Article

Identification of stably expressed housekeeping miRNAs in endothelial cells and macrophages in an inflammatory setting

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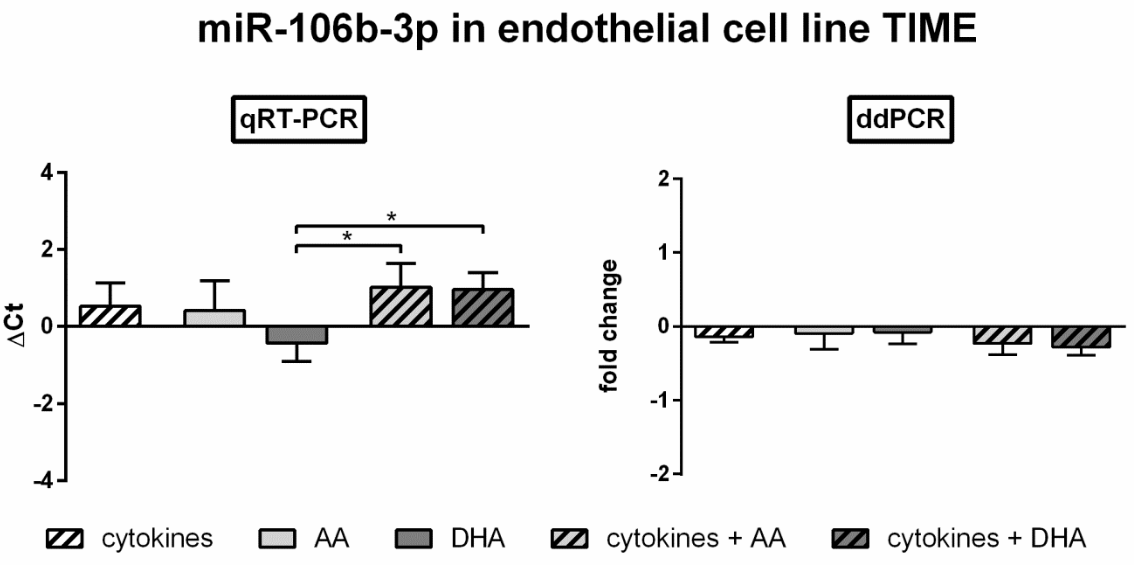

Figure S1: Expression alterations of miR-106b-3p in endothelial cell line TIME.
TIME were stimulated with a cytokine mix consisting of IL-1β, TNF-α, and IFN-γ (each in a concentration of 5 ng/ml) and/or supplemented with the unsaturated fatty acids docosahexaenoic acid (DHA, C22:6n3) or arachidonic acid (AA, C20:4n6). Relative quantification of miRNA expression (qRT-PCR) was performed by means of the miRCURY LNA SYBR Green PCR kit and appropriate miRCURY LNA miRNA PCR assay primers on a CFX96 Touch Real Time PCR detection system (N = 4, n = 2). miRNA expression is displayed as mean difference to unstimulated control on the basis of raw Ct values. Absolute quantification of miRNA expression(ddPCR) was performed by means of the EvaGreen Supermix and appropriate miRCURY LNA miRNA PCR assay primers on a QX200 ddPCR droplet reader system (N = 4, n = 2). miRNA expression was measured as RNA molecules per µl sample. Results are displayed as fold change to unstimulated control.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

