Department of Orthopedics, the Second Xiangya Hospital, Central South University, Changsha, China

IL-1β-mediated NF-κB signaling augments the osteosarcoma cell growth through modulating miR-376c/TGFA axis

Bo Liu, Yu Zhou, Xia Chen, Dan Peng*

Received October 22, 2016, accepted November 25, 2016

**Corresponding author: Dan Peng, Department of Orthopedics, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China*
xyeypd@163.com

Pharmazie 72: 419–424 (2017) *doi: 10.1691/ph.2017.6888*

Overexpression of IL-1β, one of the most well-known pro-inflammatory cytokines, is related to a plenty of diseases including cancer. Diversion of microRNAs exposed to pro-inflammatory cytokines have been noted in cancer cells, however, their functions in inflammation stress are still to be further studied. In our previous study, we reported that miR-376c inhibited the growth of osteosarcoma (OS) cells by targeting TGFA. Here, we revealed that miR-376c was downregulated in OS tissues and cells while IL-1β, NF-κB and TGFA were upregulated in OS tissues and cells. IL-1β or NF-κB could promote the OS cells growth through inducing miR-376c expression and decreasing TGFA protein levels. Furthermore, forced expression of miR-376c restored the suppression of IL-1β on the OS cells. A decrease in miR-376c and an increase in TGFA depended on IL-1β-induced NF-κB protein level, which attenuates miR-376c expression upon IL-1β reduction. Taken together, our findings indicated that IL-1β augmented miR-376c-reduction to promote OS cell growth *via* upregulating NF-κB levels. Knock-down NF-κB suppressed the expression of TGFA. Enhanced TGFA upon IL-1β induction was attenuated by NF-κB inhibition. Hence, the regulation of IL-1β/NF-κB/miR-376c/TGFA signaling in OS might present a promising strategy for the treatment of OS.

1. Introduction

Continuous exposing to inflammatory cytokines is one of the most important reasons of tumorigenesis (Rokavec and Luo 2012); thus controlling chronic inflammation is a critical approach in the prevention of tumor progression (El-Omar 2001). Actually, several inflammatory cytokine levels have been reported to be increased in the serum of cancer patients, including those of TNF-alpha, IL-6, IL-8 and VEGF in lung cancer patients (Song et al. 2013); to date, however, the functions of inflammation in tumors and cancers still remain elusive (El-Omar 2001).

The nuclear factor kappa B (NF-κB) pathway is a most important signaling pathway promoting inflammatory reactions, and is mainly based on the pro-inflammatory cytokine induced NF-κB activation and the function of NF-κB in transcriptionally activating the relative genes such as cytokines and chemokines (Pikarsky et al. 2004). Pro-inflammatory cytokines such as IL-1β trigger NF-κB activation which is usually followed by the activation of RelA- or cRel-containing complexes (Ben-Neriah and Karin 2011). In the cytoplasm, NF-κB remains inactive. It is associated with regulatory proteins including inhibitors of κB (IκB), among which the most important might be IκBα, IκBβ, and IκBε. IκBα is implicated in temporary NF-κB activation, while IκBβ is associated with persistent activation (Zandi et al. 1997). However, because of its complexity, chronic inflammation and the effect of NF-κB on inflammatory response still remains to be further explored.

Inflammation stress has effects on protein-coding gene expression, as well as on microRNAs (miRNAs) expression (Chendrimada et al. 2005). MiRNAs are a family of small, non-coding RNA molecules that are extremely conserved across species and exert crucial functions as gene expression regulators (Bartel 2004; Brown 2009). It has been reported that miRNAs function as regulators as much to 60% to the human protein coding genes, and regulate the protein levels during most biological processes, including cell growth, apoptosis and differentiation (He and Hannon 2004; Orellana and Kasinski 2015). However, whether miRNA expression was altered by chronic inflammation *via* regulating gene transcription or modulating posttranscriptional maturation still remains unknown.

In our previous study, we revealed that the growth of osteosarcoma (OS) cells was inhibited by miR-376c by directly targeting transforming growth factor-alpha (TGFA) (Jin et al. 2013). In this work, we found that IL-1β augmented miR-376c-reduction to promote OS cell growth *via* NF-κB. Knock-down of NF-κB suppressed the expression of TGFA. Moreover, enhanced TGFA upon IL-1β induction was attenuated by NF-κB inhibition. Therefore, this IL-1β/NF-κB/miR-376c/TGFA signaling pathway might play an important role in the growth regulation of OS cells.

