Serum miR-21 levels in patients with dermatomyositis

Sirs,

There are only a few reports about the relationships between microRNAs (miRNAs) and polymyositis/dermatomyositis (PM/DM): Eisenberg *et al*. found that expression of several miRNAs are up- or down-regulated in the muscle tissues of PM/DM (1). First, we also determined the miRNA levels in muscle tissues of DM patients. Total miRNA were obtained from muscle samples of 4 DM patients and 4 healthy control subjects (2). Consistent with the previous report (1), miR-21 expression in the muscle tissues was significantly elevated in DM patients (Fig. 1a).

Accordingly, we tried to evaluate the possibility that serum levels of miR-21 can be a useful marker for the diagnosis and the evaluation of disease activity of DM. Serum samples were obtained from 30 DM patients (12 men and 18 women; mean age, 54.8 years). Control serum samples were also collected from 20 healthy volunteers, 10 clinically amyopathic DM (CADM) patients, 5 PM patients, 20 systemic lupus erythematosus (SLE) patients, and 10 systemic sclerosis (SSc) patients. Institutional review board approval and written informed consent were obtained before patients and healthy volunteers were entered into this study. By the real-time PCR using hsa-miR-21 primer and total miRNA purified from serum of healthy volunteer (2), the amplification curve of miR-21 was observed, and Ct values were increased by the serial dilution of the miRNA (Fig. 1b). Thus, using our method, hsa-miR-21 was likely to be detectable and quantitative in the serum.

Then, before miRNA isolation, serum was supplemented with synthetic non-human miRNA (C. elegans miR-39, Takara) as controls providing internal reference for normalisation of technical variations between samples (3, 4). Mean serum levels of miR-21 corrected for C. elegans miR-39 levels in the same samples were significantly higher only in DM patients than those in normal subjects (Fig. 1c, p=0.041). It has been reported that serum miR-21 levels are increased in various human neoplastic disorders (5-7). Our results indicate miR-21 expression is also increased in the muscles and sera of DM patients, and may be useful for the diagnosis of DM. miR-21 expression has been previously described to be up-regulated in T lymphocytes of SLE patients (8-10), whereas serum miR-21 levels in SLE patients were higher than those in normal subjects, but not statistically significant in our study. This discrepancy may be due to the small number of patients or the different expression pattern of miR-21 between lymphocytes and serum.

When the cut-off value was set at mean value+2SD of the healthy controls, there was no significant difference between patients with elevated serum miR-21 levels (n=12) and those with normal levels (n=18) in terms of the prevalence of Gottron's sign (80.0 *vs*. 86.7%), Heliotrope rush (83.3 *vs*. 66.7%), lung involvement (50.0 *vs*. 40.0%), or internal malignancies (33.3 *vs*. 18.8%). Serum creatine kinase levels were not significantly different between patients with and without elevated miR-21 levels (1130.3 *vs*. 2861.1 U/I). However, we found mean serum IgG levels were significantly higher in patients with elevated miR-21 levels than those without (1837.4 *vs*. 1283.6 mg/dl, p=0.0041 by Mann-Whitney U-test). Serum IgG level is thought to reflect the abnormal activation of immune system and regarded as one of the disease markers in DM. Thus, serum miR-21 may also be correlated with the disease activity of DM and be involved in the pathogenesis of this disease. Another possibility is that increased miR-21 expression in DM is linked to the muscle reconstruction. Clarifying the role of miRNAs in this disease may lead to further understanding of the disease and new therapeutic approaches. Larger studies are needed by measuring serum levels of miR-21 as well as other miRNAs in increased number of patients in the future.

