miR-146a-mediated suppression of the inflammatory response in human adipocytes

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Introduction

microRNAs are a class of small (18-25 nucleotides), non-coding RNA molecules. They play an important role in the regulation of gene expression by either suppressing translation or inducing mRNA degradation. Adipocytes express at least 169 different miRNA species and 85 of them seem to be released from cells as they were detectable in cell culture supernatants [1]. Their impact ranges from regulating adipogenesis and glucose metabolism to the modulation of inflammation [2].

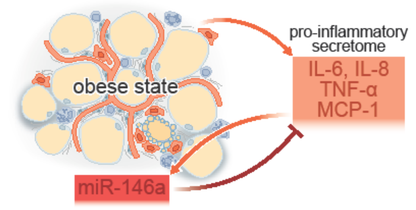
Obesity leads to the infiltration of macrophages into white adipose tissue (WAT) and local inflammation. In an Affymetrix microRNA (miRNA) array we found miR-146a expression strongly up-regulated in adipocytes under inflammatory conditions.

miR-146a plays a critical role in the inflammatory response in different cell types. In human keratinocytes miR-146a supresses the expression of IL-8, C-C chemokine ligand 20 (CCL20) and TNF-α [3]. Likewise, miR-146a regulates the inflammatory response in human monocytes [4] and human lung alveolar epithelial cells [5].
The aim of this project was to elucidate the biological function of miR-146a in human adipocytes.

Summary

Taken together, our experiments show that:

- in human adipocytes miR-146a is upregulated under inflammatory conditions
- miR-146a targets IRAK1 and TRAF6
- miR-146a counteracts the inflammation-induced activation of JNK and p38
- miR-146a reduces the inflammation-induced expression and secretion of IL-8



Model of miR-146a function in obese WAT

Therefore this work identified miR-146a as a fine tuner of the inflammatory response in human adipocytes. Considering that the tissue secretome of obese WAT is pro-inflammatory and that miR-146a is upregulated under these conditions we hypothesize that it controls the inflammatory response in a negative feedback loop. miR-146a might not only serve as a biomarker of the inflammatory state associated with obesity but also as a therapeutic agent against it.

