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Research Paper

Long Non-Coding RNAs Associated with Metabolic Traits in Human White Adipose Tissue

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ABSTRACT

Long non-coding RNAs (lncRNAs) belong to a recently discovered class of molecules proposed to regulate various cellular processes. Here, we systematically analyzed their expression in human subcutaneous white adipose tissue (WAT) and found that a limited set was differentially expressed in obesity and/or the insulin resistant state. Two lncRNAs herein termed adipocyte-specific metabolic related lncRNAs, *ASMER-1* and *ASMER-2* were enriched in adipocytes and regulated by both obesity and insulin resistance. Knockdown of either *ASMER-1* or *ASMER-2* by antisense oligonucleotides in in vitro differentiated human adipocytes revealed that both genes regulated adipogenesis, lipid mobilization and adiponectin secretion. The observed effects could be attributed to crosstalk between *ASMERs* and genes within the master regulatory pathways for adipocyte function including *PPARG* and *INSR*. Altogether, our data demonstrate that lncRNAs are modulators of the metabolic and secretory functions in human fat cells and provide an emerging link between WAT and common metabolic conditions.

1. Introduction

Long non-coding RNAs (lncRNAs) are defined as RNA transcripts longer than 200 nucleotides that do not code a polypeptide. Some of these molecules regulate various biological processes including gene imprinting, chromatin alteration and the allosteric modification of enzyme activity (Ponting et al., 2009; Quinn and Chang, 2016; Rinn and Chang, 2012). While some lncRNAs have primarily been implicated in cancer and neurodegenerative disorders (Batista and Chang, 2013; Esteller, 2011), recent studies, mostly in rodent models, also suggest the potential involvement of lncRNAs in metabolic diseases (reviewed in (Losko et al., 2016)). Thus, alterations in the nutritional status of mice (i.e. fasting, refeeding and overfeeding), result in altered expression of several lncRNAs in tissues relevant for metabolism, i.e. liver, white adipose tissue (WAT) and skeletal muscle (Yang et al., 2016), but only effects in liver were investigated in detail. LncRNAs may also control the function of pancreatic β cells of mice and humans (Akerman et al., 2017; Losko et al., 2016). However, much less is known about the potential roles of lncRNAs in modulating the function of WAT where studies have primarily focused on their effects on in vitro differentiation of adipose precursor cells (i.e. adipogenesis) (Wei et al., 2016). During human adipocyte differentiation the expression of lncRNAs co-cluster with the expression of coding genes and regulatory factors such as microRNAs and enhancers (Ehrlund et al., 2017), indicating that lncRNAs may have potentially critical functional roles in adipocytes. Furthermore, a recent genome-wide association study on cardiometabolic traits demonstrated that several WAT-expressed lncRNAs were associated with single nucleotide polymorphisms linked to cardiometabolic disease (Ballantyne et al., 2016). These studies suggest that WAT-expressed lncRNAs may affect other processes in fat cells besides adipogenesis.

WAT plays a pivotal role in metabolic disease not only based on its energy storage/release capacity but also because of its endocrine function, as it secretes numerous proteins termed adipokines (Kershaw and Flier, 2004; Sethi and Vidal-Puig, 2007). The metabolic and endocrine functions of WAT may impact the regulation of several other organs such as brain, liver and skeletal muscle (Kershaw and Flier, 2004; Sethi and Vidal-Puig, 2007). Alterations in WAT function are well-documented in obesity and insulin resistance (Guilherme et al., 2008). Moreover, WAT from different body regions is linked explicitly to metabolic disease, with visceral WAT being more pernicious than the subcutaneous depot (Wajchenberg, 2000). Nevertheless, regional differences within subcutaneous WAT could also be of importance as several investigators have shown that the abdominal region is positively, and the gluteofemoral region is negatively associated with cardiometabolic disease (Snijder et al., 2003; Yusuf et al., 2005). While the role of lncRNAs in explaining these differences are not known, it

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was recently reported that the lncRNA *HOTAIR*, with potent positive effects on adipogenesis, was expressed in gluteal but not in abdominal subcutaneous WAT (Divoux et al., 2014).

In order to assess the potential roles of lncRNAs in human WAT, we presently investigated their expression in human WAT and association with obesity and insulin resistance (Fig. 1). By combining RNA sequencing and gene microarray analyses of WAT from two clinical cohorts of obese and non-obese individuals with significant between subject variations in insulin sensitivity, we identified a set of potentially relevant lncRNAs. To identify lncRNAs that were potentially important for fat cell function we compared the expression of each gene in isolated fat cells and the stromal vascular fraction (SVF) from the same WAT biopsy using Real-time PCR. Key findings were then confirmed by probing previously generated 5′ Cap Analysis of Gene Expression (CAGE) data from human adipocyte precursor cells during differentiation from our previous study (Ehrlund et al., 2017). Finally, the functional role of the adipocyte-specific lncRNAs displaying the most prominent clinical associations was investigated in differentiated fat cells following knockdown with antisense oligonucleotides where we focused on lipid mobilization (lipolysis) and endocrine function (adiponectin release).

