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LETTER TO THE EDITOR

Identification of mouse liver mitochondria-associated miRNAs and their potential biological functions

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Dear Editor,

By analyzing the genome-wide microRNA (miRNA) expression profile using microRNA microarray and stem-loop miRNA qPCR assay, we report that unique miRNAs are enriched in mitochondria and these mitochondria-associated miRNAs may be involved in the regulation of gene expression of mitochondria and other general cellular processes such as apoptosis, proliferation and differentiation.

MiRNAs have been shown to play an essential role in modulating gene expression through translational inhibition and/or degradation of target mRNAs. As a new class of endogenous non-coding RNAs, miRNAs generally act to negatively regulate gene expression at the post-transcriptional level by base-pairing to complementary sites on the target mRNAs, thereby blocking translation or causing the degradation of the target mRNA [1]. The regulation of miRNAs generally takes place in the cytosol following miRNA-loaded RNA-induced silencing complex binding to the target mRNA [2]. Although certain mature miRNAs have been found in the nucleus [3] and the specialized processing (P) bodies of the cell cytoplasm [4], it remains unknown whether subcellular organelles such as mitochondria are associated with specific miRNAs. Since mitochondria are important subcellular organelles that contain proteins translated from mRNA transcripts derived from either the nuclear or their own genome [5], they may provide a potential site for miRNA-mediated post-transcriptional regulation. Very recently, Kren *et al.* [6] reported the detection of 15 nuclear-coded miRNAs in rat liver mitochondria. This initial study, however, did not reveal the specific enrichment of miRNAs in mitochondria. So far, there is no report about mitochondrial miRNAs in animal tissues other than liver.

To determine whether unique miRNA populations are associated with mitochondria, we isolated mitochondria from the liver of adult male C57BL/6J mice [7] (Supplementary information, Data S1). The high purity of the mitochondrial fraction was confirmed by western blot analysis using an antibody against the mitochondrial marker protein, cytochrome *c* (cyto. *c*) (Figure 1A). Morphological data also showed that the majority of isolated mitochondria are intact (Figure 1C). Western blot analysis further showed that purified mitochondria contain argonaute 2 (AGO2), a core effector of the miRNA pathway [8] (Figure 1B), suggesting a potential function of miRNAs in mitochondria.

First, we used a miRNA microarray assay [9] to survey a genome-wide miRNA profile in the isolated mitochondria and mouse liver tissue (Supplementary information, Data S1). Purified mitochondria were treated with RNase to remove mitochondria-bound RNAs before microarray assay. As shown in Figure 1D, the patterns of miRNA expression in the mitochondria and liver tissue profiles are significantly different. Supplementary information, Table S1 lists the 20 miRNAs with the highest signals in mitochondria or mouse liver fractions. These data show that mitochondria express an abundant level of miR-122, miR-805, miR-690, etc., while mouse livers have high expression levels for miR-690, miR-122, miR-451, etc. Interestingly, highly abundant miRNAs in mitochondria, such as miR-451, miR-7b and miR-26a, were not in the top 20 list of liver-expressed miRNAs, while highly abundant miRNAs in liver tissue, including miR-689 and miR-494, were expressed at low levels in liver mitochondria. These data suggest that mitochondria have a unique population of miRNAs and that the enrichment of miRNAs in mitochondria is independent of the total cellular abundance of miRNAs. After comparing the miRNA levels in mitochondria with those in mouse liver, we identified a panel of miRNAs that were selectively expressed in mitochondria with at least 2-fold higher concentration levels compared to those in liver tissue (Supplementary information, Table S2). Interestingly, although miR-122 was abundantly expressed in mitochondria, it was not selected as mitochondria-enriched miRNA due to its equally abundant expression in liver tissue. Next, we validated the mitochondria-associated miRNAs using miRNA qRT-PCR in five independent experiments. In agreement with the observation of microar-