Results

miR-146a is downregulated during adipogenic differentiation

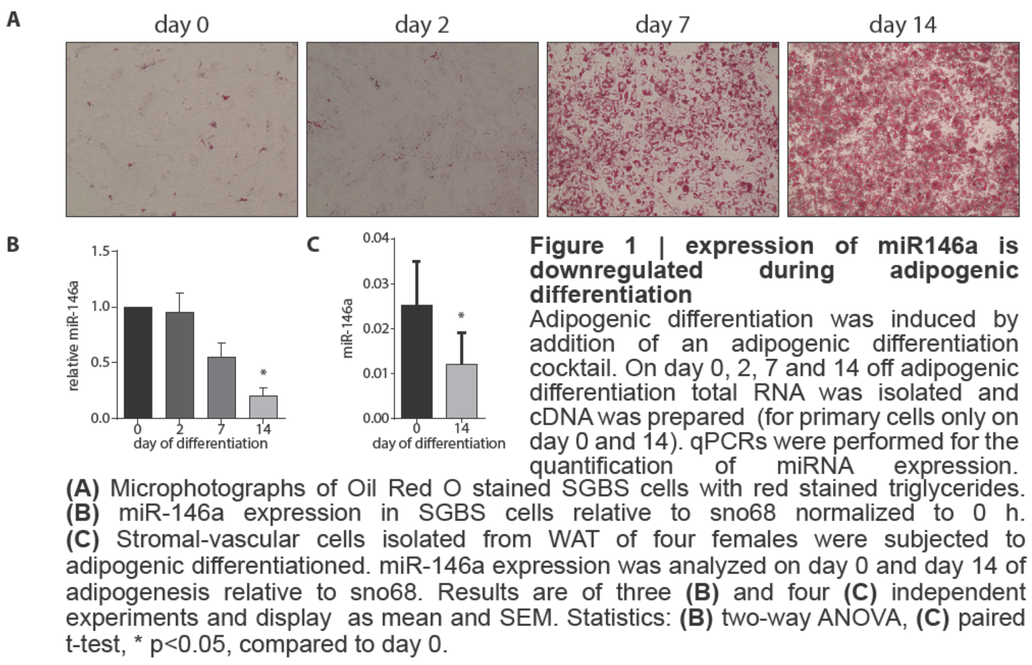


Figure 1 | expression of miR146a is downregulated during adipogenic differentiation
Adipogenic differentiation was induced by addition of an adipogenic differentiation cocktail. On day 0, 2, 7 and 14 off adipogenic differentiation total RNA was isolated and cDNA was prepared (for primary cells only on day 0 and 14). qPCRs were performed for the quantification of miRNA expression. **(A)** Microphotographs of Oil Red O stained SGBS cells with red stained triglycerides. **(B)** miR-146a expression in SGBS cells relative to sno68 normalized to 0 h. **(C)** Stromal-vascular cells isolated from WAT of four females were subjected to adipogenic differentiationed. miR-146a expression was analyzed on day 0 and day 14 of adipogenesis relative to sno68. Results are of three **(B)** and four **(C)** independent experiments and display as mean and SEM. Statistics: **(B)** two-way ANOVA, **(C)** paired t-test, * p<0.05, compared to day 0.

miR-146a is upregulated under inflammatory conditions

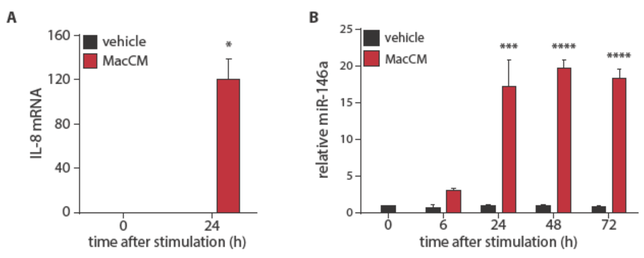


Figure 2 | MacCM stimulation induces IL-8 and miR-146a expression
Human SGBS adipocytes on day 14 of differentiation were stimulated with 10% macrophage conditioned medium (MacCM), which was obtained by stimulating THP1 macrophages with PMA for 48 h, or a vehicle control. Total RNA was isolated at 0 h and 6 h, 24 h, 48 h, and 72 h after stimulation. cDNA was prepared and qPCR was performed for the quantification of mRNA expression. **(A)** IL-8 mRNA expression at 0 h and 24 h relative to succinate dehydrogenase complex subunit A (SDHA). **(B)** miR-146a expression at 0 h, 6 h, 24 h, 48 h and 72 h relative to sno68 normalized to 0 h. Results are of three independent experiments and displayed as mean and SEM. Statistics: two-way ANOVA, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to 0 h.