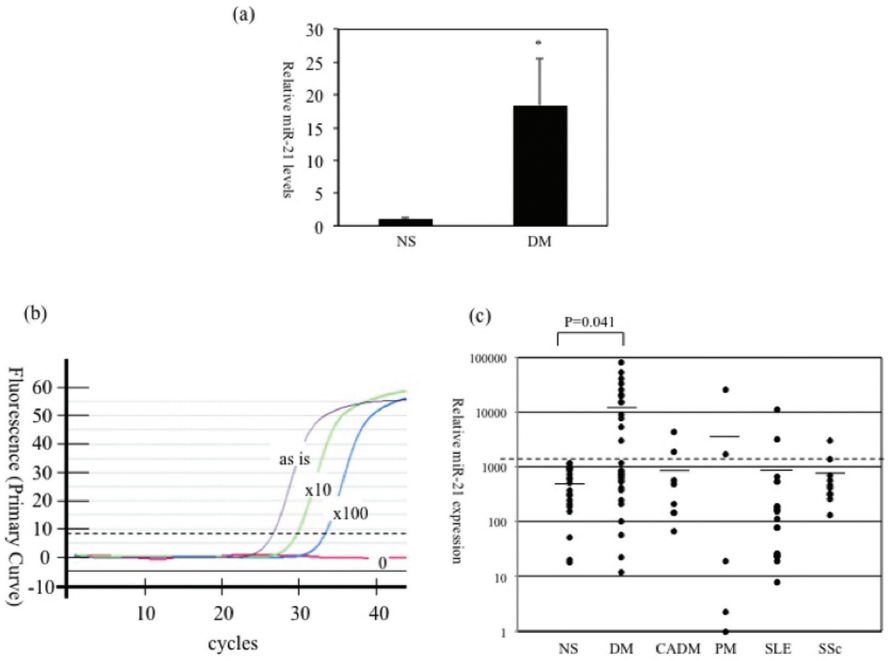

Fig. 1. **(a)** Mean relative transcript levels of miR-21 in muscle tissues from 4 normal subjects (NS) and 4 DM patients were determined by real-time quantitative PCR. *P<0.05 as compared with the value in samples from NS (1.0).
(b) miR-21 is present in serum sample. Serial dilution of cDNA (as is, 10-fold dilution, 100-fold dilation and 0) synthesised from serum miRNA was used as template for real-time PCR. Amplification curves of gene-specific transcripts are shown to illustrate the process of exponential increase of fluorescence. Horizontal dotted line indicates the threshold.
(c) Serum concentrations of miR-21 in patients with dermatomyositis (DM), clinically amyopathic dermatomyositis (CADM), polymyositis (PM), systemic lupus erythematosus (SLE), or systemic sclerosis (SSc) and in normal control subjects (NS). Serum was added with synthetic non-human miRNA (C. elegans miR-39) as a control providing an internal reference for normalisation of technical variations between samples. Then, total miRNA was purified. miR-21 levels were measured with real-time PCR, and corrected for the levels of C. elegans miR-39 in the same samples. miR-21 concentrations are shown on the ordinate. The horizontal dotted line indicates the cut-off level (mean+2SD of the values in NS). Bars show means. The minimum value was set at 1.

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References

1. EISENBERG I: Distinctive patterns of microRNA expression in primary muscular disorders. *Proc Natl Acad Sci USA* 2007; 104: 17016-21.
2. ICHIHARA A, JINNIN M, YAMANE K *et al*.: microRNA-mediated keratinocyte hyperproliferation in psoriasis vulgaris. *Br J Dermatol* 2011; 165: 1003-10.

3. KROH EM, PARKIN RK, MITCHELL PS, TEWARI M: Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods* 2010; 50: 298-301.
4. KANEMARU H, FUKUSHIMA S, YAMASHITA J *et al*.: The circulating microRNA-221 level in patients with malignant melanoma as a new tumor marker. *J Dermatol Sci*. 2011; 61: 187-93.
5. MOTOYAMA K, INOUE H, MIMORI K *et al*.: Clinicopathological and prognostic significance of PDCD4 and microRNA-21 in human gastric cancer. *Int J Oncol* 2010; 36: 1089-95.
6. ALI S, ALMHANNA K, CHEN W *et al*.: Differentially expressed miRNAs in the plasma may provide a molecular signature for aggressive pancreatic cancer. *Am J Transl Res* 2010; 3: 28-47.
7. RADOJICIC J, ZARAVINOS A, VREKOUSSIS T *et al*.: MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. *Cell Cycle* 2011; 10: 507-17.
8. PAN W, ZHU S, YUAN M *et al*.: MicroRNA-21 and microRNA-148a contribute to DNA hypomethylation in lupus CD4+ T cells by directly and indirectly targeting DNA methyltransferase 1. *J Immunol* 2010; 184: 6773-81.
9. STAGAKIS E, BERTSIAS G, VERGINIS P *et al*.: Identification of novel microRNA signatures linked to human lupus disease activity and pathogenesis: miR-21 regulates aberrant T cell responses through regulation of PDCD4 expression. *Ann Rheum Dis* 2011; 70: 1496-506.
10. GARCHOW BG, ENCINAS OB, LEUNG YT *et al*.: Silencing of microR6-21 in vivo ameliorates autoimmune splenomegaly in lupus mice. *EMBO Mol Med* 2011; 3: 605-15.