Identification of adipogenesis specific microRNAs

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GRK 1957 | ADIPOCYTE-BRAIN CROSSTALK

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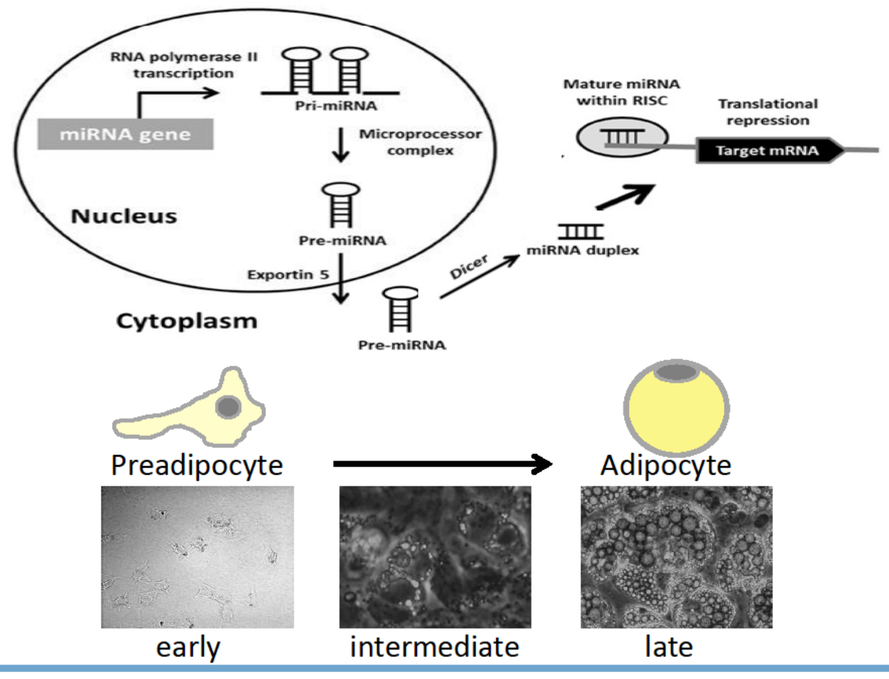
Introduction

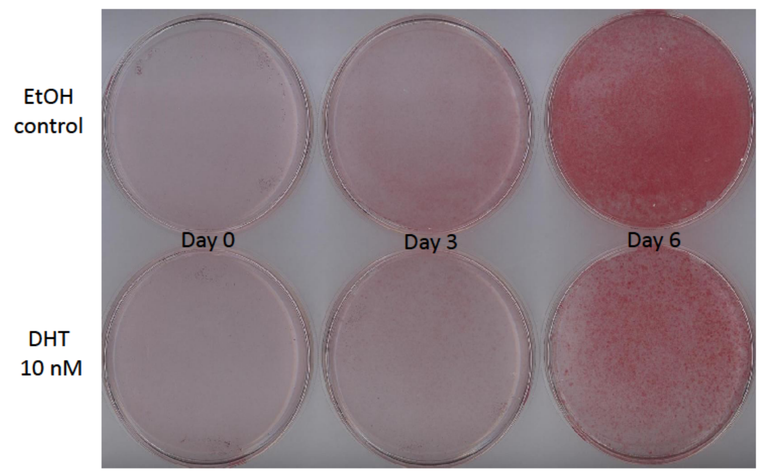
Obesity is grave and rising problem in Western societies. This disease shows a large degree of depot- and sex-specific variation and an understanding of the regulatory mechanisms underlying this variation is crucial to identify potential therapeutic targets or treatment strategies to combat obesity. MicroRNAs are recently recognized important players in adipogenesis and fat metabolism [1]. They posttranscriptionally regulate diverse biological processes, e.g. the proliferation and differentiation of cells.
Here we studied the fat depot and sex hormone-specific regulation of microRNAs during differentiation.



