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Differential expression of miR-4520a is associated with gain of function mutations in Familial Mediterranean Fever (FMF)

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Introduction

MicroRNA signature of THP1 cells revealed a 5.9-fold decreased expression of miR-4520a following siRNA-mediated knockdown of *MEFV* gene that encodes pyrin [1].

Objectives

We herein sought to validate the expression levels of miR-4520a in monocytes isolated from peripheral blood mononuclear cells (PBMCs) of FMF patients.

Methods

Dual luciferase assay was used to validate a predicted miR-4520a recognition element in the 3'UTR region of the Rheb gene. The expression levels of pyrin, miR-4520a and its putative target Rheb were validated in monocytes from FMF patients (n=9) and compared with healthy controls (n=8). Patients were off colchicine for two days (attack-free period) and monocytes were isolated from PBMCs. Total RNA together with the respective miRNA-enriched fractions were isolated from monocytes and used for mRNA and miR-4520a quantitation by real-time PCR using the 2-ΔΔCt method after normalizing to 18S RNA and RNU6B genes, respectively. Protein levels of pyrin and Rheb were detected by western blotting.

Results

The relative expression levels of miR-4520a were variable among FMF patients and not significantly different between patients and controls. However, when patients that did not harbor any mutations in *MEFV* were excluded from the analyses, the expression of miR-4520a was statistically different between FMF patients and controls ($p<0.05$), indicating an association between miR-4520a expression and mutations in the *MEFV* gene. Moreover, stratification of patients group by genotype revealed an intriguing difference in miR-4520a relative expression, with carriers of M694V variant (combined group of homozygotes, heterozygotes and compound heterozygotes) showing the highest increase ($p<0.05$). Subsequent comparison between the M694V group and healthy controls showed a significant increase in miR-4520a expression levels that remained significant even after bonferroni correction ($p<0.01$). Interestingly, one of the homozygote M694V patients with the highest fold change in miR-4520a expression (FC=7.8) experienced an FMF-attack while on study, with a concomitant decrease in miR-4520a relative expression (FC=0.45). Bio-informatic analyses showed that miR-4520a is predicted to target genes implicated in autophagy through regulation of Rheb/mTOR signaling. Expression levels of Rheb were confirmed by luciferase reporter gene assays providing further evidence that Rheb is a direct target of miR-4520a ($p<0.01$). Validation of pyrin and Rheb protein expression levels in monocytes from FMF patients is in progress.

Conclusion

Our findings provide initial evidence that Rheb is a valid target of miR-4520a and suggest that a dysfunctional pyrin due to gain of function mutations with a dosage effect [2], especially of M694V variant, may be associated with an increase in miR-4520a expression levels,

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thus contributing to deregulated mTOR signaling and subsequently IL-1β release [3].

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