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An essential role of cAMP response element-binding protein in epidermal growth factor-mediated induction of sodium/glucose cotransporter 1 gene expression and intestinal glucose uptake

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ABSTRACT

The sodium/glucose cotransporter 1 (SGLT1) is responsible for glucose uptake in intestinal epithelial cells. Its expression is decreased in individuals with intestinal inflammatory disorders and is correlated with the pathogenesis of disease. The aim of this study was to understand the regulatory mechanism of the SGLT1 gene. Using the trinitrobenzene sulfonic acid-induced mouse models of intestinal inflammation, we observed decreased SGLT1 expression in the inflamed intestine was positively correlated with the mucosal level of epidermal growth factor (EGF) and activated CREB. Overexpression of EGF demonstrated that the effect of EGF on intestinal glucose uptake was primarily due to the increased level of SGLT1. We identified an essential cAMP binding element (CRE) confers EGF inducibility in the human SGLT1 gene promoter. ChIP assay further demonstrated the increased binding of CREB and CBP to the SGLT1 gene promoter in EGF-treated cells. In addition, the EGFR- and PI3K-dependent CREB phosphorylations are involved in the EGF-mediated SGLT1 expression. This is the first report to demonstrate that CREB is involved in EGF-mediated transcription regulation of SGLT1 gene in the normal and inflamed intestine, which can provide potential therapeutic applications for intestinal inflammatory disorders.

1. Introduction

Epidermal growth factor (EGF) is an acid- and heat-stable peptide that is resistant to digestion by proteases such as pepsin, trypsin, and chymotrypsin. EGF is produced and secreted from all cells of the gastrointestinal tract, and it stimulates cell proliferation, differentiation, and survival by binding with high affinity to EGF receptors (EGFRs) and activating the receptor tyrosine kinase (RTK) activity of the receptors (Carpenter and Cohen, 1990; Marti et al., 1989; Wright et al., 1990). Activated RTK initiates relevant downstream signaling pathways that result in the modulation of multiple cellular processes (Citri and Yarden, 2006). Previous evidence has indicated that EGF plays a significant role in glucose absorption in the intestinal mucosa (Chung et al., 2002). It has been shown that EGF stimulates intracellular trafficking of pre-formed sodium-dependent glucose transporter 1 (SGLT1) protein in the apical membrane and stabilizes SGLT1 protein through interaction

Abbreviations: SGLT1, Sodium/glucose cotransporter 1; EGF, Epidermal growth factor; CRE, cAMP response element; GLUTs, Facilitative glucose transporters; SGLTs, Sodium-dependent glucose transporters; IECs, Intestinal epithelial cells; EGFRs, EGF receptors; RTK, Receptor tyrosine kinase; CREB, cAMP response element-binding protein; TNBS, Trinitrobenzene sulfonic acid; AMG, D-[^{14}C]-α-methylglucopyranoside; Vmax, Maximum velocity; MPO, Myeloperoxidase; CHX, Cycloheximide; ActD, Actinomycin D; CBP, CREB-binding protein; PI3K, Phosphatidylinositol 3-kinase; Akt, Protein kinase B.

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with EGFR (Chung et al., 2002; Weihua et al., 2008). Although EGF has been identified as a growth factor that can maintain intestinal glucose uptake, little is known regarding the regulatory mechanism of EGF-mediated SGLT1 gene expression in human intestinal cells.

Glucose is the major carbon and energy source for eukaryotic cells. Transport of glucose into mammalian cells is the rate-limiting step for its utilization; accordingly, it is a highly regulated process. Two classes of glucose transporters have been identified in humans: the facilitative glucose transporters (GLUTs) and the SGLTs (Wood and Trayhurn, 2003). SGLT1 is the primary carrier protein responsible for active transport of glucose from the lumen of the intestine across the brush border membrane of intestinal epithelial cells (IECs) (Drozdowski and Thomson, 2006). Once inside the enterocytes, these sugars are either metabolized or diffuse out of the cell through the GLUT2 facilitative transporter, located on the basolateral membrane (Wright et al., 1994). Clinical studies have shown that SGLT1 is essential for intestinal glucose absorption, as individuals with defects in SGLT1 activity exhibit severe intestinal malabsorption syndrome and diarrhea (Martin et al., 2000). In addition to its classical functions, recent evidence has indicated that SGLT1-mediated glucose uptake protects the intestinal epithelium from apoptosis and inflammation induced by microbial products (Huang et al., 2011; Palazzo et al., 2008). Impaired intestinal glucose uptake has been shown in mice and humans with intestinal inflammatory disorders (Kekuda et al., 2008; Musch et al., 2002; Sigalet et al., 2013); however, the regulatory mechanism of SGLT1 gene expression in the inflamed intestine still needs to be elucidated.