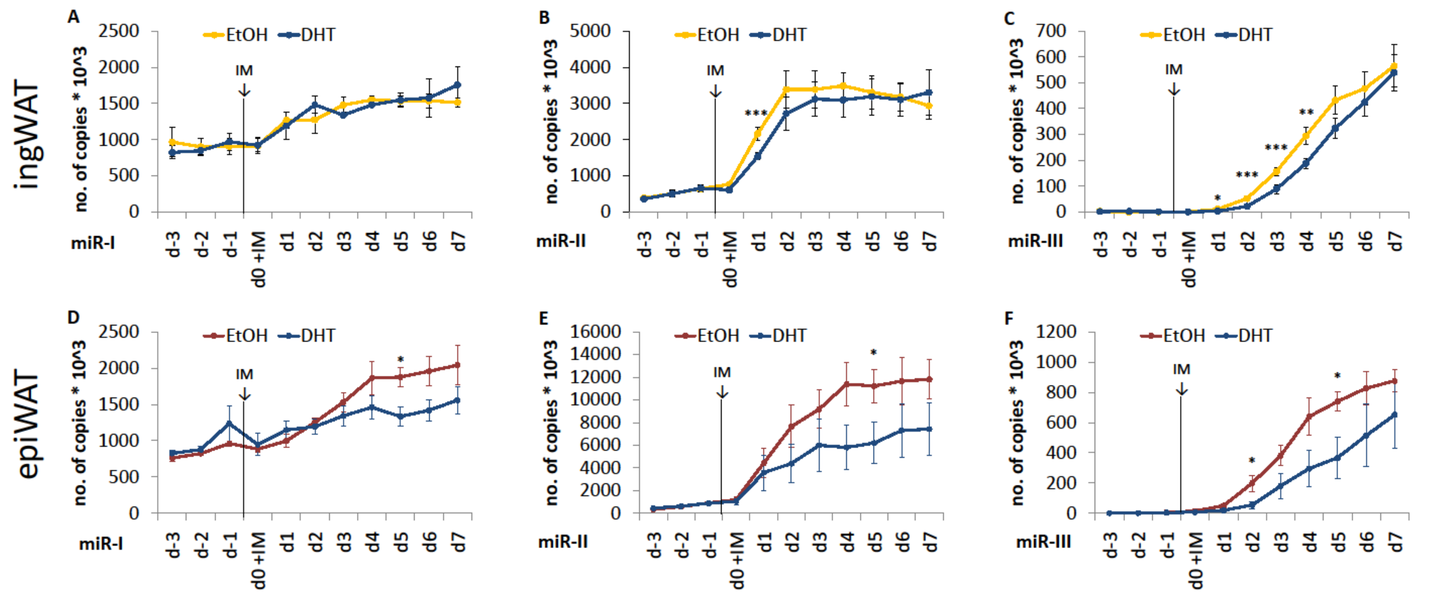
Biogenesis and function of miRNAs.
In the nucleus miRNAs are transcribed to pri-miRNAs, further processed to pre-miRNAs and exported to the cytoplasm. The exported miRNA-miRNA duplex is cleaved by Dicer into the mature miRNA (19-25nt) which is loaded onto the RISC complex. The miRISC assembly targets mRNA and represses the translation to functional protein by incomplete complementarity to the mRNA in the 3`UTR. Modified from: http://www.frontiersin.org/files/Articles/61125/fncel-07-00178-HTML/image_m/fncel-07-00178-g001.jpg

Schematically depicted adipogenesis.
Within 6-8 days pre-adipocytes differentiate *in vitro* to mature adipocytes, containing the characteristically lipid droplets. Fat depot specific characteristics and sex-hormones may influence the microRNA expression, that then can influence the proliferation and differentiation of pre-adipocytes.

