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Advanced glycation end products increase carbohydrate responsive element binding protein expression and promote cancer cell proliferation

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ABSTRACT

Diabetic patients have increased levels of advanced glycation end products (AGEs) and the role of AGEs in regulating cancer cell proliferation is unclear. Here, we found that treating colorectal and liver cancer cells with AGEs promoted cell proliferation. AGEs stimulated both the expression and activation of a key transcription factor called carbohydrate responsive element binding protein (ChREBP) which had been shown to promote glycolytic and anabolic activity as well as proliferation of colorectal and liver cancer cells. Using siRNAs or the antagonistic antibody for the receptor for advanced glycation end-products (RAGE) blocked AGEs-induced ChREBP expression or cell proliferation in cancer cells. Suppressing ChREBP expression severely impaired AGEs-induced cancer cell proliferation. Taken together, these results demonstrate that AGEs–RAGE signaling enhances cancer cell proliferation in which AGEs-mediated ChREBP induction plays an important role. These findings may provide new explanation for increased cancer progression in diabetic patients.

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1. Introduction

Patients with diabetes mellitus not only are at significantly higher risk for several forms of cancer such as liver and colorectal cancer, but also have an increased cancer-related mortality (Barone et al., 2008; Coughlin et al., 2004; Giovannucci et al., 2010). Increasing evidence suggests that the metabolic deregulation associated with diabetes, such as hyperglycemia, insulin resistance and hyperinsulinemia, may both increase the incidence of certain types of cancer and promote cancer progression (Sciacca et al., 2013; Shikata et al., 2013). Moreover, some anti-diabetic agents such as metformin may reduce cancer incidence and cancer-related mortality in multiple types of cancer (Hardie, 2013). Cancer progression is a complex process which involves cell proliferation, angiogenesis, invasion, and metastasis (Hanahan and Weinberg, 2011). In this paper, we aim to explore new mechanisms by which metabolic abnormalities in diabetic patients contribute to cancer cell proliferation during cancer progression.

Advanced glycation end products (AGEs) are produced through non-enzymatic irreversible glycation and oxidation of proteins, lipids and nucleic acid (Thorpe and Baynes, 2003). Enhanced formation of AGEs occurs particularly in conditions associated with sustained hyperglycemia such as diabetes mellitus (Bidasee et al., 2004; Brownlee et al., 1988). AGEs alter tissue function through crosslinking intra- and extra-cellular matrix proteins and through binding to their cell surface receptors, such as scavenger receptors types I and II, the receptor for advanced glycation end products (RAGE), oligosaccharyl transferase-48 (OST-48), 80K-H phosphoprotein and galectin-3 (Ott

Abbreviations: DM, diabetes mellitus; AGEs, advanced glycation end products; RAGE, receptor for advanced glycation end-products; ChREBP, carbohydrate responsive element binding protein; bHLH-LZ, basic helix-loop-helix leucine zipper; HMGB1, high mobility group box-1 protein; siRNA, small interfering RNA.

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et al., 2014; Thornalley, 1998). Among different types of receptors for AGEs, RAGE is a multiligand receptor of the immunoglobulin superfamily (Kent et al., 1985; Sorci et al., 2013; Wu et al., 2002). Binding of AGEs to RAGE results in the activation of multiple downstream signaling pathways such as the Jak/Stat, MAPK and NF-κB pathways which are involved in proliferation, inflammation, differentiation and motility (Ott et al., 2014; Xie et al., 2013; You et al., 2010). The signaling pathways activated by AGEs binding to RAGE and functional consequences are mainly dependent on the tissue or cell type (Sorci et al., 2013). In addition to AGEs, other signaling molecules such as high mobility group box-1 protein (HMGB1), S100/calgranulins, and β-amyloid can bind to RAGE and activate various downstream signaling pathways (Ott et al., 2014; Xie et al., 2013; You et al., 2010). Recent studies suggest that RAGE signaling plays an important role in liver and colorectal tumor progression (Liang et al., 2011; Pusterla et al., 2013). Moreover, AGEs may promote cancer cell proliferation through activating the RAGE signaling (Abe et al., 2004; Ishibashi et al., 2013; Kim et al., 2008). However, the underlying mechanism remains unclear.

Carbohydrate responsive element binding protein (ChREBP) is a newly identified transcriptional regulator for metabolism and proliferation in colorectal and liver cancer cells (Tong et al., 2009). The basic helix-loop-helix leucine zipper (bHLH-LZ) family transcription factor ChREBP was discovered as an important mediator of glucose-dependent induction of glycolytic and lipogenic enzyme genes in liver and adipose tissue (Billin and Ayer, 2006; Collier et al., 2007; da Silva Xavier et al., 2006; Dentin et al., 2004; Wang and Wollheim, 2002; Yamashita et al., 2001). The level of nutrients (such as glucose and fatty acid) regulates the level and activity of ChREBP in hepatocytes and adipocytes (He et al., 2004; Kawaguchi et al., 2002). The regulation of ChREBP by glucose occurs at multiple levels. Firstly, high glucose levels stimulate ChREBP gene expression (Kabashima et al., 2003). Secondly, ChREBP, which is normally localized in the cytosol, rapidly translocates to the nucleus under high glucose concentration (Davies et al., 2008). Thirdly, the DNA-binding activity of ChREBP is induced by glucose-dependent regulation (Iizuka et al., 2004). In addition to its function in regulating metabolism in normal cells, ChREBP is also required for cell proliferation in HCT116 colorectal cancer cells and HepG2 hepatoblastoma cells and plays a critical role in directing glucose metabolism into anabolic pathways such as lipid and nucleotide biosynthesis during cancer cell growth (Tong et al., 2009).

