dong L, Ma Y, et al. DNA methylation downregulated mir-10b acts as a tumor suppressor in gastric cancer. Gastric cancer: official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association. 2015; 18(1):43–54. Epub 2014/02/01. doi:10.1007/s10120-014-0340-8 PMID: 24481854.

15. Luo X, Dong Z, Chen Y, Yang L, Lai D. Enrichment of ovarian cancer stem-like cells is associated with epithelial to mesenchymal transition through a miRNA-activated AKT pathway. Cell proliferation. 2013; 46(4):436–46. Epub 2013/07/23. doi:10.1111/cpr.12038 PMID: 23869765.

16. Li Z, Yu X, Shen J, Chan MT, Wu WK. MicroRNA in intervertebral disc degeneration. Cell proliferation. 2015. Epub 2015/03/05. doi:10.1111/cpr.12180 PMID: 25736871.

17. Zhou J, Song S, Cen J, Zhu D, Li D, Zhang Z. MicroRNA-375 is downregulated in pancreatic cancer and inhibits cell proliferation in vitro. Oncology research. 2012; 20(5–6):197–203. Epub 2012/01/01. PMID: 23581226.

18. Li Z, Yu X, Shen J, Jiang Y. MicroRNA dysregulation in uveal melanoma: a new player enters the game. Oncotarget. 2015. Epub 2015/02/17. PMID:25682876.

19. Fei B, Wu H. MiR-378 Inhibits Progression of Human Gastric Cancer MGC-803 Cells by Targeting MAPK1 In Vitro. Oncology research. 2013; 20(12):557–64. Epub 2013/10/22. doi:10.3727/096504013X13775486749254 PMID: 24139413.

20. Kulkarni S, Augoff K, Rivera L, McCue B, Khoury T, Groman A, et al. Increased expression levels of WAVE3 are associated with the progression and metastasis of triple negative breast cancer. PloS one. 2012; 7(6):e38298. Epub 2012/06/30. doi: 10.1371/journal.pone.0038298 PMID: 22745662; PubMed Central PMCID: PMC3380046.

21. Kong WQ, Bai R, Liu T, Cai CL, Liu M, Li X, et al. MicroRNA-182 targets cAMP-responsive element-binding protein 1 and suppresses cell growth in human gastric adenocarcinoma. The FEBS journal. 2012; 279(7):1252–60. Epub 2012/02/14. doi: 10.1111/j.1742-4658.2012.08519.x PMID: 22325466.

22. Li Z, Yu X, Shen J, Wu WK, Chan MT. MicroRNA expression and its clinical implications in Ewing's sarcoma. Cell proliferation. 2015; 48(1):1–6. Epub 2014/12/23. doi: 10.1111/cpr.12160 PMID: 25530497.

23. Fan L, Wu Q, Xing X, Wei Y, Shao Z. MicroRNA-145 targets vascular endothelial growth factor and inhibits invasion and metastasis of osteosarcoma cells. Acta biochimica et biophysica Sinica. 2012; 44 (5):407–14. Epub 2012/04/05. doi: 10.1093/abbs/gms019 PMID: 22472569.

24. Viticchie G, Lena AM, Latina A, Formosa A, Gregersen LH, Lund AH, et al. MiR-203 controls proliferation, migration and invasive potential of prostate cancer cell lines. Cell Cycle. 2011; 10(7):1121–31. Epub 2011/03/04. PMID: 21368580.

25. Zhu X, Er K, Mao C, Yan Q, Xu H, Zhang Y, et al. miR-203 suppresses tumor growth and angiogenesis by targeting VEGFA in cervical cancer. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology. 2013; 32(1):64–73. Epub 2013/07/23. doi: 10.1159/000350125 PMID: 23867971.
31. Bian K, Fan J, Zhang X, Yang XW, Zhu HY, Wang L, et al. MicroRNA-203 leads to G1 phase cell cycle arrest in laryngeal carcinoma cells by directly targeting survivin. FEBS letters. 2012; 586(6):804–9. Epub 2012/02/07. doi: 10.1016/j.febslet.2012.01.050 PMID: 22306317.

32. Liao H, Bai Y, Qiu S, Zheng L, Huang L, Liu T, et al. MiR-203 downregulation is responsible for chemoresistance in human glioblastoma by promoting epithelial-mesenchymal transition via SNAI2. Oncotarget. 2015; 6(11):9814–28. Epub 2015/04/15. PMID: 25871397.

33. Xu M, Gu M, Zhang K, Zhou J, Wang Z, Da J. miR-203 inhibition of renal cancer cell proliferation, migration and invasion by targeting of FGF2. Diagnostic pathology. 2015; 10(1):24. Epub 2015/04/19. doi: 10.1186/s13000-015-0255-7 PMID: 25890121; PubMed Central PMID: PMC4419389.

34. Lee SA, Kim JS, Park SY, Kim HJ, Yu SK, Kim CS, et al. miR-203 downregulates Yes-1 and suppresses oncogenic activity in human oral cancer cells. Journal of bioscience and bioengineering. 2015. Epub 2015/04/26. doi: 10.1016/j.jbiosc.2015.02.002 PMID: 25910964.

