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Epigenetic priming of inflammatory response genes by high glucose in adipose progenitor cells

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ABSTRACT

Cellular metabolism confers wide-spread epigenetic modifications required for regulation of transcriptional networks that determine cellular states. Mesenchymal stromal cells are responsive to metabolic cues including circulating glucose levels and modulate inflammatory responses. We show here that long term exposure of undifferentiated human adipose tissue stromal cells (ASCs) to high glucose upregulates a subset of inflammation response (IR) genes and alters their promoter histone methylation patterns in a manner consistent with transcriptional de-repression. Modeling of chromatin states from combinations of histone modifications in nearly 500 IR genes unveil three overarching chromatin configurations reflecting repressive, active, and potentially active states in promoter and enhancer elements. Accordingly, we show that adipogenic differentiation in high glucose predominantly upregulates IR genes. Our results indicate that elevated extracellular glucose levels sensitize in ASCs an IR gene expression program which is exacerbated during adipocyte differentiation. We propose that high glucose exposure conveys an epigenetic 'priming' of IR genes, favoring a transcriptional inflammatory response upon adipogenic stimulation. Chromatin alterations at IR genes by high glucose exposure may play a role in the etiology of metabolic diseases.

1. Introduction

Chromatin remodeling plays an important role in establishing epigenetic patterns required for regulation of gene expression networks that determine cellular states. Cytosine methylation and histone post-translational modifications (hPTMs) sensitize the genome to changing environmental conditions including nutrient availability [1]. In addition, cellular metabolic pathways provide intermediates that serve as co-factors of histone modifying enzymes and can alter expression level of these enzymes [2]. Histone methyltransferases and deacetylases are metabolically responsive, altering patterns of histone H3 and H4 methylation or acetylation, respectively, on lysine (K) residues. Histone PTMs include trimethylation of H3K4 (H3K4me3), which marks transcription start sites (TSSs) of expressed and many non-expressed genes, H3K9 di- and trimethylation (H3K9me2/3) associated with inactive promoters, H3K27me3, marking inactive promoters and enhancers, and forms of H3 and H4 acetylation on promoters and enhancers, often associated with transcriptional activity. Cellular metabolic state thus confers epigenetic modifications throughout the genome, including on promoters and enhancers, key regulators of gene expression.

Mesenchymal stromal cells (MSCs) are multipotent cells found in various tissues including adipose tissue [3], and are responsive to cellular metabolic states. MSCs modulate immune and inflammatory responses, in part by secreting cytokines acting as chemoattractants for monocytes and neutrophils [4,5]. Regulatory roles of adipose tissue-derived MSCs (ASCs) in inflammation [6] are likely influenced by metabolic cues such as high circulating blood glucose in individuals with insulin resistance [7]. Elevated extracellular glucose alters metabolic states and influences H3 methylation and

Abbreviations: ASC, adipose tissue-derived MSC; ChIP, chromatin immunoprecipitation; GO, gene ontology; IR, inflammation response; MSC, mesenchymal stromal cell; TSS, transcription start site.

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acetylation [1]. Interestingly, promoters of inflammation response (IR) genes in cardiomyocytes [8] and endothelial cells [9] are modified through histone methylation in a glucose-dependent manner. These modifications may constitute an 'epigenetic memory' of an inflammatory pre-disposition [1], contributing to an increased IR after a metabolic insult. Little is known, however, on how nutrient availability and glucose levels in particular impact epigenetic modifications on genes involved in inflammatory processes in adipocyte progenitors and during adipogenic differentiation.

We show here that exposure of human primary ASCs to elevated glucose upregulates IR genes and alters H3 methylation in a manner suggesting transcriptional de-repression. Modeling of chromatin states from combinations of seven chromatin marks in 497 IR genes unveils chromatin configurations suggestive of potentially active states in promoter and enhancer elements. Accordingly, adipogenic induction in high glucose exacerbates transcriptional upregulation of IR genes. Our results suggest that elevated glucose epigenetically primes ASCs for upregulation of an inflammatory response upon adipogenic stimulation.

2. Materials and methods

2.1. Cells

ASCs were isolated from the stromal vascular fraction of human lipoaspirates after approval from the Norwegian Regional Ethics Committee (No. S06387a). Cells were cultured in DMEM/F12 (17.5 mM glucose; Gibco) containing 10% fetal calf serum (FCS) [10]. For glucose treatment, ASCs were cultured in DMEM/F12 containing 5 mM D-glucose (prepared by mixing DMEM. No. 11966025 and 21765029; Gibco), 10% FCS and supplemented with D-glucose (Sigma–Aldrich) to 15 or 25 mM. Adipogenic differentiation was as described [11,12]. Briefly, proliferating ASCs were plated confluent in DMEM/F12/10% FCS. After 24 h medium was changed to DMEM/F12/10% FCS containing 5, 15 or 25 mM glucose. Adipogenesis was initiated after another 24 h and extended to 21 days.