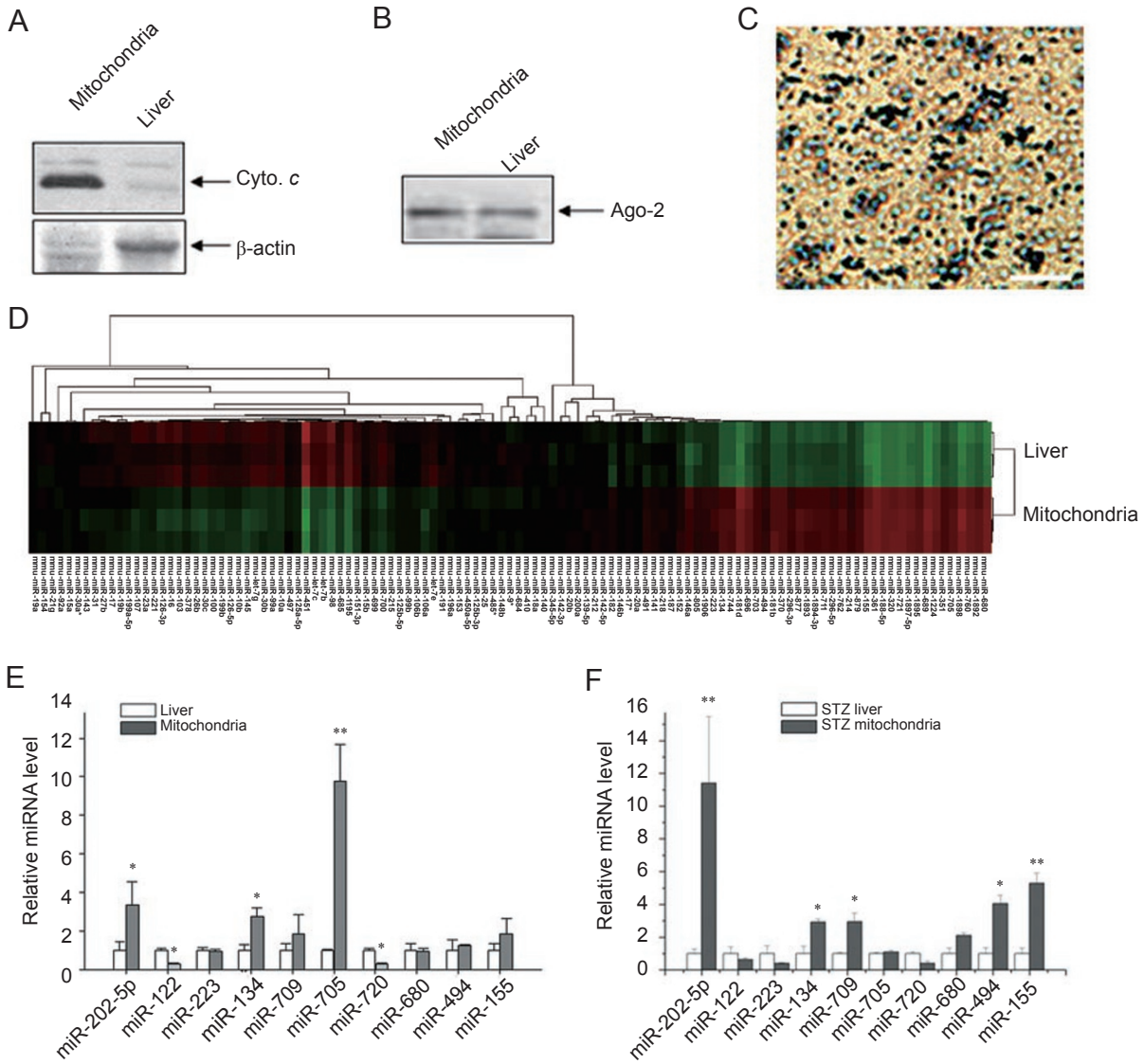

Figure 1 Different expression patterns of miRNAs between mouse liver mitochondria and liver tissue. **(A** and **B)** Western blot analysis of purified mouse liver mitochondria using antibodies against cyto. *c* and AGO2. **(C)** Morphology of purified mitochondria under light microscopy. **(D)** Microarray analysis of global miRNA expression profile in mitochondria and mouse liver tissue. **(E)** Validation of mitochondria-associated miRNAs by stem-loop miRNA qRT-PCR assay. Triplicate assays were carried out for each sample and the relative amounts of each miRNA were normalized to U6 snRNA. **(F)** Alteration of mitochondria-associated miRNAs in STZ-induced type 1 diabetic mice. Eight-week old male C57BL/6J mice received a single 150 mg/kg injection intravenously and animals were killed 14 days post-injection of STZ for liver and mitochondria isolation. Data were presented as mean ± s.d. from three independent experiments with five different samples in each experiment. **P* < 0.05; ***P* < 0.01.

ray, the relative concentrations of miR-705, miR-202-5p and miR-134 in mitochondria were significantly higher than in mouse liver (Figure 1E).

Next, we assessed the association of miRNAs with mitochondria and their potential biological relevance through determining the alteration of mitochondria-associated miRNAs in mouse livers after streptozotocin (STZ) treatment. In agreement with the previous studies showing that STZ induced type 1 diabetes and mitochondria dysfunction [10], we found that STZ treatment led to hyperglycemia (fasting blood glucose ≥12mmol/l). As shown in Figure 1F, the expression pattern of mitochondria-associated miRNAs was significantly altered in STZ-diabetic mice. The levels of miR-494, miR-202-5p, miR-

134 and miR-155 were significantly increased in mitochondria from STZ-diabetic mice, while the levels of miR-705 and miR-122 were not changed and decreased, respectively. MiRNA expression patterns in mouse liver and liver mitochondria were also altered by STZ treatment (Supplementary information, Figure S1). The results suggest that mitochondria-associated miRNAs may be involved in mitochondria dysfunctions.

Using bioinformatics tools, we predicted the target genes of mitochondria-associated miR-705, miR-494 and miR-202-5p (Supplementary information, Table S3). Several putative targets, related to mitochondria-specific functions, such as tryptophanyl-tRNA synthetase (WARS) and transcription factor A (Tfam) were derived by multiple target-predicting algorithms. The validation and characterization of these predicted target genes is necessary for better understanding the potential biological roles of mitochondria-associated miRNAs.

Taken together, the present study shows that unique miRNAs are specifically enriched within mouse liver mitochondria, and that these mitochondria-associated miRNAs may be involved in the modulation of mitochondria-specific and general cellular functions.

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(**Supplementary information** is linked to the online version of the paper on *Cell Research* website.)