2. Investigations and results

2.1. IL-1β treatment inhibits miR-376c expression and expression of TGFA is positively regulated by miR-376c

Mir-376c expression was at significant lower levels in tumor tissues than in normal tissues. Using real-time PCR analysis, we analyzed miR-376c expression in 17 paired samples (tumor and adjacent normal tissues from the same patient). We found a significantly lower level of miR-376c expression in the tumor samples relative to the levels in the adjacent normal tissues ($P < 0.0001$) (Fig. 1A). To characterize miR-376c responsible for IL-1β induction, we profiled miR-376c expression in PBS-treated OS cells and IL-1β-induced OS cells using real-time PCR assay. We examined the expression level of miR-376c in four OS cell lines and normal OS mucosa cells upon IL-1β. The results showed that miR-376c was highly downregulated upon IL-1β induction in Saos2, MG63, U2OS and SW1353 cells (Fig. 1B). Furthermore, it is also revealed that expression of TGFA mRNA showed a reverse associated with miR-376a expression in tumor tissues both with and without IL-1β induction (Fig. 1C, D). Western blot results showed that, 48 h after transfection, suppressed miR-376c in Saos2, MG63, U2OS and SW1353 cells significantly increased TGFA protein expression (Fig. 1C, D).

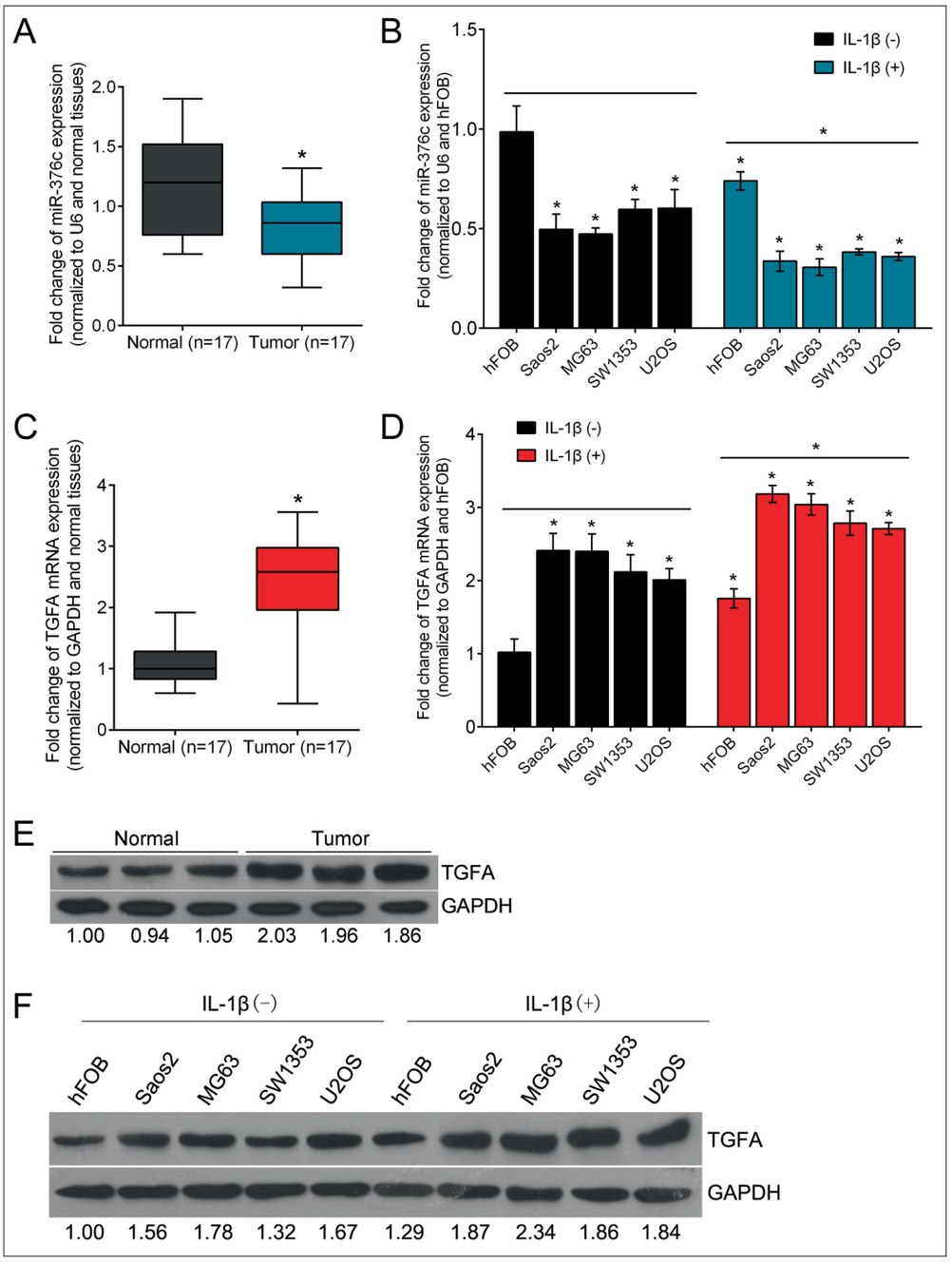

Fig. 1: IL-1β treatment inhibits miR-376c expression and expression of TGFA is positively regulated by miR-376c. MiR-376c expression was at significant lower levels in tumor tissues compared with that in normal tissues (A) and was highly downregulated upon IL-1β induction in Saos2, MG63, U2OS and SW1353 cells (B). Expression of TGFA mRNA showed a reverse associated with miR-376a expression and was at a higher level in tumor tissues both with and without IL-1β induction (C, D). Western blot results showed that, at 48 h after transfection, TGFA protein expression was significantly increased in Saos2, MG63, U2OS and SW1353cellsuponIL-1β induction (E, F). The data are presented as mean±SD of three independent experiments. *P<0.05.

2.2. *Induction of miR-376csuppresses cell growth upon IL-1β induction*

As shown in Fig. 2, we picked up Saos2 and MG63 cells with downregulated miR-376c for further study. It was shown that TGFA is among the most frequently inactivated tumor suppressor genes. Overexpression of TGFA in different mammalian tissue culture cells affects various processes including cell growth. It is plausible that miR-376c induction may suppress growth *via* the upregulation of TGFA in IL-1β-treated cells (Fig. 2A). To validate that induction of miR-376c was able to suppress cell growth upon IL-1β, we transfected Saos2 and MG63 cell lines with miR-376c mimics. It was revealed that, 48 h after transfection, induced miR-376c in Saos2 and MG63 cell lines significantly suppressed cell growth as compared with mimics NC (Fig. 2 B, C, D, and E).

2.3. *IL-1β-induced NF-κB activation is required for cell growth modulation*

IL-1β was confirmed to promote cell growth both in Saos2 and MG63 cells (Fig. 3). To explore whether NF-κB activity was presented in Saos2 and MG63cells treated with IL-1β, we transfected Saos2 and MG63 cells with NF-κB-siRNA firstly. Furthermore, cell growth showed to be inhibited on knocks-down of NF-κB, while inhibition of miR-376c expression was attenuated (Fig. 4). These results suggested that NF-κB activation is required for cell growth modulation.