IRAK1 and TRAF6 are targets of miR-146a in human adipocytes

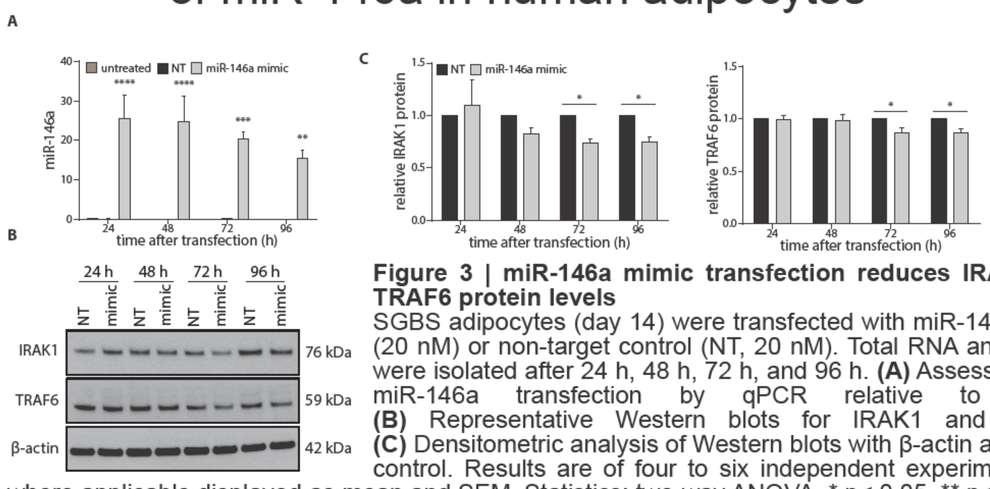


Figure 3 | miR-146a mimic transfection reduces IRAK1 and TRAF6 protein levels
SGBS adipocytes (day 14) were transfected with miR-146a mimic (20 nM) or non-target control (NT, 20 nM). Total RNA and protein were isolated after 24 h, 48 h, 72 h, and 96 h. **(A)** Assessement of miR-146a transfection by qPCR relative to sno68. **(B)** Representative Western blots for IRAK1 and TRAF6. **(C)** Densitometric analysis of Western blots with β-actin as loading control. Results are of four to six independent experiments and where applicable displayed as mean and SEM. Statistics: two-way ANOVA, * p< 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.0001, compared to corresponding NT.

