Research paper

Glucose and TNF enhance expression of TNF and IL1B, and histone H3 acetylation and K4/K36 methylation, in juvenile macrophage cells

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ABSTRACT

Hyperglycemia activates innate leukocytes such as monocytes and induces pro-inflammatory cytokine expression, resulting in increased monocyte adhesion to aortic endothelial cells. In this study, we investigated whether high glucose and/or tumor necrosis factor (TNF) would enhance pro-inflammatory cytokine expression of tumor necrosis factor (TNF) and interleukin (IL)-1β (IL1B) by altering histone modifications in U937, a juvenile macrophage cell line. The mRNA levels of TNF and IL1B in U937 cells were significantly affected by glucose concentration and TNF treatment. Mono-methylated histone H3K4 signals around TNF and IL1B were lower in cells treated with high glucose compared with low glucose. Conversely, tri-methylated histone H3K4 and H3K36 signals were higher in cells treated with high glucose compared with low glucose. TNF treatment of U937 cells cultured in high glucose enhanced histone H3K36 tri-methylation, particularly around the gene regions of TNF and IL1B. Histone acetylation was induced by treatment with TNF in high-glucose medium. The induction of acetylation and tri-methylation of K4 and K36 of histone H3 around TNF and IL1B by treatment with high glucose and/or TNF was positively associated with the induction of these genes in juvenile macrophage U937 cells.

1. Introduction

Dysfunction of innate leukocytes caused by hyperglycemia is a major trigger for the development of these diseases. Hyperglycemia activates monocytes and induces pro-inflammatory cytokine expression, resulting in increased monocyte adhesion to aortic endothelial cells (Srinivasan et al., 2003). The activation of monocytes/macrophages is positively associated with the development of many chronic diseases related to inflammation, including type 2 diabetes and its complications. A previous study demonstrated that high-glucose medium enhanced the lipopolysaccharide (LPS)-inducible expression of pro-inflammatory cytokine tumor necrosis factor (TNF), in the juvenile macrophage cell line THP-1 (Li et al., 2018). In addition, glucose fluctuations increased the production of TNF and interleukin (IL)-1β (IL1B) compared with constant high-glucose medium in these cells. We reported that postprandial hyperglycemia enhanced expression of these genes in peripheral leukocytes, and a reduction in the repeated postprandial hyperglycemia reduced their expression (Tanaka et al., 2009; Mochizuki et al., 2010; Mochizuki et al., 2011). Thus, hyperglycemia, particularly repeated postprandial hyperglycemia in people with impaired glucose tolerance or diabetes, may induce arteriosclerosis-related diseases by activating innate immune system leukocytes and associated expression of pro-inflammatory cytokines. Macrophages in adipose tissue induce a vicious circle of increasing inflammatory macrophage activation and worsening adipocyte dysfunction, including insulin resistance (Thomas and Apovian, 2017). Several studies have shown that an increased number of adipose tissue macrophages is associated with further deterioration of insulin sensitivity (Kanda et al., 2006; Hirasaki et al., 2007). Liver resident macrophages, Kupffer cells, accumulate with obesity. Kupffer cells produce pro-inflammatory cytokines that contribute to the perturbation of liver homeostasis and amplify the hepatic inflammatory response (Jager et al., 2016). Skeletal
muscle is the primary site for glucose disposition. Therefore, muscle insulin resistance has a severe effect on glucose intolerance and T2DM development (Schenk et al., 2008). A clinical study showed an increase in the number of macrophages in skeletal muscle from obese non-diabetic subjects, a finding that correlated positively with the body mass index and negatively with insulin sensitivity (Varma et al., 2009).

A large scale intervention study (UK Prospective Diabetes Study [UKPDS]) reported that strict glycemic control in the earlier stages of type 2 diabetes reduced the incidences of atherosclerosis-related diseases 10 years later (Holman et al., 2008). The UKPDS study indicated that glycemic status was held as an epigenetic memory and therefore would remain for a long period. Epigenetic memory is characterized by the regulation of gene expression, even in macrophages, by directly recruiting transcription complexes onto chromatin (Takeuchi and Akira, 2011). A previous study demonstrated that histone H3 was induced by high-glucose medium and that the modification was maintained for at least 6 days (El-Osta et al., 2008). Histone methylation is one of the most well-characterized post-translational modifications, regulating the activation and repression of transcription. The methylation of histone H3K9 induced heterochromatin structures and suppressed gene expression (Lachner et al., 2001). In contrast, the di- or tri-methylation of histone H3K4 occurred prior to histone acetylation and transcriptional activation, and induced the binding of co-transcriptional factors with histone acetylase activity to chromatin (Wang et al., 2001; Musri et al., 2006). Therefore, H3K4 methylations are thought to be important for transcriptional initiation. The acetylation of histones H3 and H4 is associated with active genes. Histone acetylation relaxes chromatin by changing the charge of the histone octamers and recruiting transcriptional machineries involved with transactivation.