The present study was designed to investigate the role and underlying mechanism of AGEs in cancer cell proliferation. We found that the AGEs–RAGE signaling pathway induced cancer cell proliferation partly through increasing the expression and activity of ChREBP. Our findings may provide new clues for the treatment of certain types of cancer in diabetic patients *via* targeting ChREBP activity.

2. Materials and methods

2.1. Materials

CellTiter 96® aqueous one solution cell proliferation assay kit was provided by Promega Corporation (USA). Protease inhibitor cocktail tablets (EDTA-free) were from Roche (Switzerland). Dulbecco's Modified Eagle's Medium (DMEM) was from Hyclone (USA). DMEM with no glucose and Opti-MEM were from Invitrogen (USA). Fetal bovine serum (FBS) was from Biochrom (Germany). Human HMGB1 was from PROSPEC (Israel). Mouse serum IgG was from Sigma (USA). All other chemicals were analytical grade. The following primary antibodies were used: anti-ChREBP (Novus, USA), anti-RAGE (Santa Cruz, USA), anti-RAGE (Abcam, UK), anti-PARP (Invitrogen, USA), anti-tubulin (Sigma, USA); anti-phospho-NF-κB p65 (Ser468) (Cell Signaling Technology, USA), anti-NF-κB p65 (Cell Signaling Technology, USA); anti-phospho-Stat3 (Tyr705) (Cell Signaling Technology, USA); anti-Stat3 (Cell Signaling Technology, USA), anti-phospho-p38 MAPK (Thr180/Tyr182) (Cell Signaling Technology, USA), anti-p38 (Santa Cruz, USA), anti-β-catenin (Cell Signaling Technology, USA) and RAGE-blocking (R&D Systems, UK) antibodies.

2.2. Cell culture

HCT116 colorectal cancer cells and HepG2 hepatoblastoma cells were cultured in DMEM supplemented with 10% FBS, 25 mmol/l glucose, 10 mmol/l HEPES, 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, 50 μmol/l β-mercaptoethanol, 100-unit penicillin/ml, and 100 μg streptomycin/ml at 37 °C in humidified 5% CO_2 atmosphere. This general culture medium is the same as 25 mmol/l glucose medium. Similar culture conditions were used for HEK293T cells, a human embryonic kidney cell line. 0 mmol/l glucose medium contains 0 mmol/l glucose DMEM supplemented with 10% FBS, 2 mmol/l L-glutamine, 100-unit penicillin/ml, and 100 g/ml streptomycin. 5.6 mmol/l glucose medium contains 0 mmol/l glucose DMEM supplemented with 5.6 mmol/l glucose, 10% FBS, 2 mmol/l L-glutamine, 100-unit penicillin/ml, and 100 g/ml streptomycin.

2.3. Preparation of AGEs

AGEs were prepared as described previously (Takeuchi et al., 2001). Briefly, 50 mg/ml BSA (Sigma, USA) was incubated under sterile conditions with 0.5 mmol/l D-glucose (Sigma, USA) for 8 weeks. The unincorporated sugar was removed by dialysis against 0.2 mmol/l PBS (pH 7.4). Nonglycated BSA was incubated under the same conditions except for the absence of D-glucose as a negative control. Preparations were tested for endotoxin using a Limulus Amebocyte Lysate (ACC, USA), and endotoxin was less than 15 EU/l.

2.4. Cell proliferation and cell viability

Cells were plated at 5×10^4 cells/well in 24-well plates and cultured for 24 h. Then, cells were cultured in no glucose DMEM supplemented with 10% FBS and 5.6 mmol/l glucose. AGEs or BSA was added to the medium and culture was continued for another 4 days for HCT116 cells or 6 days for HepG2 cells. Cell proliferation was determined by counting cells. The cell number was counted at days 1, 2, 3 and 4 for HCT116 cells or at days 2, 4 and 6 for HepG2 cells. In order to serum starve cells before AGEs treatment, HCT116 cells were cultured for 24 hours in DMEM supplemented with 0.1% FBS and 5.6 mmol/l glucose followed by AGEs or BSA treatment.

For cell cycle analysis, HCT116 cells were cultured for 24 hours in DMEM supplemented with 0.1% FBS and 5.6 mmol/l glucose followed by 24 hour AGEs or BSA treatment. 10 μM BrdU was added to the culture medium. 1 hour later, cells were fixed, permeabilized and treated with DNase I using the FITC BrdU Flow Kit (BD Pharmingen, USA). Cell cycle analysis was performed by flow cytometry using the BrdU APC Flow Kit (BD Pharmingen, USA) according to the manufacturer's instructions and analyzed using ModFit LT™ software.

Cell viability was assessed using MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetra zolium] assays. Cells were seeded into 96-well plates at a density of 5×10^3 cells per well in 100 μl for 24 hours. Then, cells were cultured in DMEM supplemented with no glucose or 5.6 mmol/l glucose. AGEs were added to the medium and culture was continued for another 72 hours. CellTiter 96® aqueous one solution cell proliferation assay kit (Promega, USA) was used for the MTS assay. Briefly, 20 μl MTS reagent was added into each well at 24-, 48- and 72-hour time points after AGEs addition. Cells were incubated at 37 °C for an additional 2 hours. Absorbance was detected at 490 nm using a plate reader (Thermo Fisher Scientific, USA). The experiment was

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