Original article

MOLECULAR METABOLISM

Altered DNA methylation of glycolytic and lipogenic genes in liver from obese and type 2 diabetic patients

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ABSTRACT

Objective: Epigenetic modifications contribute to the etiology of type 2 diabetes.
Method: We performed genome-wide methylome and transcriptome analysis in liver from severely obese men with or without type 2 diabetes and non-obese men to discover aberrant pathways underlying the development of insulin resistance. Results were validated by pyrosequencing.
Result: We identified hypomethylation of genes involved in hepatic glycolysis and insulin resistance, concomitant with increased mRNA expression and protein levels. Pyrosequencing revealed the CpG-site within ATF-motifs was hypomethylated in four of these genes in liver of severely obese non-diabetic and type 2 diabetic patients, suggesting epigenetic regulation of transcription by altered ATF-DNA binding.
Conclusion: Severely obese non-diabetic and type 2 diabetic patients have distinct alterations in the hepatic methylome and transcriptome, with hypomethylation of several genes controlling glucose metabolism within the ATF-motif regulatory site. Obesity appears to shift the epigenetic program of the liver towards increased glycolysis and lipogenesis, which may exacerbate the development of insulin resistance.

Keywords Liver; Obesity; Type 2 Diabetes; Epigenetics; Lipid; Glucose

1. INTRODUCTION

Although the prevalence of type 2 diabetes is approaching epidemic proportions, the underlying pathophysiology is still not fully understood. The etiology of type 2 diabetes is complex and multifactorial, as it is influenced by both genetic predisposition [30] and lifestyle factors, such as diet and physical activity [14]. The liver is a key glucoregulatory organ involved in the maintenance of glucose homeostasis and the development of type 2 diabetes [17,31]. In healthy individuals, insulin promotes hepatic glycogen synthesis and *de novo* lipogenesis, while inhibiting gluconeogenesis. However, in obese or type 2 diabetic patients, insulin fails to suppress hepatic glucose output, which leads to hyperglycemia via an upregulation of the gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase [27]. The underlying molecular mechanism for these shifts in hepatic metabolism is still incompletely resolved and aberrant regulation of additional metabolic pathways are likely to be involved.

Epigenetic mechanisms such as DNA methylation integrate environmental factors and genetic susceptibility by modulating transcriptional regulation without changing the underlying DNA sequence. DNA methylation is an epigenetic mark that can change in response to environmental challenges to directly modify gene expression. DNA methylation can modify gene expression in several ways, for example by altering histone interactions, influencing transcription factor binding, and/or recruitment of methyl-binding proteins [16]. Dynamic DNA methylation often occurs distal to the transcription start site, with the position co-localizing with gene regulatory elements, particularly enhancers and transcription factor-binding sites [50]. Alterations in DNA methylation at differentially methylated sites or regions have been implicated in metabolic diseases such as obesity [24,26,48] and type 2 diabetes [5,26,36,46,47]. Since DNA methylation can lead to stable alterations in the transcriptional potential, epigenetic mechanisms may partly explain the rapidly increasing prevalence of type 2 diabetes [23]. Recent evidence suggests that DNA methylation of key metabolic genes in skeletal muscle is remodeled by interventions known to

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Received December 6, 2015 • Revision received December 23, 2015 • Accepted December 27, 2015 • Available online 2 January 2016

http://dx.doi.org/10.1016/j.molmet.2015.12.004

improve insulin sensitivity such as exercise [6,36] or bariatric surgery [4]. Thus, changes in the epigenome may provide an underlying molecular mechanism for deleterious metabolic health outcomes associated with severe obesity or type 2 diabetes. Likewise, coordinated epigenetic changes may also improve metabolic health after therapeutic intervention.

Systematic studies of the DNA methylation landscape and the related transcriptome of metabolic tissues from obese and/or type 2 diabetic patients show that DNA methylation is altered in metabolic diseases [4,24,26,32,33,35,36,40,43,46,47]. For example DNA methylation at the *HIF3A* loci in blood cells is correlated with BMI in Caucasian adults [13]. Moreover, evidence from mouse models indicates that normally occurring variation in methylation levels contribute to clinically relevant hepatic traits [37]. Therefore, global epigenome and transcriptome analysis of human liver in various states of insulin resistance could offer valuable insight into regulatory mechanisms involved in the pathogenesis of type 2 diabetes.

To better understand the molecular mechanisms underlying the development of hepatic insulin resistance and type 2 diabetes, we performed a genome-wide methylome and transcriptome analysis of liver from age-matched non-obese metabolically healthy, obese non-diabetic and obese type 2 diabetic men. We found key genes involved in hepatic glycolysis and *de novo* lipogenesis were hypomethylated and activated in obese non-diabetic and obese type 2 diabetic patients compared to non-obese control subjects. Our results indicate the epigenetic landscape in liver is altered in obesity, concomitant with increased expression of genes involved in hepatic glycolysis and gluconeogenesis, as well as stearate biosynthesis. These genomic changes may contribute to the development of insulin resistance in obesity and type 2 diabetes.