Chronic inflammatory disorders in the gastrointestinal tract are caused by multiple biological environmental factors. Among them, non-immune cells play a key role in the pathogenesis of these diseases (Xavier and Podolsky, 2007). IECs maintain intestinal homeostasis by secreting antimicrobial peptides to eliminate bacterial pathogens, differentiating between beneficial and harmful microbes, and communicating with the immune system (Artis, 2008). Previous study indicated that dysregulation of NF-κB signaling specifically in IECs causes the intestinal inflammation in mice (Pasparakis, 2008). In addition, accumulating evidence suggests that increased absorption of nutrients in the gastrointestinal tract can represent a novel therapeutic approach for patients with intestinal inflammatory disorders (Critch et al., 2012; Lucendo and De Rezende, 2009). The SGLT1 protein is expressed primarily in the IECs of the gastrointestinal tract, and its activation results in decreased bacteria-induced inflammatory processes (Palazzo et al., 2008). Thus, in developing a therapeutic strategy for intestinal inflammatory disorders, the role of nutrient-absorptive IECs should be considered.

Because SGLT1 protein is critical for controlling the initial glucose uptake in humans, it is essential to gain clear insight into the regulatory mechanisms of SGLT1 gene expression. The expression of SGLT1 has been shown to be regulated at the levels of transcription and translation (Matosin-Matekalo et al., 1998; Martin et al., 2000), mRNA stability (Loflin and Lever, 2001), and protein stability (Weihua et al., 2008). In this study, we investigated the molecular mechanism underlying EGF-mediated regulation of SGLT1 gene expression using differentiated human intestinal Caco-2 cells and animal models. We identified an essential cAMP response element-binding protein (CREB) binding motif in the human SGLT1 gene promoter that is responsible for EGF-mediated induction of SGLT1 gene expression. In the inflamed intestine of mice, the decreased mucosal EGF level might lead to downregulation of CREB phosphorylation, which in turn could result in reduction of SGLT1 expression, leading to impaired intestinal glucose uptake. Thus, our results provide novel and general insight into the regulatory mechanism of SGLT1 gene expression, which can provide potential therapeutic applications for intestinal inflammatory disorders.

2. Materials and methods

2.1. Experimental animals and induction of intestinal inflammation in mice

Male C57BL/6 mice (BioLASCO Taiwan Co., Ltd, Taipei, Taiwan) were housed in ventilated cages with controlled suitable temperature (22 ± 2 °C, 30%–70% humidity), with the 12-h light/dark cycle at the animal center of National Defense Medical Center. Mice that were 6–8 weeks old with the body weight of 16–25 g were randomly divided into control and trinitrobenzene sulfonic acid (TNBS) (Sigma Chemical, St. Louis, MO, USA) treatment groups. After fasting for 18–24 h with free access to a 5% glucose solution, intestinal inflammation was induced *via* rectal instillation of 100 mg/kg of TNBS, as previously described (Qiu et al., 2011). The mice were anaesthetized with the intraperitoneal injection of pentobarbital (Siegfried AG, Zofingen, Switzerland). TNBS was instilled *via* a polyethylene PE50 catheter, which was carefully inserted into the colon (4 cm proximal to the anus). Control mice were instilled with same volume of 30% ethanol. After TNBS administration, the mice were carefully maintained at a 45° angle (head down position) for 2 min to ensure distribution of TNBS in the colon. After 4 h, the mice were given free access to food and water. After 72 h of TNBS instillation, the mice were euthanized, and tissue samples were removed for experiments. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center (IACUC-11-055), Taipei, Taiwan.

2.2. In vivo *overexpression of EGF*

The effect of EGF *in vivo* was determined by administration of recombinant mouse EGF (PeproTech, Rocky Hill, NJ, USA) (75 ng/g body weight) twice daily, as previously described (Helmrath et al., 1998), with slight modifications. After 24 h, the mice were euthanized for experiments.

2.3. Cell culture

The established human intestinal epithelial cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD). The cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose, 0.584 g/L glutamine, 10% fetal bovine serum, 3.7% sodium bicarbonate, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 1% nonessential amino acids. The cells were left to differentiate for 15–17 days after confluence. The integrity of the Caco-2 cell monolayers were monitored before every experiment by determining the transepithelial electrical resistance (TEER) of filter-grown cell monolayers by use of a commercial apparatus (Millicell ERS; Millipore, Bedford, MA) (Chang et al., 2007).

2.4. Western blot analysis

Caco-2 cells were rinsed with ice-cold PBS and lysed with RIPA buffer (Pierce, Rockford, IL, USA) with 10 mM protease inhibitor cocktail. The cell lysates were collected and centrifuged at 12,000 × *g* for 30 min at 4 °C. Next, the supernatants were harvested and subsequently normalized with the BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amount of protein samples of cell lysate (40 μg) was loaded for SDS-PAGE and subsequently transferred to PVDF paper. Nonspecific binding was blocked with PBST buffer containing 5% milk for 1 h at room temperature followed by overnight hybridization at 4 °C with the indicated primary antibodies: anti-SGLT1, anti-p-EGFR, anti-EGFR, anti-EGF, anti-p-CREB, anti-CREB, and anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with horseradish peroxidase-conjugated