Saudi Journal of Biological Sciences 25 (2018) 982–985

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

MicroRNA-4728 serves as a suppressor and antagonist of oncogenic MAPK in Burkitt lymphoma

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ARTICLE INFO

Article history:
Received 29 March 2018
Revised 4 May 2018
Accepted 6 May 2018
Available online 7 May 2018

Keywords:
miR-4728
MAPK
Burkitt lymphoma

ABSTRACT

Objective: This study identified the biological role of miR-4728 in Burkitt lymphoma (BL) process.
Methods: Ramos cells were used to analyze MicroRNA-4728 (miR-4728) biological functions. MiR-4728 expression was investigated in 14 randomly chosen tumor tissues and 12 noncancerous tissues by qRT-PCR. Cyquant assay was used to monitor cell proliferation. Colony formation assay was performed to study the effectiveness of miR-4728 on the proliferation of cells. The effects of miR-4728 on MAPK signaling pathway were detected by luciferase reporter assay. The significance of differences between groups were evaluated by SPSS.
Results: In this study, MiRNA-4728 was observed to down-regulated in BL tissues compared to the noncancerous tissues. Additionally, miR-4728 inhibited Ramos cell proliferation. Moreover, miR-4728 overexpression also decreased the MAPK signaling activity.
Conclusion: Our results suggested that miR-4728 serves as a suppressor and antagonist of oncogenic MAPK in Burkitt lymphoma. The appropriate regulation of miR-4728 might be vital to improve BL treatment.

1. Introduction

Burkitt lymphoma (BL) is a highly aggressive mature B-cell lymphoma with endemic, sporadic, and immunodeficiency-associated variants (Kluiver et al., 2006). Recently, the B-cell lymphoma classification has made it possible to recognize and successfully treat a range of disorders. However, a significant proportion of B-cell lymphoma patients still fail to respond the therapy (Di Lisio et al., 2012). Therefore, there is still a demand to identify new potential therapeutic targets.

In cancer, microRNAs (miRNAs) have been considered as important therapeutic agents, since their abnormal expression is involved in disease development, progression, metastasis, therapeutic response and patient overall survival (Monroig-Bosque Pdel et al., 2015). Although much is known about the profiling of miRNAs in many tumors, our understanding of the role of these miRNAs is incomplete, and much is left to discover. The relevance of miRNAs in lymphoid malignancies first drew our attention in 2002 (Calin et al., 2002). Since then, several studies have confirmed the importance of miRNAs in the lymphoma molecular orchestra, the mechanistic details of miR-4728 regulation are still not fully elucidated in BL.

In this study, we investigated that miR-4728 was down-regulated in human BL tumors. MAPK signaling pathway was found to be repressed after miR-4728 overexpression by the in vitro assays. It indicates that miR-4728 serves as a suppressor and antagonist of oncogenic MAPK in Burkitt lymphoma. Our study provides some hints for profound learning of miR-4728.

2. Material and Methods

2.1. Cell culture

The BL cell line, Ramos, was purchased from ATCC. The cells were maintained in RPMI-1640 medium (Cambrex Biosciences, Walkersville, MD) supplemented with ultraglutamine 1 (Cambrex Biosciences, Walkersville, MD), 100 U/ml penicillin/streptomycin, and 10% FBS (Atlanta Biologicals, Atlanta, GA) in an atmosphere containing 5% CO2 at 37 °C.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2018.05.010
1319-562X/

2.2. MiR-4728 overexpression

MiR-4728 overexpression was achieved by miR-4728 mimic (mimic-miR-4728) (life technologies, Carlsbad, CA) or miRNA control mimics (mimic-Ctrl) (life technologies) transfection according to the manufacture's instruction. The transfection was performed by antibiotic-free Opti-MEM medium (Gibco) with RNAiMAX reagent (life technologies). After 6 h transfection, the complete medium replaced the culture medium.

2.3. RNA isolation and quantitative real-time PCR

Total RNA of tissues or cells was extracted with mirVanaTM miRNA isolation kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. RNAs were reversely transcribed into cDNA with by RNA-to-cDNA kit (Applied Biosystems, Foster City, CA) after removing contaminating DNA by DNA-free DNase (Ambion, Austin, TX). The quantitative real-time PCR (qRT – PCR) assays were used to detect the gene expression with gene specific probe (Applied Biosystems, Foster City, CA). GAPDH was used as an internal control. The tissue collection was approved from the ethical committee.