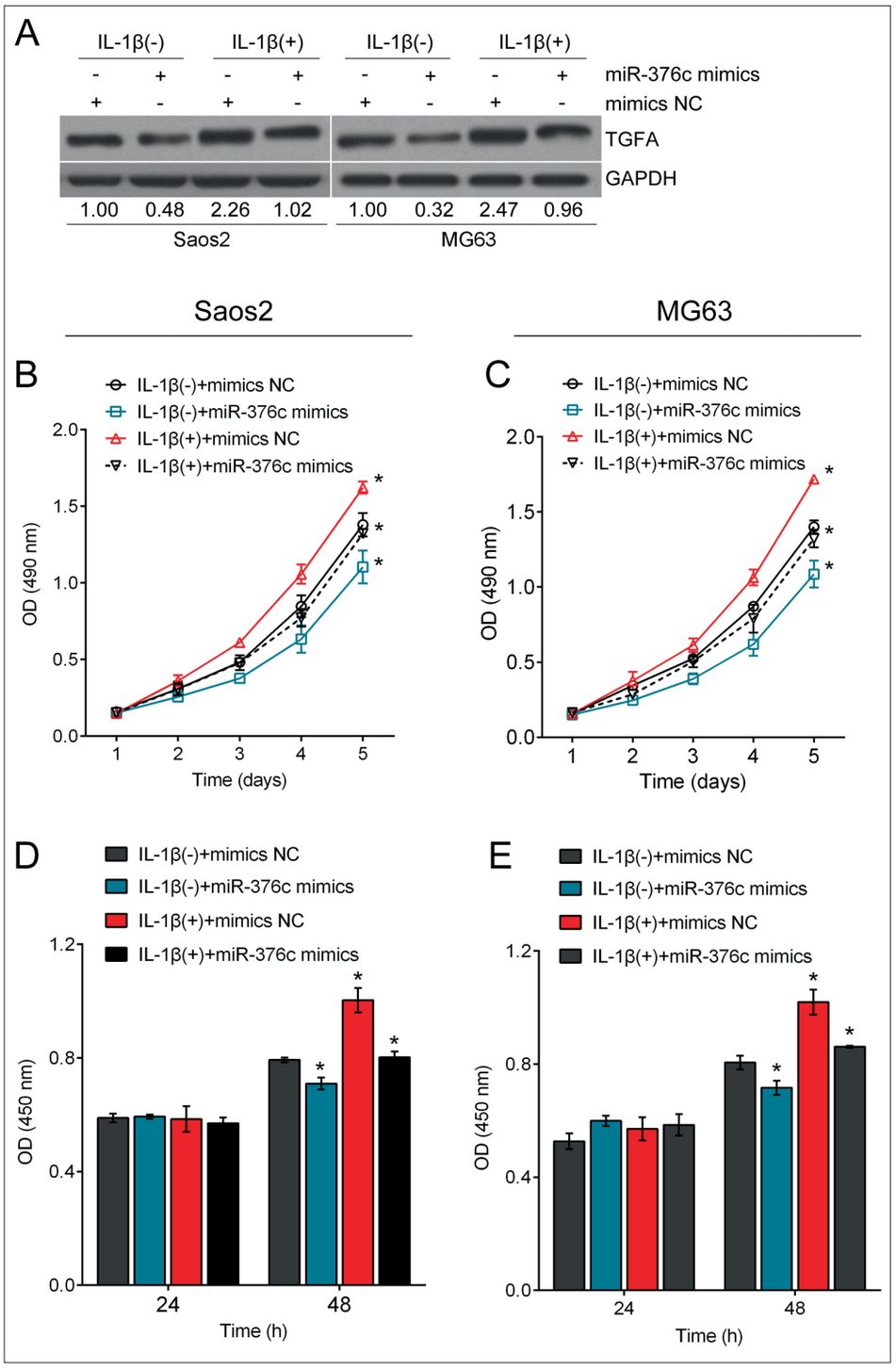

Fig. 2: Induction of miR-376c suppresses cell growth upon IL-1β induction. CCK-8 cell growth assay results showed thatmiR-376c induction may suppress growth via the upregulation of TGFA in IL-1β-treated cells (A). Knock-down of NF-κB showed the similar effect on regulation of growth by miR-376c as revealed (C, D).The data are presented as mean±SD of three independent experiments. *P<0.05.

2.4. *MiR-376c/TGFA axis modulates OS cell growth upon IL-1β/NF-κB pathway*

To determine the mechanism involved in miR-376c transactivation upon IL-1β induction, we observed that IL-1β could induced NF-κB upregulation and miR-376c repression (Fig. 5A). Moreover, results showed TGFA expression was upregulated in IL-1β treated cells *via* NF-κB activation (Fig. 5B). These results suggested that NF-κB dependent IL-1β treatment was required for OS cell growth modulation, most likely *via* inhibition of miR-376c (Fig. 5C).

3. Discussion

