Host miR-197 Plays a Negative Regulatory Role in the EV71 Infectious Cycle by Targeting the RAN Protein

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Background/Objective
microRNAs (miRNAs) are a group of endogenous, highly conserved noncoding single-stranded RNAs (~22 nucleotides (nt) in length) that are transcribed from specialized genes and undergo series processing to generate final mature miRNAs. viral gene expression and replication can be modulated by miRNAs that originate from either virus-encoded or host miRNAs. Enterovirus 71 (EV71) is a single-stranded RNA virus of the picornavirus family. Its positive-sense RNA genome comprises ~7.5 kilobases and encodes a single polyprotein, in an IRES-dependent manner, that is proteolytically cleaved to yield approximately 11 different structural and nonstructural viral proteins. EV71 is highly infectious and is sometimes associated with severe central nervous system complications.

Method
microRNA microarray
SILAC
3'UTR reporter assay

Result
We identified a cellular miR-197 that was downregulated by viral infection in a time-dependent manner. The virus-mediated miR-197 reduction was not caused by the diminished expression levels of its precursor pri-miR-197 or of the key molecules of the miRNA maturation/processing machinery. In miR-197 mimic-transfected cells, both EV71 replicon expression and IRES activity were inhibited, indicating that miR-197 targets host proteins for virus replication. Thus, we used a mass-spectrometry-based quantitative proteomics approach using stable isotope labeling with amino acids in cell culture (SILAC), aided by the TargetScan algorithm, to identify 78 putative target genes of miR-197. Among them, RAN, ITGAV, ETF1, and MAP2K1 (MEK1) were validated as genuine targets in a 3'UTR reporter assay, and specific knockdown of RAN and ETF1, but not of ITGAV and MAP2K1, inhibited viral replication. Moreover, the nuclear import of the essential replication molecules 3D/3CD and hnRNP K occurred in a RAN-dependent manner.

Conclusion
These results indicate that EV71-induced downregulation of miR-197 may constitute a newly identified mechanism that increases the expression of RAN to support the nuclear transport of both viral proteins and host proteins for viral replication.