To determine miRNA expression, the total RNA was reversely transcribed with a miRNA-specific looped RT primer (Applied Biosystems). U6 was used as an internal control. The miRNA-specific Taqman minor groove binder probes were available at Applied Biosystems.

All Taqman qRT- PCR studies were performed in triplicate on an ABI 7500 system (Applied Biosystems). Data were presented as mean ± SE (n = 3).

2.4. Cell viability assay

CyQuant assay kit (Thermo Fisher Scientific) was used to determine cell proliferation according to the manufacturer's description. Ramos cells were seeded into 96-well plates (5000 cells per well). After transfection with mimic-miR-4728 or mimic-Ctrl, the medium was replaced with the complete culture medium. The plates were frozen at the indicated time of culture. The fresh prepared CyQuant solution was added and the measurement was performed following the assay instructions. Data were presented as mean ± SE (n = 3). Cells without transfection were considered as blank control (BC).

2.5. Colony formation assay

The colony formation assay was performed to study the effectiveness of miR-4728 on the proliferation of cells. The suspended Ramos cells were seeded in the 6-well plate (BD Biosciences, Bedford, MA) (1000 cells per well). The colonies were fixed with 80% ethanol and stained with crystal violet (0.5% w/v) (Millipore, Temecula, CA).

2.6. Luciferase assay

MAPK signaling pathway activity was detected by luciferase reporter assay as previously described (Zhang et al., 2015). Ramos cells were seeded in a 96-well plate (2 × 104 cells per well). MAPK pathway-luciferase-reporter construct (SABiosciences, Frederick, MD) was co-transfected with mimic-miR-4728 or mimic-Ctrl with lipofectatmine 2000 (life technologies). The control vector of luciferase-reporter construct was used as a negative control (VC). The cells were collected for the dual luciferase reporter assay after 48 h culture (Promega). Results are calculated by dividing firefly luciferase activity by Renilla luciferase activity. Data were presented as mean ± SE (n = 3).

2.7. Statistics

The unpaired student's t-test for two groups or one-way analysis of variance (ANOVA) test, followed by Tukey's multiple comparison for multiple groups were used to compare the significance of differences between the mean of different groups. SPSS (SPSS Inc. Chicago, IL) was used to calculate the significance and a value of $P < 0.05$ indicated the statistical significance. Data is shown as means ± SE (n = 3).

3. Results

3.1. MiR-4728 level is down-regulated in BL tissues

MiR-4728 plays was found to be involved in many biological functions and abnormally expressed in multiple type of human cancers (Persson et al., 2011; Newie et al., 2014; Schmitt et al., 2015). In this current study, we first characterized miR-4278 expression levels in the BL tissues and noncancerous tissues. We randomly chose 14 tumor tissues and 12 noncancerous tissues for the miR-4278 characterization. As shown in Fig. 1, miR-4278 expression was found to be dramatically decreased in the tumor tissues than noncancerous tissues by more than 50%. This result suggests that down-regulation of miR-4728 may be involved in the progression of BL.

3.2. MiR-4728 suppresses human Ramos cell proliferation

To evaluate the biological functions of miR-4728, we overexpressed the miR-4728 in Ramos cells (Fig. 2A). MiR-Ctrl was used as a negative control for miR-4278. After transfection of miR-4278 or miR-Ctrl mimics, the cell proliferation was determined by Cyquant assay at different indicated time points. Ramos cell proliferation was observed to be significantly decreased after miR-4278 up-regulation than the miR-Ctrl and untreated cells