As a crucial pro-inflammatory cytokine, interleukin-1 (IL-1) is produced by malignant or micro environmental cells (Kasza 2013). IL-1 has also an effect on tumorigenesis and tumor invasiveness as a pleiotropic cytokine; therefore, it represents a possible applicant for a modulator that can tilt the balance between inflammation and immunity toward the induction of antitumor responses (Roy et al. 2006). IL-1α and IL-1β are crucial agonists of IL-1. In their secreted forms, IL-1α and IL-1β exert the very similar biological

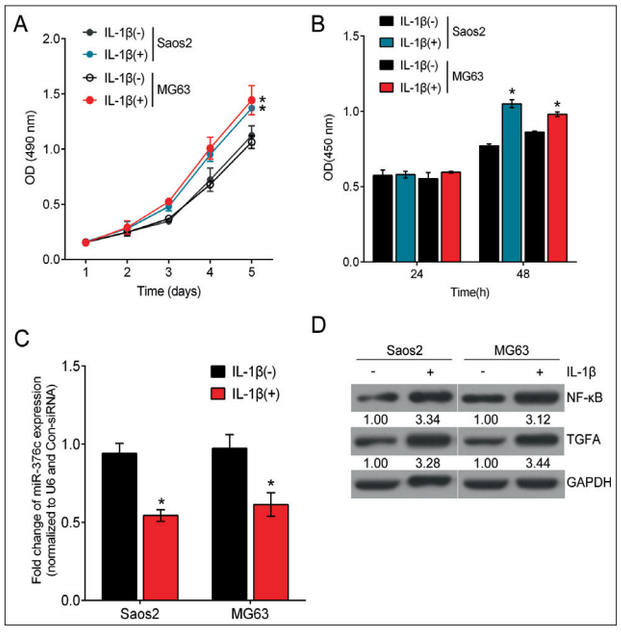

Fig. 3: IL-1β treatment effect on cell growth modulation. The cell growth and invasion were determined using CCK-8 cell growth assay at five time points (on day 1, 2, 3, 4 and 5) and DNA synthesis in proliferating cells was determined by measuring BrdU incorporation (A, B). Saos2 or MG63 cells were transfected with scrambled miR lentivirus (without miR-376c) (miR-SRC), miR-376c lentivirus, added with IL-1β or without IL-1β. The data are presented as mean±SD of three independent experiments. *P<0.05.

