Microrna Mir-17 Promotes Tumor Growth Of Synovial Sarcoma By Inhibiting P21waf1/cip1

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Disclosures:

Introduction: Synovial sarcoma is high-grade malignancy and accounts for approximately 5-10% of the soft tissue sarcoma, which mainly occurs surrounding para-articular regions in adolescents and young adults. Surgical resection and combination chemotherapy has resulted in about 60% five-year survival rate, however that of ten-year became miserably low; development of effective therapeutics linking to an improved prognosis is therefore urgently implored. MicroRNAs can function as tumor suppressors or as oncogenes and also as potential specific cancer biomarkers; however, the function of microRNAs in synovial sarcoma has still unclear.

Methods: All procedures in this study were approved by the ethical committee of Hokkaido University Graduate School of Medicine.

(in vitro study) Following infection of OncoMir library in synovial sarcoma cells, we identified miR-17 from large colonies formed by Fuji cells in colony formation assay (Figure 1). We established miR-17-overexpressing synovial sarcoma cell lines, and the cell proliferation and colony forming ability were analyzed. To clarify the miR-17-targeting genes, we tested the prediction of miR-17 target gene using a PicTar algorithm, and luciferase reporter assay was performed to assess the miR-17-dependent direct regulation. Furthermore, the effect of anti-miR-17 reagent on cell growth of Fuji and HS-SYII cells was examined.

(in vivo study) The levels of miR-17 in seven synovial sarcoma patient’s samples were investigated by qRT-PCR. To evaluate the effect of miR-17 on in vivo tumorigenic activity of synovial sarcoma, miR-17-overexpressing Fuji cells were subcutaneously injected into nude mice, and the tumor volume was measured twice a week. Histopathological evaluation of the formed tumors was performed. Statistical analyses were performed with Student’s t-test. P values <0.05 were considered statistically significant.

Results: (in vitro study) miR-17 was endogenously expressed in three synovial sarcoma cell lines Fuji, HS-SYII, and SYO-1 and also at the various extent in all of human synovial sarcoma samples tested (Figure 2). Overexpression of miR-17 in Fuji and HS-SYII cells significantly increased cell growth and colony forming ability (Figure 2). Of note, miR-17 was induced by SYT-SSX, a specific oncprotein in synovial sarcoma (Figure 3). PicTar algorithm predicted p21Waf1/Cip1 as the direct targeting molecule of miR-17 with the reliability of scoring of 4.19. Forced expression of miR-17 notably attenuated the levels of p21 protein in Fuji cells in p53-independent manner. It was noteworthy that marked p21 expression upon Doxorubicin treatment was also strikingly repressed by miR-17 overexpression (Figure 4). Luciferase reporter assay demonstrated that miR-17 directly targets the 3’-UTR of p21 mRNA (Figure 5). Treatment of anti-miR-17 in Fuji and HS-SYII cells led to the decreased cell growth, consistent with upregulation of p21 protein (Figure 6).

(in vivo study) Tumor volume formed in mice in vivo is significantly increased by miR-17 overexpression, with a marked increase in MIB-1 index (Figure 7).

Discussion: In this study, the findings provide the first evidence that miR-17 plays an essential role in human synovial sarcoma. Forced expression of miR-17 leads to promote colony formation capacity, cell proliferation, and tumorigenicity in vivo by directly targeting the p21-3’UTR. In addition, we here revealed that significant involvement of SYT-SSX chimeric protein for inducing miR-17. In conclusion, miR-17 promotes the tumor growth in synovial sarcoma by targeting p21 mRNA, which may be critical for the tumorigenicity of synovial sarcoma.

Significance: We here disclosed the significance of miR-17 on tumorigenicity of synovial sarcoma in SYT-SSX dependent manner, targeting p21 mRNA. miR-17 might be therefore potent molecular target in the treatment of human synovial sarcoma.

Acknowledgments: We thank Dr. Akira Kawai and Dr. Hiroshi Sonobe for the use of SYO-1 and HS-SYII cells, respectively, Dr. Shigetsugu Hatakeyama for the use of a Luminometer, Ms. Kazuko Shimizu for pathological assistance, and all members of our laboratory for helpful discussions. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology, from the Japan Society for the Promotion of Science, and from the Ministry of Health, Labor, and Welfare of Japan, as well as a grant from the Japan Science and Technology Agency.


Figure 2

(A) miR-17 expression is elevated in these immortalized human cell lines. PU.1, MS-5, TXX1, and NCI-H460, and also in the various subclones of the primary human immortal sarcoma samples tested (MS-5C). miR-17 correlates with increased sarcoma colony formation capacity and cell proliferation, as well as enhanced tumor growth and metastasis.

(B) Immortalized human sarcoma cell lines (i.e., SV40-T, PU.1, MS-5, TXX1) were inoculated with miR-17 expressing and control vectors, respectively, and the stably transfected cells were analyzed for colony formation capacity and cell proliferation.

Figure 3

(A) Overexpression of SYT-SSX2 chimeric protein increased expression of miR-17 than their control by semiquantitative RT-PCR in Fuji cells. These results suggest that SYT-SSX2 chimeric protein can regulate miR-17 by some specific signaling pathways.
Fig. 4. miR-17 significantly decreased the levels of the p21 protein in FUsI cells. miR-17 regulates the level of p21 signaling. FUSI cells were infected with miR-17 lentivirus or control lentivirus. Expression of the p21 protein was evaluated by Western blot. As shown, the levels of p21 were significantly decreased in the presence of miR-17, but not in the presence of control.

Fig. 5. miR-17 directly targets p21-3'UTR in synovial sarcoma. (A) Overexpression of miR-17 inhibits p21-3'UTR luciferase activity in FUSI cells. Dual luciferase assays in FUSI cells. The reporter constructs containing p21-3'UTR or not in downstream of the firefly luciferase gene. The constructs were cotransfected with the miR-17 overexpression plasmid or with the control vector. Relative luciferase activity was normalized to that of firefly luciferase. When miR-17 was cotransfected with p21-3'UTR in FUSI cells, p21-3'UTR luciferase activity showed significant inhibition, compared with their control group. (B) Dual luciferase assay in stably transduced FUSI miR-17 cells. The almost same result was obtained.
Fig. 6. Anti-miR-17 agent suppressed cell proliferation and increased expression of the p21 protein. (A) Then, we co-transfected the luciferase reporters vector with miR-17 expression vectors or with the control vector. The Renilla luciferase reporter vector pRL-CMV (Promega, Madison, WI, USA) was also transfected for transfection efficiency. Firefly and Renilla luciferase activity was measured. When MD cells were treated by anti-miR-17 agent, the cell growth was suppressed, compared with their control. (B) When MD cells were treated by anti-miR-17 agent, the expression of the p21 protein was increased, compared with treated with their control.

Fig. 7. Anti-miR-17 agent suppressed the expression of p53 in vivo. HIV-1 vector containing the intact copy of p53 or P75 in MD cells and their control. After 29 days after injection, the xenografts of P53 MD cells became clearly larger than the control. Finally, in 50 days after injection, the size of P53 MD cells xenografts is significantly larger than their control mice. These indicate that overexpression of miR-17 increases the expression of p53 and suppresses the expression of p53.