2. Materials and Methods

2.1. Cohorts

The study included two cohorts consisting of 108 women who were recruited from the general adult population in the Stockholm (Sweden) area (Supplementary Table 1). Cohort 1 comprised 15 lean and 13 obese women who were matched for age. Cohort 2 consisted 80 obese age-matched women with or without insulin resistance and has been described in detail before (Arner et al., 2016). Obesity was defined as body mass index (BMI) > 30 kg/m^2. The study was approved by the regional ethics board, and written informed consent was obtained from each subject. Subjects in cohort 1 were investigated in the morning after an overnight fast when abdominal subcutaneous WAT was obtained by fine-needle aspiration (Kolaczynski et al., 1994). One part of WAT was frozen and stored at −70 °C for subsequent RNA extraction. The remaining tissue was subjected to collagenase treatment and isolated fat cells were used to measure lipolysis and lipogenesis as described in detail (Lofgren et al., 2005). The basal rates as well as rate stimulated by isoprenaline, a synthetic catecholamine (lipolysis), and insulin (lipogenesis) were measured. In cohort 2, abdominal subcutaneous and visceral (from greater omentum) WAT was obtained at the beginning of bariatric surgery. A venous blood sample was collected alongside for clinical chemistry measures as described (Arner et al., 2016). For comparisons between different fractions of the WAT, Samples of abdominal subcutaneous adipose tissue (about 100–200 g) were obtained from cosmetic liposuction of 11 healthy female subjects. The mean and (range) of age and BMI were 38 (20–50) years and 26 (22–29) kg/m^2, respectively.

2.2. Mapping of Affymetrix Probesets to the Annotation of FANTOM CAGE Associated Transcriptome (FANTOM-CAT)

To compare our previously published microarray data (GSE101492) (Arner et al., 2016) with the RNA-seq data (generated from this study) on the same set of gene models, we remapped the Affymetrix HTA 2.0 probe sets onto the FANTOM-CAT gene models (robust level, n = 53,220 genes) (Hon et al., 2017). Briefly, coordinates of the transcript exons of each FANTOM-CAT gene on hg19 were obtained from

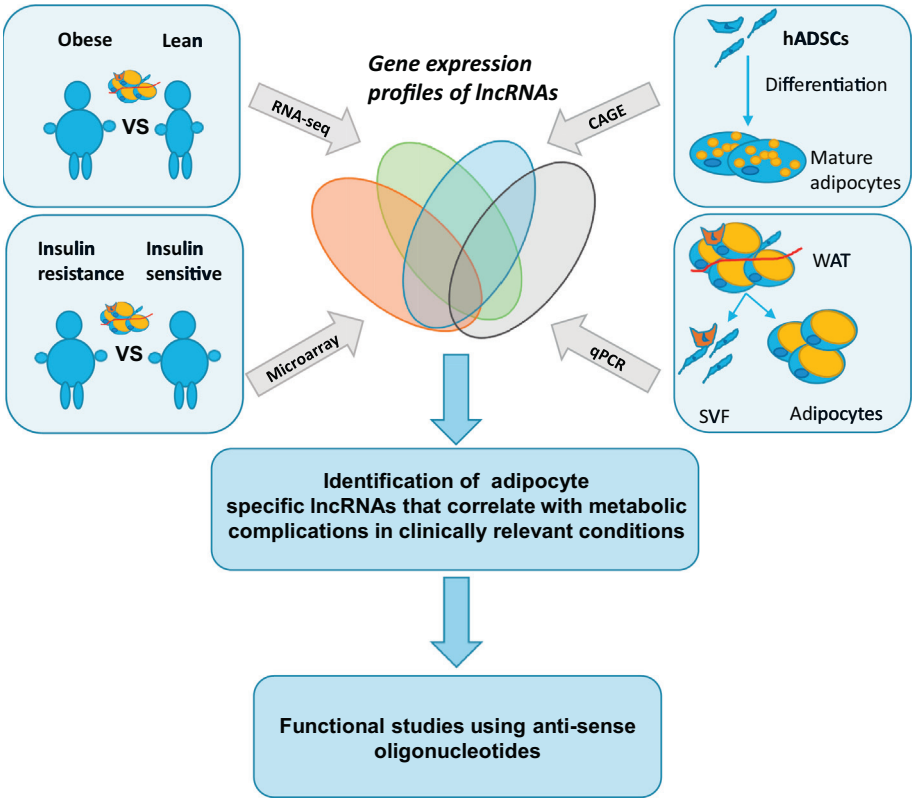

Fig. 1. Flowchart represents the strategy used for gene expression analysis. These initial data used in the analysis were obtained from two well-characterized clinical cohorts; Affymetrix microarray data from visceral and subcutaneous adipose tissue of 80 obese individuals with or without insulin resistance and adipose RNA-sequencing data from 15 lean and 13 obese subjects. Identified candidate long non-coding RNAs (lncRNAs) for further studies. This involved global expression during human in vitro adipogenesis determined by single molecule RNA-seq using 5′-Cap analysis gene expression (CAGE) and expression analysis in different cellular fractions of adipose tissue using quantitative Real-time PCR. hADSCs = human adipose tissue-derived stem cells; WAT = white adipose tissue; SVF = stromal vascular fraction of adipose tissue.

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