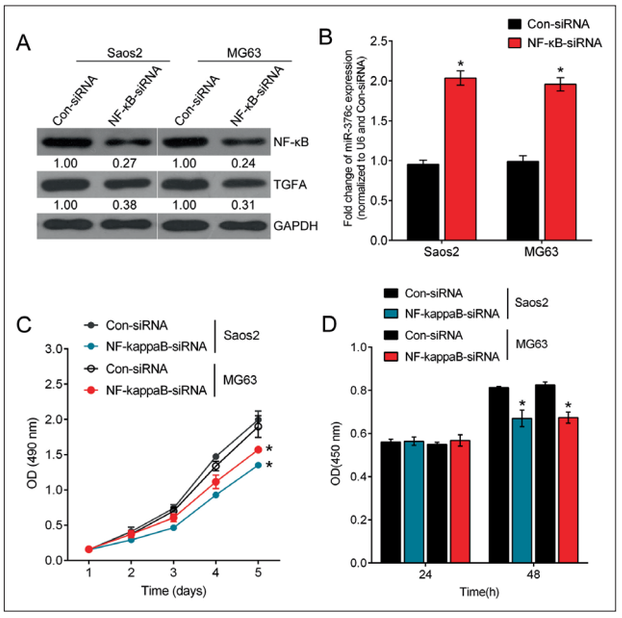

Fig. 4: IL-1β-induced NF-κB activation is required for cell growth modulation. Furthermore, upregulation of TGFA expression and inhibition of miR-376c expression showed to be attenuated on knock-down of NF-κB (A, B). Consequently, cell growth showed to be downregulated on knock-down of NF-κB. These results suggested that NF-κB activation is required for cell growth modulation (C, D). The data are presented as mean±SD of three independent experiments. *P<0.05.

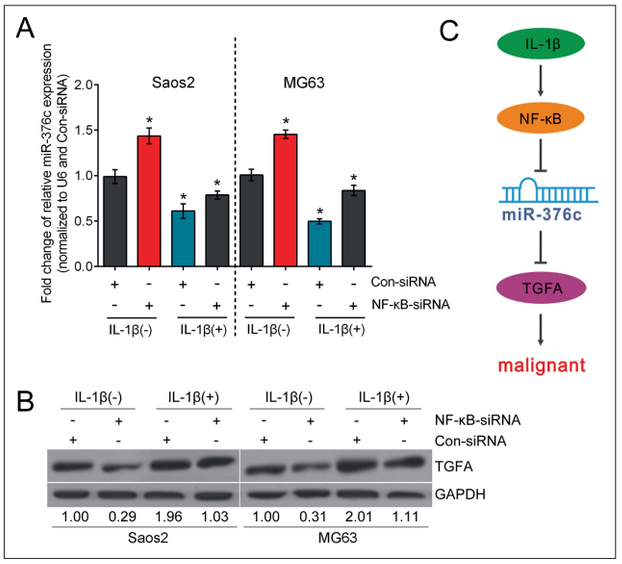

Fig. 5: MiR-376c/TGFA axis modulated cell growth upon IL-1β/NF-κB pathway. IL-1β could induce NF-κB upregulation thus resulted in miR-376c repression (A). Moreover, results showed TGFA expression was upregulated in IL-1β treated cells via NF-κB activation (B). These results suggested that NF-κB dependent IL-1β treatment was required for TGFA upregulation, most likely *via* its inhibition of miR-376a (C). The data are presented as mean±SD of three independent experiments.

effects *via* binding to very similar receptors (Cebo et al. 2001). However, IL-1α and IL-1β are different in their compartmentalization within the cell or the microenvironment (Trebec-Reynolds et al. 2010). IL-1β is active and could mediate inflammation only in its secreted form, and inflammation promotes carcinogenesis, tumor invasiveness and immune suppression (Carmi et al. 2013). Recently, in clinical trials in patients bringing out various diseases with inflammatory manifestations, some new IL-1β antagonists have been used (Zhang 2011). In the present study, we confirmed that miR-376c was downregulated in IL-1β-treated tissues and cells. A better understanding of the integrative role of IL-1β signaling pathways in the malignant process will enable the application of novel IL-1β modulation approaches in cancer patients.