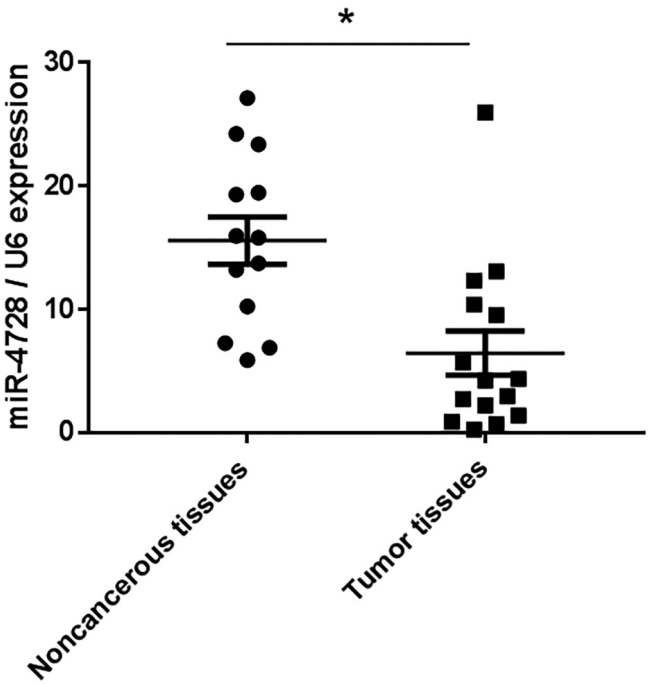

Fig. 1. miR-4278 is down-regulated in cancer tumors than non-tumor tissues. Taqman qRT-PCR was used to determine the expression level of miR-1297 in human BL tissues. Data is represented as the mean ± SE, *P < 0.05, vs noncancerous-tissues.

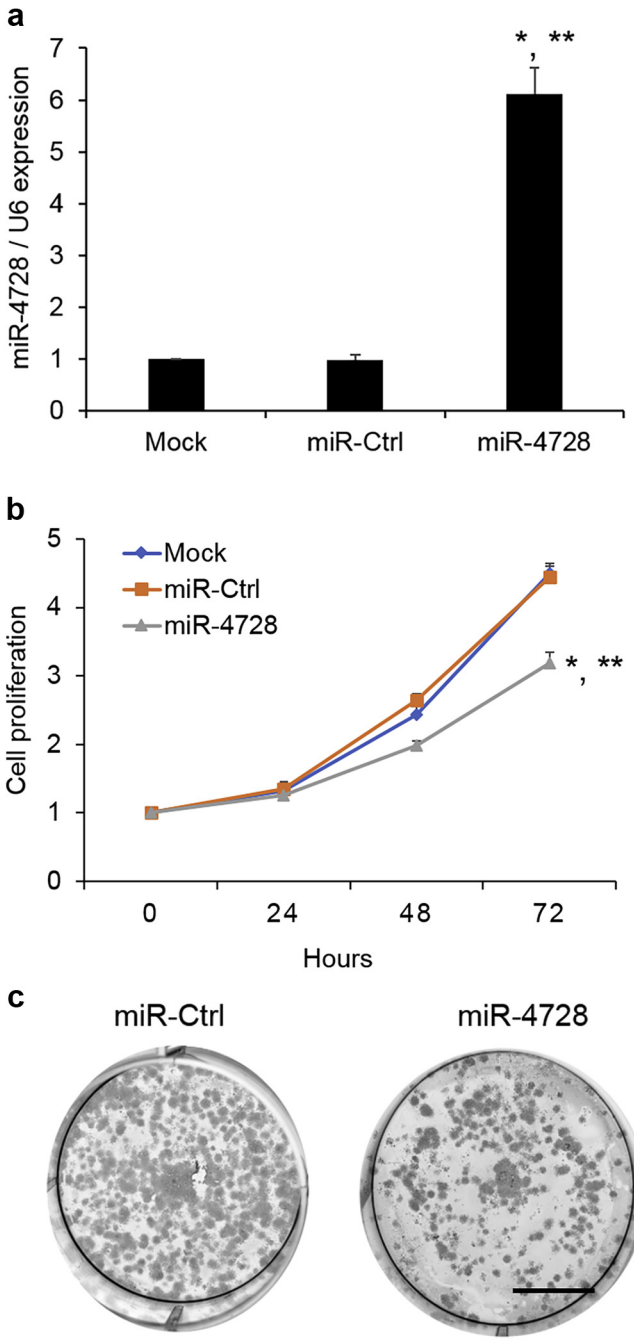

Fig. 2. Effects of miR-4728 on Ramos cell proliferation. Ramos cells were transfected with miR-4728 mimic and miR-Ctrl. (A) the expression level of miR-1297 in Ramos cells after transfection; (B) Cyquant assay was used to monitor the cell proliferation of Ramos cells after miR-4728 overexpression; (C) colony formation assays of Ramos cells expressing either miR-Ctrl or miR-4728. Data represents the mean ± SE (n = 3). $^{*}P < 0.05$, vs Mock; $^{**}P < 0.05$, vs miR-Ctrl. Scale bar: 20 μm.

(Mock) (Fig. 2B). The colony numbers were also dramatically reduced after miR-4728 overexpression (Fig. 2C)

3.3. MiR-4728 negatively regulates MAPK signaling pathway

Subsequently, we further deciphered the underlying regulatory mechanism associated with the MAPK signaling pathway. MAPK signaling pathway has been shown to be associated with a variety of cellular activities including cell proliferation, differentiation, survival, death and transformation (McCubrey et al., 2006; Torii et al., 2006; Dhillon et al., 2007). In breast cancer, miR-4728 has been found as an antagonist of oncogenic MAPK signaling (Schmitt et al., 2015). Therefore, we examined the possibility that miR-4278 could affect MAPK signaling pathway in BL. MAPK dual-luciferase reporter assay was used to determine the signaling activity. MiR-4728 was found to reduce the MAPK luciferase reporter activity by more than 40% compared with mimic-Ctrl in Ramos cells (Fig. 3). The MAPK luciferase reporter assay result indicated that miR-4728 negatively regulates MAPK activity in Ramos cells.

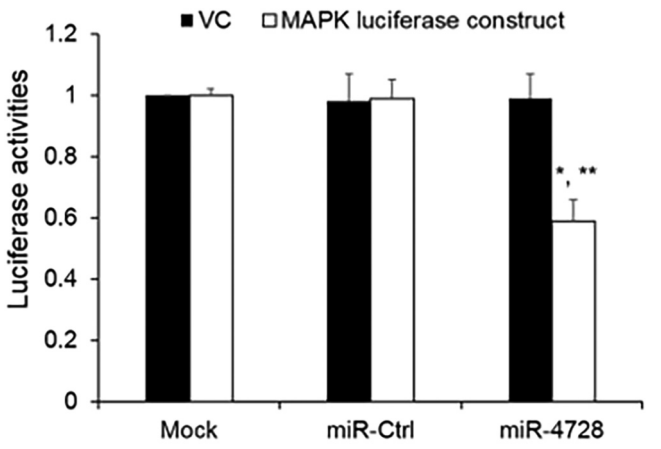

Fig. 3. Effect of miR-4728 regulation on MAPK signaling in Ramos cells. Ramos cells were co-transfected with MAPK luciferase reporter and its relative vector control (VC). Luciferase activity was normalized to Renilla.

4. Discussion

Accumulating evidence suggests that plenty of miRNAs play fundamental and important roles in many biological processes, and the alteration of miRNAs is associated with a wide range of human diseases. The potential of miRNAs as diagnostic biomarkers and therapeutic targets in cancers is obvious a particular miRNA expression profile can distinguish cancers according to diagnosis and developmental stage of the tumor to a greater degree of accuracy than traditional gene expression analysis (Liu et al., 2005). Recently, miRNAs are attractive in research as oncogenes or tumor suppressor genes in the processes of tumorigenesis (Kasinski et al., 2011; Iorio et al., 2012), but still few dysfunctional miRNAs were found in BL. Particularly, the regulatory roles of miR-4728 in BL is still needed to clarify.

MiR-4728 has been found to play important roles in multiple types of cancers. For instance, loss of miR-4728 expression was found in breast cancer and correlates with worse patient overall survival (Schmitt et al., 2015). It is also verified that miR-4728 could act as a marker of HER2 status in breast cancer (Li et al., 2015). MiR-4728 was also found to function as a tumor suppressor in ulcerative colitis-associated colorectal neoplasia through regulation of focal adhesion signaling (Pekow et al., 2017). In this study, miR-4728 expression was found to be down-regulated in human BL tumor tissues compared with the non-cancerous tissues. Further biological functional study of miR-4728 in vitro indicated that miR-4728 overexpression could significantly suppress the proliferation of human BL cell line, Ramos. These results suggested that miR-4728 is involved in the progression of BL.

MAPK is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the nucleus of the cell. MAPK signaling pathways are evolutionary conserved kinase modules that well-known for its function to control cellular processes such as growth, proliferation, apoptosis, and so on (Dhillon et al., 2007). Although miR-4728 was found to be as an

antagonist of oncogenic MAPK signaling in breast tumors, whether MAPK signaling is regulated by miR-4728 remains poorly understood in BL. In this study, our data suggests that MAPK signaling is involved in the miR-4728 regulatory network.