Regarding pro-inflammatory cytokines, chromatin immunoprecipitation (ChIP) analysis indicated that LPS induced phosphorylation and acetylation of histone H3 and the recruitment of NF-κB to TNF in human monocyte-derived dendritic cells (Ghosh et al., 2010). However, it is still unclear whether the induction of pro-inflammatory cytokine genes by high glucose and the inflammatory cytokine TNF is regulated by histone modification. Therefore, in this study, we examined whether the co-administration of high glucose and TNF would alter the expression of IL1B and TNF and promote histone methylation and acetylation of histone H3 around the IL1B and TNF genes in the juvenile macrophage cell line U937.

2. Materials and methods

2.1. Cell culture

U937 cells from the American Type Culture Collection (Rockville, MD, USA) were cultured in Roswell Park Memorial Institute medium (RPMI 1640) containing 20 mM HEPES (pH 7.4), 1% antibiotic-antimycotic mixed solution (Nacalai Tesque, Kyoto, Japan), 1% non-essential amino acids (Invitrogen, Waltham, MA, USA), 2 mM L-glutamine (Invitrogen) with 10% fetal calf serum (FCS) at 37 °C and 5% CO2. The cells were seeded at a density of 1.4 × 10^6 cells/mL in culture plates (Iwaki, Tokyo, Japan) with 5 mM glucose RPMI 1640 for 4 days and divided into four groups: 5 mM or 25 mM glucose with 5 ng/mL human TNF-α (PeproTech, Rocky Hill, NJ) or vehicle (0.1% bovine serum albumin). Low glucose (5 mM) and high glucose (25 mM) are equivalent to 90 mg/dL (corresponding to a fasting blood glucose concentration in healthy subjects) and 450 mg/dL (corresponding to a non-fasting blood glucose concentration in diabetic subjects without drug therapy), respectively. Previous studies have demonstrated that TNF in the medium is secreted from primary macrophages at a concentration of 2-5 ng/mL (Dominguez-Gutierrez et al., 2018; Sampaio et al., 2018). Therefore, we used 5 ng/mL of TNF as the physiological concentration. After 16 h of culture in each medium, the cells were collected for analysis. A previous study showed that 24 h of TNF stimulation after 3 days of glucose pre-treatment enhanced the mRNA expression of IL1B in U937 cells (Fujimoto et al., 2010). In this study, we chose a shorter period because we sought to examine the earlier effects of co-treatment with high glucose and TNF.

2.2. Quantitative reverse transcription (qRT)-PCR

Total RNA was extracted by the acidified guanidine thiocyanate method (Chomczynski and Sacchi, 2006). Total RNA samples were converted into cDNA by reverse transcription using SuperScript™ III reverse transcriptase (Invitrogen) according to the manufacturer’s instructions. To quantitatively estimate the mRNA levels of TNF and IL1B, PCR amplification was performed using a Light-Cycler instrument (Roche Diagnostics, Basel, Switzerland). PCR primers used were: forward, 5'-CACGCTCTTCTCCTTCTCGAT-3' and reverse, 5'-GCCAGAGGGCTGATTAGAGA-3' for TNF; and forward, 5'-CTGCTGCGGTGTGAAGA-3' and reverse, 5'-TGGGTAATTTCGAGGATCTACA-3' for IL1B.

2.3. ChIP assay

Cells were incubated in fixation solution (1% formaldehyde, 4.5 mM HEPES pH 8.0, 9 mM NaCl, 0.09 mM EDTA, 0.04 mM ethylene glycol tetra-acetic acid) in 10% FCS/RPMI for 30 min at 37 °C. Reactions were terminated by adding glycin e to a final concentration of 150 mM. After washing in FACS solution (phosphate-buffered saline PBS, 2% bovine serum, 0.05% NaN3), the samples were sonicated in sodium dodecyl sulfate (SDS) lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1% SDS, 0.5 mM phenylmethylene sulfonylfluoride) to generate DNA fragments of 200–500 bp. The ChIP assay was performed as previously (Inamochi et al., 2014), using antibodies (0.4 μg each) against mono-methylated histone H3K4 (Abcam, London, UK), di-methylated histone H3K4 (Abcam), tri-methylated histone H3K4 (Merck Millipore, Burlington, MA, USA), mono-methylated H3K36 (Abcam), di-methylated histone H3K36 (Abcam), tri-methylated histone H3K36 (Abcam) and acetylated histone H3 (Abcam). The precipitated DNA was subjected to real-time PCR using primers corresponding to the indicated sites in the promoter/enhancer and gene body regions. The cycle threshold (CT) values of the ChIP signals detected by real-time PCR were converted to percentages of the signal for the input DNA using the delta-delta method (Livak and Schmittgen, 2001), with the formula 100 × [(CT input − CT IP sample)]. The primer sequences used in ChIP assays are listed in Supplemental Table 1.