MiR-376c has been found to be modulated in many types of solid tumors, including liver cancer (Iwaki et al. 2013), prostate cancer (Wijayakumara et al. 2015) and ovarian cancer (Ye et al. 2011). In our previous study, miR-376c has been identified to inhibit the growth of OS cells by directly targeting TGFA (Jin et al. 2013). Compared to the scramble control, miR-376c mimics transfected Saos-2 cells exhibited a notable reduction of migratory ability. The consistent effect on invasive ability was also noticed in parallel invasion assay. Meanwhile, we tested cell growth and motility under the condition of overexpression of TGFA and results indicated a forced expression of TGFA increased OS cell growth, migration and invasion. In the present study, we performed real-time PCR to analyze miR-376c expression in 17 paired samples (tumor and adjacent normal tissues from the same patient) while we examined the expression level of miR-376c in four OS cell lines and normal OS mucosa cells upon IL-1β. The results showed that miR-376c was highly downregulated upon IL-1β induction in OS cells and in tumor tissues. Furthermore, expression of TGFA mRNA and protein was reversely associated with miR-376a expression in tumor tissues and IL-1β treated cells.

To further reveal the role and mechanism ofIL-1β in modulation miR-376a and TGFA expression, we picked up Saos2 and MG63 cells with downregulated miR-376c for further study. To understand the effect of miR-376c on OS upon IL-1β, miR-376c mimics were used to enhance its biological function in the Saos2 and MG63 OS cell lines. It was found that exogenous miR-376crepressed cell survival upon IL-1β. It was revealed that, at 48 h after transfection, induced miR-376c in IL-1β treated Saos2 and MG63 cell lines significantly suppressed cell growth as compared with mimics NC. Based on the above findings, miR-376c might be a potential therapeutic target for OS. To explore the effect of miR-376c on OS before it advances further into the clinical application stage, two essential questions persist: how miR-376c affects the outcome of OS, and how miR-376c is downregulated in OS. In many previous

studies, NF-κB activation was revealed to be triggered by proinflammatory cytokines such as IL-1β and usually leads to the activation of RelA- or cRel-containing complexes (Zandi et al. 1997) . To explore whether NF-κB activity was present in Saos2 and MG63cells treated with IL-1β, we transfected Saos2 and MG63 cells with NF-κB-siRNA. As predicted, cell growth showed to be inhibited on knocks-down of NF-κB, while inhibition of miR-376c expression was attenuated, which suggested that NF-κB/miR-376c/TGFA pathway suppressed the growth of OS cells. Our data might provide new insights into the pathophysiological mechanism of OS and suggest a novel drug target for OS.

Among the intracellular nuclear transcription factors, NF-κB is the most crucial one, and it plays a critical role in the transcriptional regulating process of many genes that are affected by diverse stimuli (Mizumoto et al. 2011; Zuo et al. 2011). Evidence showed that, *via* transcriptional regulation, NF-κB could contribute to tumorigenesis (Pikarsky et al. 2004; Zuo et al. 2011). As NF-κB is a regulator to miRNA expression, meanwhile, some miRNAs are also implicated in NF-κB expression directly or indirectly (Bian et al. 2001; Gantier et al. 2012; Song et al. 2012; Zuo et al. 2011). We were interested in miRNA regulation of the NF-κB signal pathway and found that NF-κB induced miR-376c expression to significantly decrease, whereas the knockdown of NF-κB promoted miR-376c expression. Therefore, we conclude that NF-κB promotes miR-376c expression. To determine whether miR-376c's effects on OS growth are downstream of NF-κB, we used gain-of- and loss-of-function assays to examine the role of miR-376c in OS cells. MTT and BrdU assays showed that miR-376crepressed Saos2 and MG63 cell growth compared with the control, which is consistent with NF-κB's ability to suppress the growth of human cervical cell lines.

Furthermore, we revealed a significant effect of IL-1β on miR-376c and TGFA expressions *via* NF-κB activation. To confirm these effects of IL-1β *via* NF-κB activation, we performed real-time PCR analysis finding that in IL-1β treated OS cells miR-376c expression was obviously inhibited, whereas miR-376c expression inhibition was attenuated on knock-down of NF-κB. Moreover, by performing western blot analysis, it was revealed that TGFA expression was upregulated in IL-1β treated OS cells, and the upregulation was attenuated on knock-down of NF-κB by contrast. Taken together, we confirmed that NF-κB dependent IL-1β treatment upregulated TGFA expression, most likely *via* its inhibitory effect on MiR-376c, thus modulating OS cells growth.

In conclusion, our observations indicated that IL-1β-mediated NF-κB signaling augments the OS cell growth *via* modulating MiR-376c/TGFA axis and provided a potential implication for the clinical management of OS.

4. Experimental

4.1. Tissue specimens, cell lines and cell transfection

We collected 17 paired primary OS tissues and the corresponding adjacent normal tissues. All clinic specimens were collected from patients who received a surgical operation at the Second Xiangya Hospital, Central South University (Changsha, China). The clinic specimens were stored in liquid nitrogen at -80 °C. The project of the present study is under the approval of the Ethic Committee of the Second Xiangya Hospital, Central South University.

hFOB, human osteoblast cell line, and four human OS cell lines, including Saos2, MG63, U2OS, SW1353 were purchased from the ATCC, USA, cultured in RPMI-1640 medium (Invitrogen, CA, USA) adding 10% FBS (Gibco, CA, USA) as the supplement, incubated at 37 °C in a humidified atmosphere with 5% CO_2.

