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MACF1 POSITIVELY REGULATES OSTEOBLAST DIFFERENTIATION VIA β-CATENIN SIGNALLING

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Introduction: Microtubule-actin crosslinking factor 1 (MACF1) is a key cytoskeletal linker and plays a critical role in numerous cells [1]. We have previously demonstrated MACF1 to be highly expressed in osteoblastic cells and participated in the osteoblasts response to environmental stimuli [2]. However, the role of MACF1 in osteoblast function is not well understood. Studies show that MACF1 is critical for embryo development by activating Wnt/β-catenin signalling, which also plays a key role in regulating osteoblast differentiation. We therefore aimed to investigate whether MACF1 regulates osteoblast differentiation through Wnt/β-catenin signalling in this study.

Subjects and Methods: Stable MACF1-knockdown MC3T3-E1 preosteoblasts constructed by lentivirus-mediated short-hairpin RNA (shRNA) targeting *MACF1* gene were adopted. The MC3T3-E1 preosteoblasts infected by control-shRNA were used as a control. Alizarin red s staining was applied to detect mineralised nodules formation. Real time PCR was used to detect the mRNA expression of alkaline phosphatase (*ALP*), runt-related transcription factor 2 (*Runx2*), and T-cell factor 1 (*TCF1*). The luciferase reporter assay was further used to detect the TCF1 transcriptional activity. Moreover, the β-catenin and GSK-3β levels were measured by western blot with and without lithium chloride treatment.

Results: MACF1-knockdown (MACF1-KD) significantly inhibited mineralised nodules formation and osteogenic gene expression. The translocation of β-catenin into the nucleus was decreased by MACF1-knockdown and the downstream TCF1 transcriptional activity and *Runx2* expression were significantly suppressed. However, 6 hour treatment of 30 mM LiCl inhibited GSK-3β activation, induced β-catenin nuclear translocation and partly restored the decreased expression of TCF1 and Runx2. These findings showed that MACF1 deficiency inhibited osteoblast differentiation and the nuclear translocation of β-catenin, suggesting that MACF1 positively regulates osteoblast differentiation and may act through GSK3β/β-catenin signalling.

Discussion and Conclusion: The present study uncovers, for the first time an important role of MACF1 in osteoblasts and suggests that MACF1 may positively regulate osteoblast differentiation via β-catenin/TCF1-Runx2 signalling and acts upstream of GSK-3β/β-catenin signalling.

Funding/support: This work was supported by the National Natural Science Foundation of China (31400725, 31570940), the Project Funded by China Postdoctoral Science Foundation (2015T81051), and the Project Supported by Natural Science Basic Research Plan in Shaanxi Province of China (2015JM3078,2015JQ3076).

Acknowledgements: The authors thank Dr Hong Zhou (The University of Sydney, Australia) for generously providing MC3T3-E1 cell line and Dr Pengsheng Zheng for gifting Top flash luciferase plasmid and *Renilla* luciferase vector (Xi'an Jiaotong University).

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c-Kit-Gαi COMPOUNDS MEDIATED SCF DOWNSTREAM SIGNALLING PATHWAYS ACTIVATED IN THE PROLIFERATION AND SURVIVAL OF OSTEOSARCOMA CELLS

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Background and objective: Osteosarcoma is the most common primary malignancy of bone. Abnormal expression and activation of the receptor tyrosine kinases (RTKs) play a vital role in the occurrence and development of osteosarcoma. Studies show that c-Kit, a type III RTK, which is activated by its ligand, stem cell factor (SCF), and the newly-identified signalling mediator Gαi were both over-expressed in human OS tissues and cell line. The two also formed a signalling complex. Further studies showed that c-Kit-Gαi recruited the adaptor protein Gab1 (Grb2-associated binder 1) to transduce downstream signalling. This study was designed from a perspective of signalling pathway to investigate the role of c-Kit-Gαi complex in the proliferation of osteosarcoma.

Methods: The expression of Gαi1/3, c-kit, and Gab1 of OS cell lines (U2OS, MG-63), osteoblasts (MC3TC-E1), and bone cells (OB-6) were detected by western blot. MG63 cells were incubated with 0, 0.1, 1, 5, 25, and 50ng/ml of SCF for 24 and 48 hours. The cell proliferation was detected by Brdu method and the cell survival was detected by MTT. The MG63 cells were cultured with 50ng/ml SCF for 0, 5, 10, 20, and 30 minutes, western blot was used to detect the expression of T- Kit, P-Kit, T-Gab1, P-Gab1, T-AKT, Akt473, P-S6K, P-PLC, T-PLC, T-S6, P-S6, T-ERK, and P-ERK. We used the shRNA-mediated silencing or genetic knockout of Gαi in the MG-63 cells, and further detection of the activation conditions of Akt-mTOR and Erk-MAPK downstream signalling (T-AKT, Akt473, Akt308, GSK3α/3β, mTOR, S6K, S6, ERK) in the cells induced by SCF.