2.4. Statistical analysis

The results are expressed as means ± standard error of the mean. Statistical analysis was performed using two-way analysis of variance to assess the interaction between glucose concentration and TNF treatment, followed by the Tukey’s test when appropriate. A value of p < 0.05 was considered statistically significant.

3. Results

3.1. Incubation of U937 cells with TNF under high-glucose conditions enhances the mRNA expression of pro-inflammatory cytokines

We determined the TNF and IL1B mRNA levels in U937 cells cultured in low (5 mM)- or high (25 mM)-glucose medium with or without 5 ng/mL TNF. The mRNA expression of TNF was significantly affected by glucose concentration (F = 32.67, p < 0.0001) and TNF treatment (F = 20.23, p = 0.0002), and there was interaction between the two (F = 6.241, p = 0.0213). The mRNA expression of IL1B was also significantly affected by glucose concentration (F = 58.32, p < 0.0001) and TNF treatment (F = 12.59, p = 0.0020), but without an interaction (F = 0.884, p = 0.3584) (Fig. 1).
3.2. Incubation with TNF under high-glucose conditions alters histone H3K4 methylation around TNF and IL1B

We performed ChIP assays for regions around TNF and IL1B using antibodies targeting methylated histone H3K4 in U937 cells. The mono-methylated histone H3K4 signals around TNF and IL1B were lower in cells cultured with high-glucose medium than in those cultured with low-glucose medium (Fig. 2). As shown in Table S2, mono-methylated histone H3K4 levels were significantly affected by glucose concentration in all regions measured. Treatment with TNF increased the mono-/di-methylated histone H3K4 signals from the promoter to the gene body region of TNF and IL1B. The simple main effect was that TNF treatment significantly increased the mono-/di-methylated histone H3K4 signals, especially under low-glucose culture conditions (Fig. 2). Interaction effects between glucose and TNF in the di-methylated histone H3K4 were observed in the upstream region (−400 bp) of TNF and in the gene body region (+5000 bp) of IL1B (Table S3). The tri-methylated histone H3K4 signals around TNF and IL1B were higher in cells cultured with high-glucose medium with TNF. As shown in Table S4, these signals on the gene body regions of TNF and IL1B were affected by glucose concentration and TNF treatment but showed no interaction. The tri-methylated histone H3K4 signals on the promoter/enhancer and downstream regions of TNF and IL1B were similar to those for mono-di-methylation, and increased with TNF treatment under low-glucose culture conditions (Fig. 2). Interaction effects in the tri-methylated histone H3K4 between glucose and TNF were observed in the upstream regions (−400 bp in TNF and −500 bp in IL1B) but not in the gene body regions of these genes (Table S3).

3.3. Incubation with TNF under high-glucose conditions alters histone H3K36 methylation around the TNF and IL1B genes

As shown in Table S5, the mono-methylated histone H3K36 signals around IL1B, with the exception of a region 5000 bp from the transcription start site, were affected by TNF treatment. The di-methylated histone H3K36 signals around IL1B were likely to be increased by TNF treatment and decreased in low-glucose medium (Fig. 3), but showed no significant interaction (Table S6). The tri-methylated histone H3K36 signals in the gene body regions of TNF and IL1B were highly abundant (Fig. 3). The tri-methylated histone H3K36 signals around TNF (+1000 and +2000 bp) and IL1B (+5000 bp) were significantly affected by glucose concentration and TNF treatment (Table S7), and were highest in cells cultured with high-glucose medium with TNF (Fig. 3). Interaction effects in the tri-methylated histone H3K36 were observed in the upstream region (−1000 bp) of TNF (Table S7).