miR-146a reduces the activation of JNK and p38

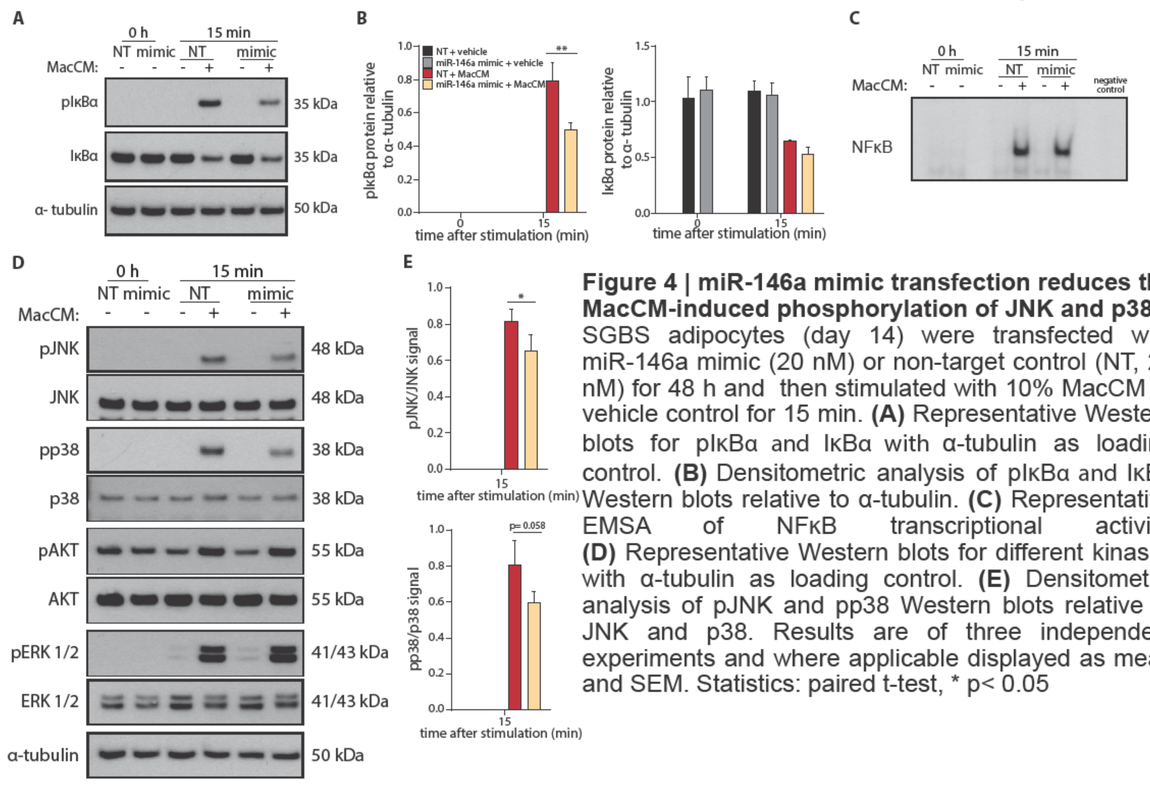


Figure 4 | miR-146a mimic transfection reduces the MacCM-induced phosphorylation of JNK and p38
SGBS adipocytes (day 14) were transfected with miR-146a mimic (20 nM) or non-target control (NT, 20 nM) for 48 h and then stimulated with 10% MacCM or vehicle control for 15 min. **(A)** Representative Western blots for pIκBα and IκBα with α-tubulin as loading control. **(B)** Densitometric analysis of pIκBα and IκBα Western blots relative to α-tubulin. **(C)** Representative EMSA of NFκB transcriptional activity. **(D)** Representative Western blots for different kinases with α-tubulin as loading control. **(E)** Densitometric analysis of pJNK and pp38 Western blots relative to JNK and p38. Results are of three independent experiments and where applicable displayed as mean and SEM. Statistics: paired t-test, * p< 0.05

miR-146a dampens the inflammatory response in human adipocytes

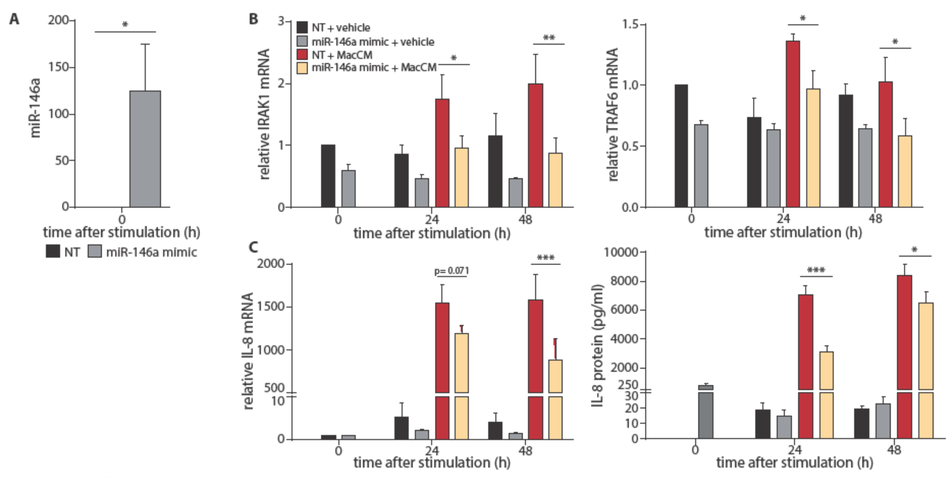


Figure 5 | miR-146a mimic transfection reduces IL-8 expression and secretion
SGBS adipocytes (day 14) were transfected with miR-146a mimic (20 nM) or non-target control (NT, 20 nM) for 48 h and then stimulated with 10% MacCM or vehicle control. RNA and media supernatants were collected at 0 h as well as 24 h and 48 h after stimulation. **(A)** Assessement of miR-146a transfection by qPCR relative to sno68. **(B)** IRAK1 and TRAF6 mRNA expression relative to SDHA. **(C)** IL-8 mRNA expression relative to SDHA and secreted IL-8 protein measured in media supernatants by ELISA. Results are of four (qPCR data) or three (ELISA) independent experiments and displayed as mean and SEM. Statistics: **(A)** paired t-test, **(B)** and **(C)** two-way ANOVA, * p< 0.05, ** p< 0.01, *** p< 0.001.

References

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Funding & Contact

This project was funded by the ESPE-Research Unit Award 2013-2015. If you have any question please contact pamela.fischer@uniklinik-ulm.de or julian.roos@uniklinik-ulm.de.

ESPE 2015
67--P1
Fat Metabolism, Obesity
Julian Roos
DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: 54th Annual Meeting 1-3 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology