MicroRNA-21 is Overexpressed in Malignant Peripheral Nerve Tumor Cell and Regulate Cell Proliferation by Targeting PDCD4

+Yoshida, A; Morimoto, Y; Itani, S; Sasaki, T; Hasei, J; Kinusida, T; Ozaki, T
+Okayama University Graduate School, Okayama, Japan
Senior author ayo@md.okayama-u.ac.jp

INTRODUCTION

Tumors of peripheral nerves sometimes occur in everywhere and present several malignancies. Benign tumors were mainly categorized as schwannoma or neurofibroma, and malignant tumors were as malignant peripheral nerve sheath tumor (MPNST). About 80% of MPNSTs indicate pathologically high-grade, and are high incidence of local recurrence (40-65%) and distant metastasis (40-68%). The 5-year survival for tumors at all sites is about 50%. Neurofibromatosis type 1 (NFI) is an autosomal dominant neurocutaneous disorder, and about 5% of patients with NFI, neurofibromas progress to MPNSTs 1). MicroRNAs (miRNAs) are noncoding RNAs of approximate 22nt in length that function as post-transcriptional regulators. MicroRNAs have been implicated not only in the development of primary tumors, but also in affecting progression and the metastatic phase of the disease2).

In our current study, we examined the expression profiling of miRNA in neurogenic tumors, and predicted that some miRNAs would be a target of gene tumor progression by cluster analysis. This results showed that the differences in miRNA expression patterns between MPNSTs and NFs, such as miR-21, miR-135b, miR-125b, miR-155 and miR-301. The aim of this study is to clarify the function of miRNA-21 in the MPNSTs.

MATERIALS AND METHODS

miRNA expression of clinical tissue specimens
RNA was extracted from 13MPNSTs and 13 NFs, and 5 nerves of fresh frozen tissues using ISOGEN (NIPPON GENE). For miRNA expression, the quantitative real-time RT-PCR analysis was performed using mirVana qRT-PCR miRNA Detection Kit (Ambion) and mirVana qRT-PCR Primer Sets (miR-21, miR-135b, miR-125b, miR-155 and miR-301). All PCR reactions were run in duplicate and gene expression, relative to RUN6B, calculated using the 2(−△△Ct) method.

Transfection with mir-21 oligonucleotides
For transfection of miRNA antisense, MPNST cell line, YST-1 was used. YST-1 cells were transfected with Anti-miR-21 miRNA inhibitor (miR-21i) (Ambion) at 30 nmol-L(-1) final concentration, using NeoFx (Ambion). MiR inhibitor#1 (NCi) (Ambion) was used for negative control. For qRT-PCR analysis, total RNA was extracted 48 h after transfection; for Western blot analysis, cell lysate was prepared 48 h after transfection.

Cell proliferation and caspase-3/7 assay
After 48 hours of incubation, the cells were subjected to the CellTiter-Glo Luminescent Cell Viability assay (Promega) and the Caspase-Glo3/7 assay (Promega). The rate of absorbance was determined using a multiplate reader and the results were presented as fold change when compared with their respective untreated controls.

Western blot analysis
Total protein was isolated from YST-1 cells transfected with miRNA antisense. Rabbit anti-programmed cell death 4 (PDCD4) antibody (ROCKLAND), mouse anti-Phosphatase and tensin homologue deleted on chromosome ten (PTEN) antibody (Santa Cruz) and mouse monoclonal anti-beta actin antibody (Sigma) were used. The blots were visualized using ECL plus (Amersham Biosciences). The band intensity was calculated by Image J as compared with mock control.

Statistical methods
Differences between groups were compared using Student’s t-test for continuous variables. P < 0.05 was considered to be significant.

RESULTS
In the qRT-PCR analysis, miR-21, miR-135b, miR-125b, miR-155 and miR-301 expression were significantly different between NF and MPNST. In particular, miR-21 and miR-135b expression were higher in MPNST than NF and normal nerve. MiR-125b and miR-135b expression were lower in MPNST and NF than normal nerve (data not shown).

To assess the expression of miR-21 in clinical specimens, we performed qRT-PCR. The expression of miR-21 was significantly higher in MPNST than NF and normal nerve, respectively (p<0.01 and p<0.05) (Fig.1). In the miR-21i transfected cells, it was indicated that reduced the expression level of miR-21 in YST-1 transfected with miR-21i, effectively (p<0.05) (data not shown). Caspase assay showed that apoptosis in YST-1 cells transfected with miR-21i, NCi and mock were about 150%, 115% and 100%, respectively (p<0.05) (Fig.2a). The group of transfected with miR-21 antisense indicated to increase apoptosis. The group was also decreased cell proliferation (Fig.2b), suggesting that miR-21 could accelerate MPNST cell proliferation.

The protein expression of PDCD4 (3.2-fold) and PTEN (1.3-fold) were higher in transfected with miR-21i (Fig.3). These data suggested that PDCD4 expression is possibly regulated by miR-21 in YST-1 cell.

DISCUSSION
We identified that 5 miRNAs was significant different between NF and MPNST by clustering of expression profiling. These miRNAs may be involved in tumorgenesis or differentiation in peripheral nerve tumors. MiR-21 was significantly higher expression in MPNSTs than in NFs and normal nerves. The expression of miR-21 in some cancers is higher than that in normal tissue, and the miRNA is known to act as oncogene2, 3). PDCD4 was identified as a novel tumor suppressor gene and it was found to be downregulated in several types of human cancer4). PTEN is a tumor suppressor gene, associated with negative regulation in the PKA kinase pathway5). Therefore, inhibition of miR-21 was responsible for the upregulation of PDCD4 and PTEN, and was induced caspase activity and was decreased cell proliferation in YST-1 cell. These data suggest that these proteins induce apoptosis in MPNST cell.

MiR-21 may have an important role as tumor progression factor in MPNST through its target gene, PDCD4 and PTEN.

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