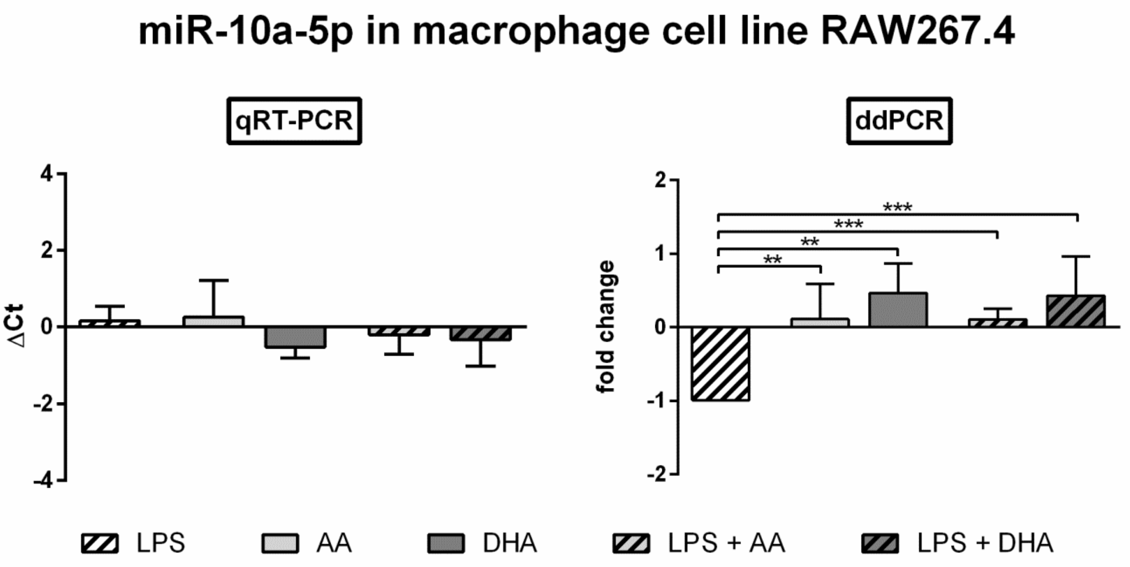

Figure S2: Expression alterations of miR-10a-5p in macrophage cell line RAW264.7.
RAW264.7 were stimulated with LPS (1 µg/ml) and/or supplemented with the unsaturated fatty acids docosahexaenoic acid (DHA, C22:6n3) or arachidonic acid (AA, C20:4n6). Relative quantification of gene expression (qRT-PCR) was performed by means of the miRCURY LNA SYBR Green PCR kit and appropriate miRCURY LNA miRNA PCR assay primers on a CFX96 Touch Real Time PCR detection system ($N = 4$, $n = 2$). miRNA expression is displayed as mean difference to unstimulated control on the basis of raw Ct values. Absolute quantification of miRNA expression (ddPCR) was performed by means of the EvaGreen Supermix and appropriate miRCURY LNA miRNA PCR assay primers on a QX200 ddPCR droplet reader system ($N = 4$, $n = 2$). miRNA expression was measured as RNA molecules per µl sample. Results are displayed as fold change to unstimulated control.
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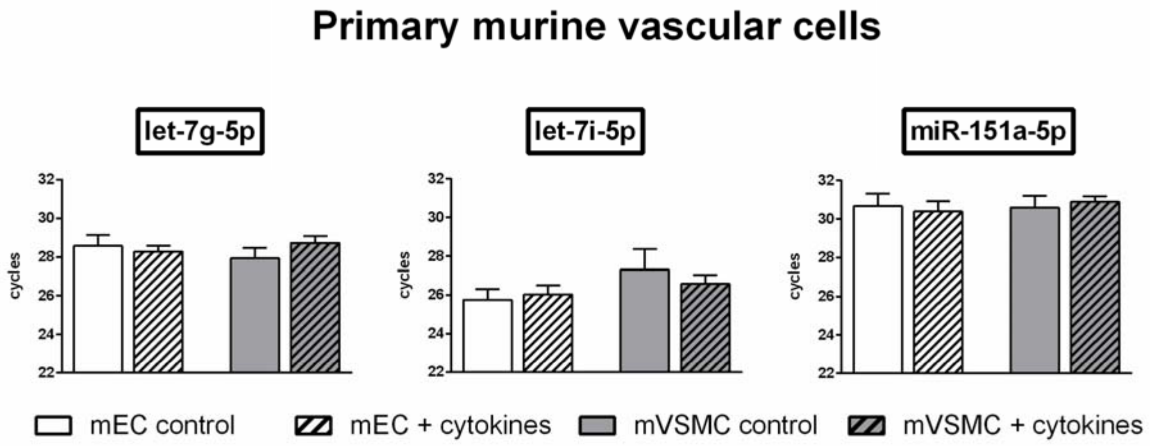

Figure S3: Expression rates of let-7g-5p, let-7i-5p, and miR-151a-5p in primary murine vascular cells. Murine endothelial cells (mEC) were isolated from murine mesenteries and vascular smooth muscle cells (mVSMC) from lungs of C57BL/6 mice. Cells were stimulated with a cytokine mix consisting of IL-1β, TNF-α, and IFN-γ (each in a concentration of 5 ng/ml). Relative quantification of miRNA expression was performed by means of the miRCURY LNA SYBR Green PCR kit and appropriate miRCURY LNA miRNA PCR assay primers on a CFX96 Touch Real Time PCR detection system (N = 4, n = 2). miRNA expression is displayed as mean difference to unstimulated control on the basis of raw Ct values.