Collectively, we have shown that miR-4728 is down-regulated in the BL tumor tissues and affects Ramos cell proliferation. In addition, we have also shown that MAPK signaling is involved in the regulatory network of miR-4728 in BL. These results suggested that appropriate regulation of miR-4728 might possess therapeutic potentials for BL treatment.

Conflict of interest

The authors declare no conflict of interest.

References

Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F., Croce, C.M., 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA 99, 15524–15529.

Dhillon, A.S., Hagan, S., Rath, O., Kolch, W., 2007. MAP kinase signalling pathways in cancer. Oncogene 26, 3279–3290.

Di Lisio, L., Martinez, N., Montes-Moreno, S., Piris-Villaespesa, M., Sanchez-Beato, M., Piris, M.A., 2012. The role of miRNAs in the pathogenesis and diagnosis of B-cell lymphomas. Blood 120, 1782–1790.

Iorio, M.V., Croce, C.M., 2012. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol. Med. 4, 143–159.

Kasinski, A.L., Slack, F.J., 2011. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. Nat. Rev. Cancer 11, 849–864.

Kluiver, J., Haralambieva, E., de Jong, D., Blokzijl, T., Jacobs, S., Kroesen, B.J., Poppema, S., van den Berg, A., 2006. Lack of BIC and microRNA miR-155 expression in primary cases of Burkitt lymphoma. Genes Chromosomes Cancer 45, 147–153.

Li, H., Zhou, X., Zhu, J., Cheng, W., Zhu, W., Shu, Y., Liu, P., 2015. MiR-4728-3p could act as a marker of HER2 status. Cancer Biomark 15, 807–814.

Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A., Downing, J.R., Jacks, T., Horvitz, H.R., Golub, T.R., 2005. MicroRNA expression profiles classify human cancers. Nature 435, 834–838.

McCubrey, J.A., Lahair, M.M., Franklin, R.A., 2006. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. Antioxid Redox Signal 8, 1775–1789.

Monroig-Bosque Pdel, C., Rivera, C.A., Calin, G.A., 2015. MicroRNAs in cancer therapeutics: "from the bench to the bedside". Expert Opin. Biol. Ther. 15, 1381–1385.

Newie, I., Sokilde, R., Persson, H., Grabau, D., Rego, N., Kvist, A., von Stedingk, K., Axelson, H., Borg, A., Vallon-Christersson, J., Rovira, C., 2014. The HER2-encoded miR-4728-3p regulates ESR1 through a non-canonical internal seed interaction. PLoS One 9, e97200.

Pekow, J., Hutchison, A.L., Meckel, K., Harrington, K., Deng, Z., Talasila, N., Rubin, D. T., Hanauer, S.B., Hurst, R., Umanskiy, K., Fichera, A., Hart, J., Dinner, A.R., Bissonnette, M., 2017. miR-4728-3p functions as a tumor suppressor in ulcerative colitis-associated colorectal neoplasia through regulation of focal adhesion signaling. Inflamm Bowel Dis. 23, 1328–1337.

Persson, H., Kvist, A., Rego, N., Staaf, J., Vallon-Christersson, J., Luts, L., Loman, N., Jonsson, G., Naya, H., Hoglund, M., Borg, A., Rovira, C., 2011. Identification of new microRNAs in paired normal and tumor breast tissue suggests a dual role for the ERBB2/Her2 gene. Cancer Res. 71, 78–86.

Schmitt, D.C., Madeira da Silva, L., Zhang, W., Liu, Z., Arora, R., Lim, S., Schuler, A.M., McClellan, S., Andrews, J.F., Kahn, A.G., Zhou, M., Ahn, E.Y., Tan, M., 2015. ErbB2-intronic microRNA-4728: a novel tumor suppressor and antagonist of oncogenic MAPK signaling. Cell Death Dis. 6, e1742.

Torii, S., Yamamoto, T., Tsuchiya, Y., Nishida, E., 2006. ERK MAP kinase in G cell cycle progression and cancer. Cancer Sci. 97, 697–702.

Zhang, L., Huang, C., Guo, Y., Gou, X., Hinsdale, M., Lloyd, P., Liu, L., 2015. MicroRNA-26b modulates the NF-kappaB pathway in alveolar macrophages by regulating PTEN. J. Immunol. 195, 5404–5414.