MiR-376c mimics were used to achieve miR-376c overexpression (GeneCopoecia, Guangzhou, China). TGFA ORF expression clone (GeneCopoecia, Guangzhou, China) was used to achieve TGFA overexpression. NF-κB-siRNA (Cell Signaling, MA, USA) was used to achieve NF-κB knockdown. Lipofectamine2000 (Invitrogen, CA, USA) was used for the indicated transfection. After being plated in 96-well or 6-well plates, cells were then transfected with indicated miRNA mimics or ORF expression clone, cultured for 24 h, consequently used for the next experiments.

4.2. RNA extraction and real-time PCR assays

We extracted total RNA from target cells by using Trizol reagent (Invitrogen). Mature miR-376c expression was determined using a Hairpin-it TM miRNAs qPCR kit (Genepharma, Shanghai, China). TGFA expression was monitored by using SYBR green qPCR assay. RNU6B expression was used as an endogenous normalization. Data were analyzed by using $2^{-\Delta\Delta CT}$ method.

4.3. CCK-8 cell growth assay

The proliferation rates of OS cells were monitored by using Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich, MO, USA). We seeded 0.5×10^4 target cells in each well of the 96-well plate for 24 h. The target cells were then transfected with the indicated miRNA mimics or siRNA, with or without IL-1β treatment, and further incubated for 1, 2, 3, 4 and 5 days respectively. 1 h before the endpoint, 10 μl CCK-8 reagents was added into each well. A microplate reader was used to determine OD_{490nm} value in each well.

4.4. BrdU incorporation assay

DNA synthesis in the target cells was monitored by measuring 5-bromo-2-deoxyuridine (BrdU) incorporation. 24 h or 48 h after cell transfection with the indicated miRNA mimics or siRNA, BrdU assays were performed. The transfected cells were seeded in 96-well culture plates at a density of 2×10^3 cells/well, cultured for 24 h or 48 h, incubated with a final concentration of 10 μM BrdU (BD Pharmingen, CA, USA) for 2 h to 24 h. At the end of the incubation period, the medium was removed, the cells were fixed for 30 min at RT, incubated with peroxidase-coupled anti-BrdU-antibody (Sigma-Aldrich) for 60 min at RT, washed three times with PBS, incubated with peroxidase substrate (tetramethylbenzidine) for 30 min, and the absorbance values were measured at 490 nm. Background BrdU immunofluorescence was determined in cells not exposed to BrdU but stained with the BrdU antibody.

4.5. Western blot analysis

Immunoblotting was performed to detect the expression of NF-κB and TGFA in OS cell lines. Cultured or transfected cells were lysed in RIPA buffer with 1% PMSF. Protein was loaded onto a SDS-PAGE minigel and transferred onto PVDF membrane. After probed with 1:1000 diluted rabbit polyclonal NF-κB and TGFA antibody (Abcam, MA, USA) at 4 °C overnight, the blots were subsequently incubated with HRP-conjugated secondary antibody (1:5000). Signals were visualized using ECL Substrates (Millipore, MA, USA). β-Actin was used as an endogenous protein for normalization.

4.6. Luciferase reporter assay

Saos2, MG63, U2OS, SW1353 cells were transfected with miR-376c and pGL3 luciferase reporter constructs harboring the miR-376c target sequence. After 24 h, the activities of firefly luciferase and renilla luciferase in the cell lysates were measured with the Dual-Luciferase Assay System (Promega, Madison, WI, USA). For the luciferase transcription reporter assay, miR-376c gene promoter sequences (WT or site deletion) were cloned into the promoter region of the pGL3-Basic vector, and luciferase activity was measured as described above.

4.7. Statistical analysis

All data from three independent experiments were expressed as mean±SD and processed using SPSS17.0statistical software. The expression of miR-376c in OS tissues and their matched adjacent normal bone and myeloid tissues were compared by Wilcoxon's paired test. The difference among the groups in migration and invasion assay was estimated by Student's t-test or one-way ANOVA. A P value of<0.05 was considered to be statistically significant.

Acknowledgement: This paper was supported by the program of Hunan Provincial Department of science and technology support (project number: 2015JC3045).

Conflicts of interest: None declared.

References

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281-297.

Ben-Neriah Y, Karin M (2011) Inflammation meets cancer, with NF-kappaB as the matchmaker. Nat Immunol 12: 715-723.

Bian X, McAllister-Lucas LM, Shao F, Schumacher KR, Feng Z, Porter AG, Castle VP, Opipari AW, Jr. (2001) NF-kappa B activation mediates doxorubicin-induced cell death in N-type neuroblastoma cells. J Biol Chem 276: 48921-48929.