3.4. Incubation with TNF under high-glucose conditions alters histone H3 acetylation around TNF and IL1B

The acetylated histone H3 signals around TNF (−1000, −400, +1000, +2000 and +2000 bp) and IL1B (−1000, +1000, +2000 and +5000 bp) were significantly affected by TNF treatment (Table S8). The simple main effect was that the acetylated histone H3 signals on the gene body region of TNF (+1000 bp) were significantly increased by co-treatment with TNF in high-glucose medium (Fig. 4). Interaction effects in the tri-methylated histone H3K36 were observed in the gene body region (+1000 bp) of TNF (Table S8).

4. Discussion

The production of TNF and IL1B is enhanced in the circulating blood of Type 1 diabetes subjects (Fatima et al., 2016). These pro-inflammatory cytokines induce insulin resistance and inflammation in many tissues (Akash et al., 2018). It was reported that TNF produced by monocytes enhanced IL1B production by an autocrine feedback loop (Gane et al., 2016). Therefore, we considered that TNF would enhance the production of pro-inflammatory cytokines including TNF and IL1B induced by high-glucose medium. In this study, we co-treated U937 cells with high-glucose medium and TNF, a diabetic factor. We found that treatment for 16 h with high glucose or high glucose plus TNF enhanced the mRNA expression of TNF and IL1B in U937 cells. Previous studies demonstrated that high (25 mM) glucose treatment for 24 h increased the release of TNF from the juvenile macrophage cell line THP-1 (Cordero-Herrera et al., 2017). Further studies should be examined to determine whether the protein expression levels of TNF and IL1B in U937 cells are enhanced by co-treatment with high glucose and TNF. Treatment of mouse primary macrophages with high glucose enhanced the mRNA expression of TNF and protein concentration in the culture medium (Pan et al., 2012). In addition, high-glucose stimulation of primary macrophages, which are isolated from buffy coat samples of healthy subjects, induced the expression of M1 macrophage cell surface markers such as CD11c, but no significant differences were observed in the secretion level of TNF between cells with and without high glucose treatment (Torres-Castro et al., 2016). The results concerning primary macrophages in previous studies correspond to the results in U937 cells in this study. We need to examine whether co-administration with high glucose and TNF enhances the expression of IL1B and TNF in primary macrophages and alters histone modifications of IL1B and TNF in primary macrophages. Interestingly, histone H3 acetylation and H3K4 trimethylation of TNF in the gene body regions and mRNA expression in TNF-treated U937 cells were dramatically increased by co-incubation with high glucose. In addition, an association was observed between glucose and TNF following the induction of histone H3 acetylation and...
mRNA. These results indicate that TNF expression could be coordinately enhanced by TNF and glucose. The incidence of complications such as macrovascular and microvascular diseases is higher in type 2 diabetes than in type 1 diabetes (Karabouta et al., 2008) and may be caused by the induction of both TNF and glucose in type 2 diabetes.

In this study, incubation with high glucose reduced the mono-methylation of histone H3K4 and enhanced broad tri-methylation around TNF and IL1B in U937 cells. In addition, tri-methylation of H3K36 and histone acetylation around TNF and IL1B in U937 cells were enhanced by co-administration of high glucose and TNF. In particular, the enhanced modifications occurred in the gene body regions of TNF and IL1B. A recent study reported that di-/tri-methylation of histone H3K4 instead of histone H3K27/36 around the IL6 and TNF genes was increased in LPS-stimulated macrophages (Xu et al., 2015). However, epigenetic changes around inflammation-related genes were only observed in the promoter/enhancer regions. Our study is the first to report histone H3 acetylation and H3K36 tri-methylation in the gene body region, rather than the promoter/enhancer region, that was enhanced by TNF in high-glucose medium. Recent studies demonstrated that histone acetylation in the gene body region enhanced the transcriptional elongation step by recruiting positive transcription elongation factor b (P-TEFb) (Qi et al., 2011). In addition, histone H3K36 di-/tri-methylations terminate excessive transcriptional elongation reactions by inducing DNA methylation in the gene body region (Krogan et al., 2003). Furthermore, histone H3K4 di-/tri-methylations recruited chromo-ATPase/helicase-DNA binding domain 1 (CHD1) in the SWI/SNF complex, whereby CHD1 relaxes chromatin including the gene body (Pray-Grant et al., 2005). These results indicate that treatment
with high glucose and TNF might enhance mRNA transcriptional elongation efficacy by increasing acetylation and tri-methylation at K4 of histone H3. In addition, the induction of H3K36 methylation by co-treatment with TNF and high glucose may be related to repression of the excessive induction of TNF and IL1B mRNA in U937 cells. Whether transcriptional elongation efficacy and recruitment of proteins related to transcriptional elongations including P-TEFb and CHD1 were altered by this co-treatment should be examined in future studies.

