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Data in Brief

miRNAs modified by dietary lipids in Caco-2 cells. A microarray screening



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ABSTRACT

We performed a screening of miRNAs regulated by dietary lipids in a cellular model of enterocytes, Caco-2 cells. Our aim was to describe new lipid-modified miRNAs with an implication in lipid homeostasis and cardiovascular disease [1,2]. For that purpose, we treated differentiated Caco-2 cells with micelles containing the assayed lipids (cholesterol, conjugated linoleic acid and docosahexaenoic acid) and the screening of miRNAs was carried out by microarray using the µParaflo®Microfluidic Biochip Technology of LC Sciences (Huston, TX, USA). Experimental design, microarray description and raw data have been made available in the GEO database with the reference number of GSE59153. Here we described in detail the experimental design and methods used to obtain the relative expression data.

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Specifications	
Organism/cell line/tissue	Human Caco-2 cells (enterocytes)
Sex	N/A
Sequencer or array type	μParaflo®Microfluidic Biochip Technology of LC Sciences (Huston, TX, USA)
Data format	Raw data
Experimental factors	Micelles containing cholesterol, CLA or DHA vs. empty micelles
Experimental features	Differentiated Caco-2 cells were treated with micelles containing cholesterol (48 h) or micelles containing DHA or CLA (24 h). Control cells were treated with empty micelles. miRNA-enriched total RNA was isolated with miRNeasy mini kit (Qiagen, Madrid, Spain). The expression of miRNAs contained in the miRBase version 17 was analyzed by microarray using the $\mu Paraflo@Microfluidic Biochip Technology of LC Sciences (Huston, TX, USA). Raw data were normalized, processed and analyzed for statistical significance using t-test and applying multiple testing correction$
Consent	N/A
Sample source location	N/A

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59153.

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2. Experimental design, materials and methods

For differentiation, Caco-2 cells were plated on polycarbonate Transwell® filter inserts of 0.4 µm pore size (Costar, Madrid, Spain) in 6-well plates. Approximately 14 days after confluence cells develop morphologic and physiologic characteristics of normal enterocytes [3]. Cholesterol (Chol), CLA and DHA (Sigma, Madrid, Spain) were delivered to cells as micelles with Lyso-phosphatidylcholine (Lyso-PC; Sigma, Madrid, Spain) and sodium taurocholate (Tau; Sigma, Madrid, Spain) [4]. Briefly, Chol (250 μM), CLA (200 μM) or DHA (200 μM) were diluted in n-hexane (Scharlab; Barcelona, Spain) and dried under a nitrogen stream. Lyso-PC (200 µM) was also diluted in n-hexane and mixed with the dried Chol, CLA or DHA, respectively. The mixture was then dried under a nitrogen stream. Tau (5 mM) was diluted in ethanol 95% and added to the Lyso-PC/lipid mixture and then evaporated under a nitrogen stream. The resulted micelles were diluted in a serum-free medium and mixed vigorously until clear. As control, we used empty micelles made with Lyso-PC and Tau. Four control and cholesterol samples, three DHA samples and two CLA samples were analyzed.

MicroRNA-enriched total RNA was isolated with miRNeasy mini kit (Qiagen, Madrid, Spain) following manufacturer's instructions. Total RNA concentration was quantified with a Nanodrop and the integrity was confirmed in a 1% agarose gel.

The expression of microRNAs contained in the miRBase version 17 was analyzed by microarray using the µParaflo®Microfluidic Biochip Technology of LCsciences (Huston, TX, USA). A total of 1 µg of miRNA-enriched RNA was used for miRNA microarray. At least three samples of each condition were analyzed (except for CLA, for which we processed

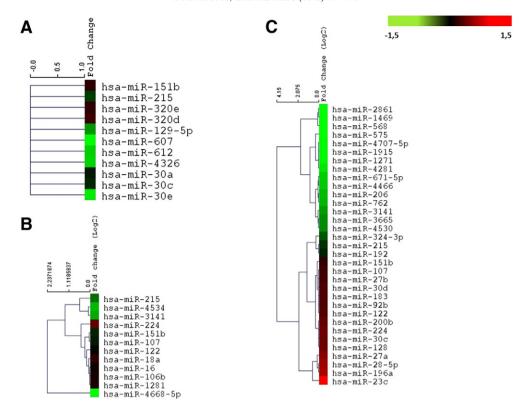


Fig. 1. Cluster analysis of differentially expressed miRNAs with cholesterol (A), CLA (B) or DHA (C) comparing with empty micelle treated cells, miRNA expression was measured by microarray and relative expression was calculated as the Log2 (test intensity/control intensity) after background subtraction and LOWESS normalization.

two biological replicates). Raw data corresponding to signal intensity were corrected for background subtraction and normalized using the LOWESS (locally weighted regression) method. Relative miRNA levels were calculated as the Log2 of test intensity/control intensity. Data were processed to merge replicates and discard discordant replicates (1-fold Standard deviation). The resulting fold change (FC) was Log2 transformed and analyzed for statistical significance using t-test and applying multiple testing corrections. Microarray data has been submitted to the GEO database complying with the Minimal Information About a Microarray Experiment (MIAME) guidelines (GSE59153). Significant microRNAs were clustered using average linkage algorithm and Euclidean distance correlation with TigerMEV software (Boston, MA, USA) (Fig. 1).

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