Brown RH (2009) Medicine. A reinnervating microRNA. Science 326: 1494-1495.

Carmi Y, Dotan S, Rider P, Kaplanov I, White MR, Baron R, Abutbul S, Huszar M, Dinarello CA, Apte RN, Voronov E (2013) The role of IL-1beta in the early tumor cell-induced angiogenic response. J Immunol 190: 3500-3509.

Cebo C, Dambrouck T, Maes E, Laden C, Strecker G, Michalski JC, Zanetta JP (2001) Recombinant human interleukins IL-1alpha, IL-1beta, IL-4, IL-6, and IL-7 show different and specific calcium-independent carbohydrate-binding properties. J Biol Chem 276: 5685-5691.

Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, Nishikura K, Shiekhattar R (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436: 740-744.

El-Omar EM (2001) The importance of interleukin 1beta in Helicobacter pylori associated disease. Gut 48: 743-747.

Gantier MP, Stunden HJ, McCoy CE, Behlke MA, Wang D, Kaparakis-Liaskos M, Sarvestani ST, Yang YH, Xu D, Corr SC, Morand EF, Williams BR (2012) A miR-19 regulon that controls NF-kappaB signaling. Nucleic Acids Res 40: 8048-8058.

He L, Hannon GJ (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 5: 522-531.

Iwaki J, Kikuchi K, Mizuguchi Y, Kawahigashi Y, Yoshida H, Uchida E, Takizawa T (2013) MiR-376c down-regulation accelerates EGF-dependent migration by targeting GRB2 in the HuCCT1 human intrahepatic cholangiocarcinoma cell line. PLoS One 8: e69496.

Jin Y, Peng D, Shen Y, Xu M, Liang Y, Xiao B, Lu J (2013) MicroRNA-376c inhibits cell proliferation and invasion in osteosarcoma by targeting to transforming growth factor-alpha. DNA Cell Biol 32: 302-309.

Kasza A (2013) IL-1 and EGF regulate expression of genes important in inflammation and cancer. Cytokine 62: 22-33.

Mizumoto Y, Kyo S, Kiyono T, Takakura M, Nakamura M, Maida Y, Mori N, Bono Y, Sakurai H, Inoue M (2011) Activation of NF-kappaB is a novel target of KRAS-induced endometrial carcinogenesis. Clin Cancer Res 17: 1341-1350.

Orellana EA, Kasinski AL (2015) MicroRNAs in cancer: a historical perspective on the path from discovery to therapy. Cancers (Basel) 7: 1388-1405.

Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431: 461-466.

Rokavec M, Luo JL (2012) The transient and constitutive inflammatory signaling in tumorigenesis. Cell Cycle 11: 2587-2588.

Roy D, Sarkar S, Felty Q (2006) Levels of IL-1 beta control stimulatory/inhibitory growth of cancer cells. Front Biosci 11: 889-898.

Song L, Liu L, Wu Z, Li Y, Ying Z, Lin C, Wu J, Hu B, Cheng SY, Li M, Li J (2012) TGF-beta induces miR-182 to sustain NF-kappaB activation in glioma subsets. J Clin Invest 122: 3563-3578.

Song XY, Zhou SJ, Xiao N, Li YS, Zhen DZ, Su CY, Liu ZD (2013) Research on the relationship between serum levels of inflammatory cytokines and non-small cell lung cancer. Asian Pac J Cancer Prev 14: 4765-4768.

Trebec-Reynolds DP, Voronov I, Heersche JN, Manolson MF (2010) IL-1alpha and IL-1beta have different effects on formation and activity of large osteoclasts. J Cell Biochem 109: 975-982.

Wijayakumara DD, Hu DG, Meech R, McKinnon RA, Mackenzie PI (2015) Regulation of Human UGT2B15 and UGT2B17 by miR-376c in Prostate Cancer Cell Lines. J Pharmacol Exp Ther 354: 417-425.

Ye G, Fu G, Cui S, Zhao S, Bernaudo S, Bai Y, Ding Y, Zhang Y, Yang BB, Peng C (2011) MicroRNA 376c enhances ovarian cancer cell survival by targeting activin receptor-like kinase 7: implications for chemoresistance. J Cell Sci 124: 359-368.

Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M (1997) The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell 91: 243-252.

Zhang H. 2011. Anti-IL-1beta therapies. Recent Pat DNA Gene Seq 5: 126-135.

Zuo QP, Liu SK, Li ZJ, Li B, Zhou YL, Guo R, Huang LH (2011) NF-kappaB p65 modulates the telomerase reverse transcriptase in the HepG(2) hepatoma cell line. Eur J Pharmacol 672: 113-120.