Results: The expression of c-Kit, Gαi1/3, and Gab1 in cultured OS cell lines (U2OS, MG-63) was significantly higher than those of osteoblasts (MC3TC-E1) and bone cells (OB-6). In MG-63 cells, the exogenous SCF increased the phosphorylation of c-Kit, Gab1, Akt473, S6K, PLC, S6, and ERK, activated signalling pathways, and then promoted cell proliferation and survival. shRNA-mediated silencing or genetic knockout of Gαi dramatically inhibited the phosphorylation of Akt473, Akt308, GSK3α/3β, mTOR, S6K, S6, and ERK, which was activated by SCF.

Conclusion: The c-Kit-Gαi complex transduces Akt-mTOR and Erk-MAPK downstream signalling activated by SCF, and then plays a key role in maintaining and promoting the survival and proliferation of osteosarcoma cells. The target therapy aimed at the complex will provide a new approach to curb and treat osteosarcoma.

Funding/support: This study was sponsored by a grant from the National Natural Science Foundation of China (no. 81171712), Jiangsu Province's Key Provincial Talents Program (2011—15), and Jiangsu Province's Key Medical Department in 2011.
http://dx.doi.org/10.1016/j.jot.2016.06.092

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OSTEOGENIC CAPABILITY OF BMP9 IS RESTORED BY ATRA VIA ACTIVATE p38MAPK PATHWAY IN OSTEOSARCOMA CELLS

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Introduction: Osteosarcoma (OS) is the most frequent primary bone sarcoma, which is generally regarded as a differentiation disease that is caused by genetic and epigenetic disruptions of terminal differentiation of osteoblasts. Bone morphogenetic protein 9(BMP9) is probably the most osteogenic differentiation potent inducer, but whether it can induce osteogenic differentiation of OS is unclear. All-trans retinoic acid (ATRA) can restore normal osteogenesis in OS. In this study, we aim to identify the relationship between ATRA and BMP9 in inducing osteogenesis differentiation of OS.

Subjects and Methods: 143B, OS cell line, were used to explore the mechanisms of regulating differentiation and proliferation during treatment with ATRA and (or) BMP9. The level of protein expression was tested by western blot assay. The levels of mRNA expression were tested by Semi-quantitative RT-PCR (sqPCR). ALP activity was assessed by a modified Great Escape SEAP Chemiluminescence assay. The proliferation effects were tested by crystal violet staining.

Results: In 143B cells, BMP9 could not up-regulate the expression of osteogenesis markers (such as Runx-2, Dlx-5, ALP, OPN, and OCN) but conversely, it could promote proliferation of cells. Then, we found that ATRA successfully enhanced these markers, and it significantly inhibited proliferation of cells. The expression levels of BMP9 were up-regulated after ATRA treatment. Cells stimulated by treating with ATRA and BMP9 exhibited higher expression levels of osteogenesis markers, than that treated by ATRA or BMP9, and also the effect of anti-proliferation of ATRA was enhanced. During differentiation induction, the expression level of P-p38 was up-regulated with ATRA treatment, which was higher than that with ATRA or BMP9 treatment. The osteogenesis markers induced by ATRA or ATRA and BMP9, were down-regulated by treated with SB (p38 inhibitor SB203580). Meanwhile, SB could promote the proliferation of 143B cells; it also reversed the anti-proliferation of ATRA.

Discussion and Conclusion: According to the results of all above, we find that BMP9 fails to induce osteogenic differentiation of 143B cells alone, which is inconsistent with the previous report that BMP2 can promote differentiation of OS, indicating the existence of possible differentiation of BMP9 defects in OS. We found that ATRA successfully induces osteoblastic differentiation of osteosarcoma cells *in vitro* and it significantly inhibited proliferation of 143B cells, which is consistent with other reports. Meanwhile, ATRA up-regulated the expression levels of BMP9 in OS. The expression levels of osteogenic-related genes were enhanced when BMP9 combined with ATRA. Thus, we suppose that in the osteogenic potential restoration of BMP9 in OS, ATRA may play a critical role. During differentiation induction, the p38MAPK pathway may have an important role in the control of osteogenesis-related genes expression. In conclusion, this study demonstrates that ATRA can restore the osteogenic capability of