35. Li Z, Yu X, Wang Y, Shen J, Wu WK, Liang J, et al. By downregulating TIAM1 expression, microRNA-423 suppresses gastric cancer invasion and growth. Oncotarget. 2014. Epub 2015/02/06. 25654811.

36. Yu X, Li Z, Liu J. MiRNAs in primary cutaneous lymphomas. Cell proliferation. 2015. Epub 2015/03/05. doi: 10.1111/cpr.12179 PMID: 25736784.

37. Cai CK, Zhao GY, Tian LY, Liu L, Yan K, Ma YL, et al. miR-15a and miR-16-1 downregulate CCND1 expression, suppressing proliferation and invasion of Hela cells. Oncology reports. 2015; 32(3):1051–6. Epub 2015/03/19. doi: 10.3892/or.2015.3656 PMID: 25621610; PubMed Central PMID: PMC4419389.

38. Huang G, Nishimoto K, Zhou Z, Hughes D, Kleinerman ES. miR-20a encoded by the miR-17-92 cluster increases the metastatic potential of osteosarcoma cells by regulating Fas expression. Cancer research. 2012; 72(4):908–16. Epub 2011/12/22. doi: 10.1158/0008-5472.CAN-11-1460 PMID: 22186140; PubMed Central PMID: PMC3288434.

39. Jones KB, Salah Z, Del Mare S, Galasso M, Gaudio E, Nuovo GJ, et al. miRNA signatures associate with pathogenesis and progression of osteosarcoma. Cancer research. 2012; 72(7):1865–77. Epub 2012/02/22. doi: 10.1158/0008-5472.CAN-11-2663 PMID: 22350417; PubMed Central PMID: PMC3328547.

40. Zhao S, Yao DS, Chen JY, Ding N. Aberrant expression of miR-20a and miR-203 in cervical cancer. Asian Pacific journal of cancer prevention: APJCP. 2015; 14(4):2289–93. Epub 2013/06/04. PMID: 23725129.

41. Li T, Gao F, Zhang XP. microRNA-203 enhances chemosensitivity to 5-fluorouracil by targeting thymidylate synthase in colorectal cancer. Oncology reports. 2015; 33(2):607–14. Epub 2014/12/09. doi: 10.3892/or.2014.3646 PMID: 25482885.

42. Taiapaleennaki H, Browne G, Akech J, Zustin J, van Wijnen AJ, Stein JL, et al. Targeting of Runx2 by miR-135 and miR-203 Impairs Progression of Breast Cancer and Metastatic Bone Disease. Cancer research. 2015; 75(7):1433–44. Epub 2015/01/31. doi: 10.1158/0008-5472.CAN-14-1026 PMID: 25634212; PubMed Central PMID: PMC4383679.

43. Haier A, Grunewald TG, Orth M, Reiss C, Kneitz B, Spahn M, et al. Loss of tumor suppressor mir-203 mediates overexpression of LIM and SH3 Protein 1 (LASP1) in high-risk prostate cancer thereby increasing cell proliferation and migration. Oncotarget. 2014; 5(12):4144–53. Epub 2014/07/02. PMID: 24980827; PubMed Central PMID: PMC4147312.

44. Li Z, Du L, Dong Z, Yang Y, Zhang X, Wang L, et al. MiR-203 suppresses ZNF217 upregulation in colorectal cancer and its oncogenicity. PloS one. 2015; 10(1):e0116170. Epub 2015/01/27. doi: 10.1371/journal.pone.0116170 PMID: 25621639; PubMed Central PMID: PMC4306553.

45. Xiang J, Bian C, Wang H, Huang S, Wu D. MiR-203 down-regulates Rap1A and suppresses cell proliferation, adhesion and invasion in prostate cancer. Journal of experimental & clinical cancer research. 2015; 34(1):8. Epub 2015/02/01. doi: 10.1186/s13046-015-0125-x PMID: 25636908; PubMed Central PMID: PMC4321708.

46. Wang T, Gilkes DM, Takano N, Xiang L, Luo W, Bishop CJ, et al. Hypoxia-inducible factors and RAB22A mediate formation of microvesicles that stimulate breast cancer invasion and metastasis. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(31):E3234–42. Epub 2014/06/19. doi: 10.1073/pnas.140041111 PMID: 24938788; PubMed Central PMID: PMC4128139.

47. Zhang Y, Zhao FJ, Chen LL, Wang LQ, Nephew KP, Wu YL, et al. MiR-373 targeting of the Rab22a gene suppresses tumor invasion and metastasis in ovarian cancer. Oncotarget. 2014; 5(23):12291–303. Epub 2014/12/03. PMID: 25460499; PubMed Central PMID: PMC4323008.

48. Yang Z, He M, Wang K, Sun G, Tang L, Xu Z. Tumor suppressive microRNA-193b promotes breast cancer progression via targeting DNAJC13 and RAB22A. International journal of clinical and experimental pathology. 2014; 7(11):7563–70. Epub 2015/01/01. PMID: 25550792; PubMed Central PMID: PMC4270560.