In this study, we examined interactions between glucose and TNF on mRNA expression and histone modifications. We detected interactions in di-/tri-methylation of histone H3K4 and di-methylation of histone H3K36 around the upstream (promoter/enhancer) region and acetylation of histone H3 in the gene region. There were no interactions between glucose and TNF on the TNF and IL1B mRNAs, nor were there any interactions observed for most of the histone modification positions on TNF and IL1B. Therefore, we propose that glucose and TNF independently affect expression of TNF and IL1B and the modifications of histones around the genes. Why interactions were observed in the di-/tri-methylation of histone H3K4 and the di-methylation of histone H3K36 it should be examined. A recent study demonstrated that the induction of IL1B and TNF by titanium in the mouse macrophage cell line RAW264.7 was inhibited by (+)-JQ1 (Torres-Castro et al., 2016), which is an inhibitor of acetyl-lysine recognition motifs, or bromodomains (Filippakopoulos et al., 2010). It was reported that histone H3 K4/K36 di-/tri-methylation is detected in active chromatin similar to histone H3 acetylation (Musri et al., 2006; Yoh et al., 2008). Histone acetylation and H3K4 methylation in gene body regions enhance transcriptional elongation, whereas H3K36 methylation terminates

Fig. 3. Effects of stimulation with high glucose and tumor necrosis factor (TNF) on histone H3K36 methylation around TNF and IL1B in U937 cells. Data are expressed as means ± standard error of the mean for 4–6 plates per group. Statistical analysis was performed using two-way ANOVA to assess the association between the glucose concentration and TNF treatment, followed by Tukey’s test when an association was observed. *p < 0.05, significant difference between low glucose and high glucose groups within the same treatment. #p < 0.05, significant difference between the bovine serum albumin (BSA) and TNF groups within the same glucose concentration.
excessive transcriptional elongation caused by histone acetylation (Baubec et al., 2015). Therefore, enhanced histone H3 acetylation and K4/K36 methylation by treatment with high glucose and TNF in U937 cells are likely related to excess mRNA levels of IL1B and TNF. We should examine whether (+)-JQ1 treatment reduces the expression of IL1B and TNF in U937 cells treated with high glucose and TNF. In addition, we should examine whether the induction of histone H3 acetylation and/or H4/K36 methylation of IL1B or TNF by the introduction of histone acetyl-transferases or histone deacetylases using the CRISPR-Cas9 system (Liao et al., 2017) alters the histone modification of IL1B and TNF, as well as their mRNA expression in U937 cells.

Incubation with high glucose reduced the mono-methylation of histone H3K4 levels around TNF and IL1B, but treatment of TNF tended to increase that. Given that mono-methylation of histone H3K4 decreased and tri-methylation of histone H3K4 increased, the change in mono-methylation level might have been due to different effects of glucose and TNF on U937 cells. If so, this would indicate that the pro-inflammatory effects of high glucose and TNF stimulation are not exerted through the same signaling pathway. High-glucose conditions and TNF stimulation increased not only mRNA expression of IL1B and TNF but also histone H3 acetylation around TNF, and high glucose and TNF were observed to interact. To determine whether their effect is additive or synergistic, further experiments with varying glucose and TNF concentrations will be required. In addition, the duration of the histone modifications around TNF and IL1B in U937 cells treated with high-glucose medium and TNF remain unclear. We found that the gene expression levels of Il1b and Tnf in peripheral leukocytes were increased by sucrose administration to rats with impaired glucose tolerance; these expression levels, but not blood glucose concentrations at fasting, were higher in rats with repeated sucrose administration for 4 days than in those without (Tanaka et al., 2009). These results indicate that information related to repeated hyperglycemia remains as a legacy effect on the expression of pro-inflammatory cytokines at fasting. However, whether the modifications of histones are directly related to these legacy effects on pro-inflammatory cytokine production by repeated hyperglycemia remains unclear. In addition, the duration of the histone modifications in cells treated with high-glucose medium and TNF after TNF is withdrawn and the culture medium is changed to low glucose are unknown.

5. Conclusion

The induction of acetylation and tri-methylation of K4 and K36 of histone H3 on transcribed regions of TNF and IL1B by treatment with high glucose and/or TNF was positively associated with the induction of these genes in U937, a juvenile macrophage cell line.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2020.100034.

Author contribution

Kazue Honma: Writing – Original draft preparation/Reviewing and editing. Chie Machida: Investigation. Kazuki Mochizuki: Writing – Original draft preparation, Investigation. Toshinao Goda: Conceptualization, methodology.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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