Adipose-derived exosomal miRNAs orchestrates gene regulation in the liver: is this the missing link in NAFLD?

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It has long been known that adipose tissue have functions beyond the storage of lipids. More particularly, adipocytes secrete hormones and other molecular cues including adipokines that actively influence metabolism at distant tissue sites. However, until now researchers have not understood how these molecules are targeted to distant organs and more importantly, how they regulate metabolic homeostasis. Scientist at Joslin Diabetes Center and Harvard Medical School in Boston now have revealed a mechanism whereby small pieces of genetic material called microRNAs (miRNAs), act as messenger molecules that regulate gene expression in other organs such as the liver.

miRNA's are snips of non-coding RNA produced intracellularly and secreted into the circulation either as free entities, or packaged into small vesicles called exosomes (1). They mediate their effects either by mRNA cleavage, translational repression or mRNA destabilization following binding to target transcript sequences (2). Using genetically engineered mice deficient for the miRNA-processing enzyme (Dicer), in their adipose tissue (ADicerKO), the researchers demonstrated that the levels of exosomal miRNA in the circulation was significantly lower compared to control animals. These animals also had less adipose tissue and showed some degree of insulin resistance. Transplanting thermogenic brown adipose tissue (BAT), and to a lesser extent also energy-storing white adipose tissue (WAT) into ADicerKO animals, restored exosomal miRNA levels and also their capacity to process glucose. These experiments elegantly indicated that adipose tissue is an important source of circulating exosomal miRNAs and that distinct adipose depots contribute differently to the type of exosomal miRNAs as well as the capacity to regulate whole body metabolism.

Thomou *et al*., then studied whether these miRNA's effectively direct gene regulation in distant tissues such as the liver. They focused specifically on Fibroblast growth factor 21 (FGF21) and observed that this parameter was elevated within the liver of ADicerKO mice. They identified miR-99b as the predicted regulator of FGF21 expression. Further analysis showed that miR-99b was reduced in circulating exosomes from ADicerKO mice, and its levels were restored by BAT transplantation. They then developed tools to measure the exosomal miRNA-dependent hepatic expression of FGF21 by transfecting ADicerKO mice with a *Fgf21*-luciferase reporter (FGF21-3'UTR reporter). In a control condition, these animals featured a bright luminescent signal from the liver when subjected to *in vivo* imaging, correlating with the absence of miRNAs and their inability to repress hepatic Fgf21. When the mice were transferred with exosomes from WT mice or exosomes from KO mice electroporated with miR-99b, the hepatic Fgf21 expression was successfully repressed as visualized by the dampened intensity of the luminescence signal

(originating from the FGF21-3'UTR reporter). It seems therefore that in healthy conditions miRNA-99b is needed to keep Fgf21 repressed and in its absence the repressive effect is lost resulting in elevated hepatic Fgf21. Another important observation that the group of Kahn made using a similar *in vitro* system, was that the regulation of Fgf21 was dependent on exosomal delivery and could not be recapitulated with naked miR-99. It is therefore not only the regulatory molecule and its actions that are important for the observed effects, but also the barcode information provided by its packaging that is crucial in directing these vesicles to the desired tissue site. Additionally, packaging of the miRNA within exosomal vesicles may increase miRNA stability and in this way aid their delivery to distant sites in an intact state.

Although FGF21 has emerged as an important regulator of metabolism and its association with lipodystrophy has been established (3), it is plausible that many more targets and tissues may be involved since multiple other miRNAs were dampened in ADicerKO mice and restored upon fat transplantation. Additionally, it may be interesting to evaluate whether the absence of miRNA's also unleash the expression of genes such as collagens, matrix metalloproteinases and targets of hepatic fibrosis relevant to the progression diseases such as non-alcoholic fatty liver disease (NAFLD). We therefore are probably only beginning to understand the magnitude of other processes that are regulated by such an inter-organ communication system.

The authors' most striking result stems from yet another masterly developed method whereby they directly measured adipose-liver cross-talk using a human miRNA. More specifically, they engineered adipocytes to produce a human specific miRNA in donor animals (has_miRNA). In acceptor animals, they engineered hepatocytes to express the molecular target for the human miRNA with a luminescent reporter (hsa_miR-302f 3′ UTR reporter). Injecting isolated exosomes from the donor cohort (containing the human miRNA) into acceptor animals resulted in a dramatic reduction in liver cell luminescence (originating from the hsa_miR-302f 3′ UTR reporter), indicative of target binding and repression of gene expression. This is very strong data confirming for the first time that adipose tissue, through exosomes, can directly 'talk' to liver cells and regulate gene expression.

Extrapolating these observations to known human diseases the authors demonstrated that patients with congenital lipodystrophy (with a generalized loss of adipose tissue) or HIV-associated lipodystrophy (with reduced Dicer in adipose tissue) show a similar reduction in exosome-derived miRNAs suggesting a direct involvement of altered miRNA's in the metabolic derangements associated with these conditions. This finding also raises the question whether altered

miRNA/exosome signaling may be involved in obesity, the metabolic syndrome or NAFLD. Adipose tissue-liver cross talk has been suggested to occur during NAFLD and factors originating from adipose tissue (but also from the liver) have been described as potential drivers of disease progression. Previously our group and others showed in NAFLD that adipose tissue inflammation preceded the development NASH and suggested that early events or secreted factors in adipose tissue are prerequisites for the progression of Non Alcoholic Fatty Liver (NAFL) to Non Alcoholic Steatohepatitis (NASH) (4, 5). However, direct evidence for such a mechanism was lacking until now and it will be intriguing to address the involvement of similar inter-cellular communication mechanisms during NAFLD progression (Fig. 1). Complexing the situation however, a number of other potential exosomal or non-exosomal messenger molecules may contribute to the regulation of metabolism, besides miRNAs (Fig. 1) (6). Additionally, due to their imperfect target binding, miRNAs could potentially have off-target effects that need to be considered and analyzed in future studies. Nevertheless, these intriguing findings may potentially identify new pathways in adipose tissue crosstalk that may be targeted through gene therapy restoring defective microRNAs signaling to downstream organs such as the liver.

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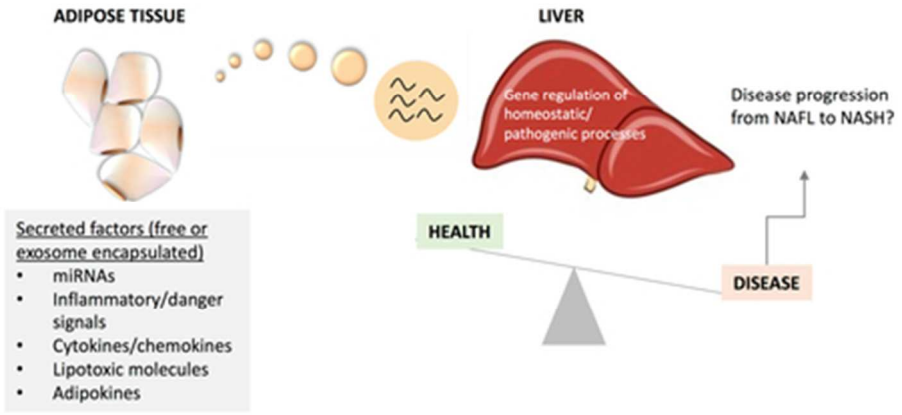
Figure legends

Figure 1. Dysregulated signaling within adipose tissue as potential mechanism for driving disease progression in the liver.



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