Table S1: Summary of miRNAs discussed as data normalizers in different human studies

Condition	miRNA	Source	Reference
Bladder cancer	miR-16-5p, miR-193a-5p	Serum	doi:10.3892/mmr.2015.3428
	miR-101, miR-125a-5p, miR-148b, miR-151-5p, miR-181b, miR-874	Tissue	doi:10.1371/journal.pone.0039309
Breast cancer	miR-16, miR-425	Blood	doi:10.1371/journal.pone.0083718
	let-7a, miR-16	Tissue	doi:10.1186/1471-2199-9-76
	miR-16-5p, miR-29a-3p	Tissue	doi:10.3390/ijms17020156
Cervical cancer	miR-23a, miR-191	Tissue	doi:0.3858/emm.2011.43.6.039
	miR-151-5p, miR-152-3p, miR-423-3p	Tissue	doi:10.1016/j.tranon.2018.12.010
Chronic kidney disease	miR-16	Urinary exosomes	doi:10.1371/journal.pone.0183435
Colorectal cancer	miR-191-5p	Serum	doi:10.1371/journal.pone.0083025
	miR-25-3p, miR-93-5p, miR-106b-5p	Serum	doi:10.1038/srep35611
	let-7g, miR-27a, miR-193a-5p	Tissue	doi:10.1371/journal.pone.0150593
	miR-16, miR-345	Tissue	doi:10.1186/1471-2407-10-173
	miR-345, miR-520d, miR-1228	Tissue, plasma, exosomes	doi:10.1038/s41598-017-08784-3
Diffuse large B-cell lymphoma	miR-24	Tissue	doi:10.1136/jclinpath-2012-201021
Dihydrotestosterone treatment	miR-16, miR-1228-3p	Prostate cells lines RWPE-1, LNCaP, 22RV1, DU145, PC-3	doi:10.1038/s41598-018-19458-z
Esophageal cancer	miR-28-5p, miR-34a-5p, miR-186-5p	Tissue	doi:10.1007/s12032-016-0860-7
Gastric cancer	miR-16, miR-93	Serum	doi:10.1007/s10620-011-1981-7
	miR-101-3p, miR-140-3p	Tissue	doi: 10.1016/j.gene.2017.04.016
Heart failure / hypertension	let-7i, miR-16	Serum	doi:10.21037/jtd.2018.04.128
Hepatitis B	miR-22*, miR-26a, miR-221	Serum	doi:10.1007/s12033-011-9414-6
	miR-24-3p, miR-151a-5p, miR-425-5p	Cell line HepG2	doi:10.1186/s13104-016-1848-2
Kidney cancer	miR-28, miR-103, miR-106a	Tissue	doi:10.1016/j.ab.2011.06.009
Low/high estrogen levels	miR-188-5p, miR-222-3p	Plasma	doi:10.1186/s13104-017-2636-3
Lung cancer	miRNA-192	Pleural effusion	doi:10.3892/ol.2014.2404
Major depressive disorder	miR-93-5p, miR-101-3p	Plasma	doi:10.1016/j.jad.2013.12.035
Neuronal differentiation	miR-26b, miR-103, miR-106b	Cell lines SK-N-BE(2)C, neuro-2a, NG108-15, PC12; ENStem-A neural progenitor cells	doi:10.1016/j.neuroscience.2011.10.024
Ovarian cancer	miR-191-5p	Tissue	doi:10.1111/jcmm.12927
Prostate cancer	miR-191	Urine sediments	doi: 10.1155/2015/973597
	miR-130b	Tissue	doi:10.3858/emm.2010.42.11.076
Stable coronary artery disease	miR-4516, miR-6090	Plasma	doi:10.5603/CJ.2016.0109
Thyroid cancer	miR-99a-5p, miR-151a-3p, miR-197-3p, miR-214-3p	Aspiration smears	doi:10.3892/or.2016.5113
Training status	miR-320d	Plasma	doi:10.1038/s41598-019-38505-x
Vulvar cancer	miR-93-5p, miR-425-5p	Plasma	doi:10.1177/1010428317717140