2.2. Antibodies

Antibodies were anti-H3K4me3 (003-050, Diagenode), anti-H3K9me3 (056-050, Diagenode), anti-H3K27me3 (069-050, Diagenode), and anti-α-tubulin (T5326, Sigma–Aldrich). An irrelevant IgG was used in control ChIPs (12-370, Millipore).

2.3. Western blotting

Cells were lysed in Laemmli buffer and proteins separated by SDS-PAGE and blotted on Immobilon-FL (Millipore). Membranes were blocked with Odyssey blocking buffer (Licor), incubated with antibodies and proteins visualized using IRDye-800-coupled secondary antibodies (Licor) on an Odyssey imaging system.

2.4. Reverse transcription (RT)-qPCR

Total RNA was extracted using GenElute™ Mammalian Total RNA Miniprep Kit (Sigma–Aldrich) and reverse-transcribed (iScript cDNA synthesis kit; Biorad). RT-qPCR was done in triplicates using the LightCycler® 2.0 System (Roche) and indicated primers (Supplementary Table 1). mRNA levels were normalized to *SF3A1* expression by the comparative CT method.

2.5. Expression microarrays

Total RNA was isolated and processed using Illumina TotalPrep RNA Amplification Kit (Ambion) for labeling and hybridization to Illumina HumanWG-6 v3 Expression BeadChips. Data were analyzed and quartile normalized using Illumina's GenomeStudio v2010.1, Gene Expression module v1.6.0. We called transcripts detected with $P \geq 0.05$ as 'absent' and those detected with $P < 0.05$ as 'present'. For analysis of glucose effects, fold expression change cut-off for up- and down-regulated genes was set to 1.5. 'Inflammation response' GO terms were identified by searching GO database 2014-02-01 with AmiGO (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi) for all terms related to GO:0006954. Analysis of enriched GO terms was done using DAVID 6.7 with $P < 0.001$. Microarray data are deposited under NCBI GEO GSE48774.

2.6. Chromatin immunoprecipitation

ChIP was done as described [13]. Briefly, 3×10^6 cells were cross-linked, lysed and sonicated. ChIP was done in duplicates using 2.5 μg antibody. ChIP DNA was analyzed by qPCR on a MyiQ Real-time PCR Detection System as follows: 95 °C 3 min, and 40 cycles of 95 °C 30 s, 60 °C 30 s, and 72 °C 30 s. Primers (Supplementary Table 2) were designed to amplify promoter regions based on HG18 genome assembly.

2.7. Analysis of chromatin states

ChIP-seq datasets of hPTMs used for chromatin state modeling were obtained from a previous study [14] using a similar source of ASCs and differentiation protocol. hPTM and CTCF enrichment data were converted into chromatin states in 200-base pair bins throughout the genome using ChromHMM [15]. Options were selected to learn a 15-state model using the Baum–Welch training algorithm as described [12]. To determine enrichment of gene regions (defined as gene ± 10 kb to accommodate promoter and (putative) enhancer elements, due to the nature of the hPTMs examined) in each chromatin state, we computed the ratio of coverage of a given state (chromatin state (cs)1-15) per base in the gene region normalized to coverage of that chromatin state in the whole genome. Heat maps of chromatin state enrichment per gene region were generated as a percentage of coverage of the gene region normalized by the length of the gene region. Genes with similar chromatin state profiles were clustered by hierarchical clustering using hclust and enrichment of states as variables [12]. The tree was generated using cutree. Scripts were written in Perl or R and plots generated using ggplot2 in R.

2.8. Statistics

Mean values from RT-qPCR and ChIP qPCR experiments were normalized to reference levels (fold change and fold enrichment values, respectively), and one sample t-test was used with $P < 0.05$ (IBM SPSS Statistics v19.0).

3. Results and discussion

3.1. Exposure of ASCs to high glucose alters histone methylation on promoters of inflammatory genes

To get a first appreciation of a glucose effect on expression of genes involved in inflammation responses, we examined transcripts of genes with a connection to hyperglycemia and diabetes, encoding cytokines and chemokines implicated in immune cell chemotaxis. We performed this analysis in human ASCs from three non-obese donors. Purified ASCs were cultured for 21 days (after thawing stocks) in 5, 15 or 25 mM glucose. Genes examined included *RARRES2* (chemerin), *CCL2* (monocyte chemoattractant

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