Results

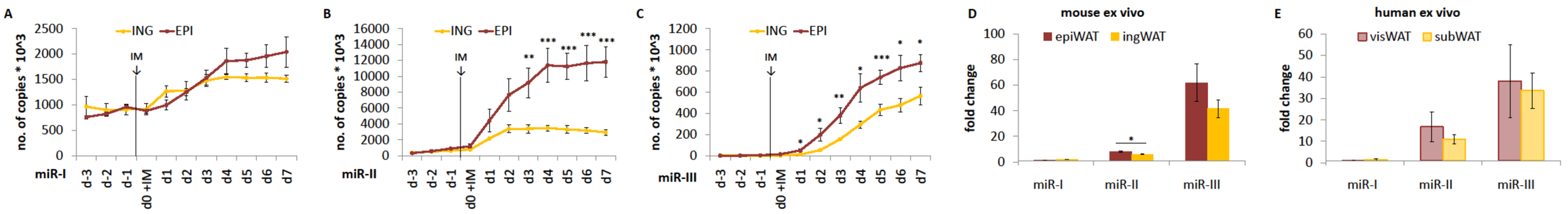


DHT attenuates lipid accumulation in epiWAT.
Oil Red O staining reveals less lipid content in DHT stimulated cells compared to controls (EtOH) at days 3 and 6 of fat cell differentiation, indicating a decelerated differentiation to mature adipocytes.



DHT regulates microRNA expression during adipocyte differentiation.
MicroRNA expression profiles of microRNAs miR-I (A,D), miR-II (B,E) and miR-III (C,F) in ingWAT (A-C, yellow) and epiWAT (D-F, red) cell lines in control conditions (EtOH) and chronically stimulated with DHT (blue). DHT (significantly) decreases the expression of miR-II and miR-III, indicating a regulation of these microRNAs during differentiation. All datasets were normalized to miR-191 (housekeeping).

MicroRNAs are differently expressed between white fat depots.



Comparing *in vitro* microRNA expression profiles between ingWAT and epiWAT reveals significantly different expression for mir-II (B) and miR-III (C), but not for miR-I (A). Furthermore *ex vivo* fold change expression analysis of these microRNAs between pre-adipocytes and mature adipocytes indicates fat depot specific differences of microRNA expression in mice (D) and humans (E). Fold change was calculated by 2^ΔΔCt method. All datasets were normalized to miR-191.

Summary & Conclusion

✓ Stimulation with sex-hormone DHT….
…. decelerates epiWAT adipocyte differentiation
…uncovers microRNA regulation during adipogenesis

✓ MicroRNA expression varies (significantly) between ingWAT and epiWAT fat depots *in vitro* and *ex vivo* in mice and humans

➢ MicroRNAs are specifically expressed in adipocyte differentiation and their expression can be influenced by sex-hormones and fat depot specific characteristics.

Literature

[1] P. Arner and A. Kulyté MicroRNA regulatory networks in human adipose tissue and obesity. Nat Rev Endocrinol. 2015 May;11(5):276-88

Material & Methods

Cell culture: Inguinal (ing) and epididymal (epi) white adipose tissue (WAT) SV-40 immortalized pre-adipocytes were seeded in differentiation medium (DM). 24 hours later chronic stimulation with 10 nM male sex-hormone dihydrotestosterone (DHT) or EtOH (control) started. Confluent cells were induced for 24 hours with induction medium (IM = DM + IBMX, indomethacine, dexamethasone). Cells maintained in DM until they reached mature adipocyte phenotype *Mice & Humans:* IngWAT and epiWAT from C57BL/6 mice and subcutaneous and visceral WAT from men were *ex vivo* digested with collagenase A; pre-adipocytes and mature adipocytes were separated by centrifugation. *RT-qPCR:* Universal cDNA Synthesis Kit II, miRCURY LNA™ RT PCR primer sets and ExiLENT SYBR® miRCURY LNA™ microRNA PCR (Exiqon) were used to the manufactures instructions and recommendations. Data sets were normalized to miR-191-5p. *Western Blot:* Equal concentrations of protein lysates were separated in SDS-PAGE and transferred to a PVDF membrane, which were blocked and then probed with primary antibodies overnight at 4 °C: C/EBP-β, Pref-1, A-FABP (Santa Cruz), GAPDH (Cell Signaling), β-actin (Sigma). *Oil-Red-O:* Lipid content was stained with freshly prepared Oil Red O working solution for 1 hour in formalin fixed cells.

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