2. RESULTS

2.1. Obese and type 2 diabetic signature of the hepatic methylome and transcriptome

Liver biopsies were obtained from non-obese men during cholecystectomy and from obese non-diabetic and obese type 2 diabetic men during Roux-en Y gastric bypass surgery. Anthropometric and clinical parameters of the study cohort are presented (Table 1). Age was not different between the cohorts. Body weight, body mass index (BMI) and waist circumference did not differ between the obese non-diabetic and obese type 2 diabetic patients, but was increased compared to the non-obese participants. While plasma triglycerides, high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL) levels were unchanged between the cohorts, fasting serum glucose, glycated hemoglobin (HbA_{1c}) and homeostatic model assessment – insulin resistance (HOMA-IR) levels were increased in the obese type 2 diabetic patients compared to the non-obese controls and obese non-diabetic patients.

To identify novel regulatory mechanisms involved in the pathogenesis of obesity and type 2 diabetes, we determined the hepatic methylome of 7 non-obese metabolically healthy, 7 obese non-diabetic and 8 obese type 2 diabetic Caucasian men of similar age using the Illumina Infinium 450K Bead Chip. We also assessed the hepatic transcriptome using Affymetrix gene arrays of liver biopsies obtained from 18 individuals (6 from each group). Samples from 17 of the subjects were subjected to both the transcriptome and methylome analysis. Methylome data were filtered for detection and analyzed according to the Beta Mixture Quantile dilation (BMIQ) method [29]. Employing a multigroup comparison, 5834 CpG loci were identified to be differentially methylated among the three groups (FDR < 0.25). Hierarchical clustering and principal component analysis (PCA), based on the differentially methylated sites, revealed a clear separation of the three phenotypic groups, with the obese non-diabetic group being well-separated from the obese type 2 diabetic and non-obese group (Figure 1A,B). In total, 5682 CpG sites mapping to 3058 individual genes were differentially methylated in obese non-diabetic individuals versus non-obese controls ($p < 0.05$; Tables S1 and S2). Moreover, 2255 CpG sites mapping to 1388 individual genes were differentially methylated between obese type 2 diabetic individuals compared to non-obese controls ($p < 0.05$; Table Tables S1 and S2). In particular, the vast majority of CpG sites displayed decreased DNA methylation in obese non-diabetic and obese type 2 diabetic individuals, compared to non-obese controls (97 and 92%, respectively; Figure 1C). The distribution of the absolute differences in DNA-methylation between obese non-diabetic versus non-obese controls and obese type 2 diabetic individuals versus non-obese controls is shown in Figure 1D.

To investigate whether obesity and/or type 2 diabetes is associated with alterations in global DNA methylation, and/or alterations in the methylation profile at specific genomic positions, the distribution of altered CpG sites was mapped according to defined CpG categories and in relation to the nearest gene. Although distinct changes in DNA methylation were associated with obesity and type 2 diabetes (Figure 1), global DNA methylation with regard to CpG categories was similar between the three cohorts (Figure S1A). Additionally, genomic distribution of the differentially methylated sites with regard to the nearest gene was similar between the cohorts (Figure S1B). In general, differential DNA-methylation was observed at specific loci, rather than spread over larger chromosomal areas (data not shown), as is often observed in various cancers [45].

PCA analysis based on overall gene expression, separated the three groups into distinct clusters (Figure 2A). Analysis of differences in gene expression profiles between the groups revealed that 448 transcripts were differentially expressed in obese non-diabetic individuals versus non-obese controls (FDR ≤ 0.25; Table S3). Moreover, 1285 transcripts were differentially expressed in obese type 2 diabetic individuals versus non-obese controls (FDR ≤ 0.25; Table S3). Only 11 transcripts were identified as differentially expressed in obese non-diabetic individuals versus obese type 2 diabetic individuals.

Canonical pathway analysis was employed to identify pathways in the liver that are altered in obesity and type 2 diabetes. Interestingly, there was substantial overlap between pathways altered in obese non-diabetic individuals compared to non-obese controls and pathways altered in obese type 2 diabetic individuals compared to non-obese controls (Figure 2B). For example, genes involved in stearate biosynthesis, AMPK signaling and PI3K/AKT signaling were affected in both obese non-diabetic, as well as obese type 2 diabetic individuals compared to non-obese controls (Figure 2C). Genes involved in gluconeogenesis and glycolysis were specifically altered in liver from obese non-diabetic individuals compared to non-obese controls (Figure 2D; upper panel). Moreover, transcripts involved in PXR/RXR activation were specifically altered in obese type 2 diabetic individuals compared to non-obese individuals (Figure 2D; lower panel).

2.2. Correlation of the obese and type 2 diabetic hepatic methylome and transcriptome

Figure 3A,B displays CpG sites with altered DNA-methylation and where the expression of the associated gene is changed for obese non-diabetic individuals versus non-obese controls and obese type 2 diabetic individuals versus non-obese controls, respectively. Overall, we identify 36 and 61 genes with significantly altered expression

