Free Radical Biology and Medicine 68 (2014) 247–257

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Signaling steps in the induction of genomic damage by insulin in colon and kidney cells

Eman Maher Othman [a,b], Henning Hintzsche [a], Helga Stopper [a,*]

[a] Institute of Pharmacology and Toxicology, University of Wuerzburg, D-97078 Wuerzburg, Germany
[b] Department of Analytical Chemistry, Faculty of Pharmacy, University of El-Minia, 61519 El-Minia, Egypt

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form
20 November 2013
Accepted 9 December 2013
Available online 16 December 2013

Keywords:
Diabetes
Insulin
Genotoxicity
Mitochondria
NADPH oxidase
Free radicals

ABSTRACT

Diabetes mellitus (DM), a disease with almost 350 million people affected worldwide, will be the seventh leading cause of death by 2030. Diabetic patients develop various types of complications, among them an increased rate of malignancies. Studies reported the strong correlation between DM and several cancer types, of which colon and kidney cancers are the most common. Hyperinsulinemia, the high insulin blood level characteristic of early diabetes type 2, was identified as a risk factor for cancer development. In previous studies, we showed that an elevated insulin level can induce oxidative stress, resulting in DNA damage in colon cells in vitro and in kidney cells in vitro and in vivo. In the present study, we elucidate the signaling pathway of insulin-mediated genotoxicity, which is effective through oxidative stress induction in colon and kidney. The signaling mechanism is starting by phosphorylation of the insulin and insulin-like growth factor-1 receptors, followed by activation of phosphatidylinositide 3-kinase (PI3K), which in turn activates AKT. Subsequently, mitochondria and nicotinamide adenine dinucleotide phosphate oxidase (NADPH) isoforms (Nox1 and Nox4 in colon and kidney, respectively) are activated for reactive oxygen species (ROS) production, and the resulting excess ROS can attack the DNA, causing DNA oxidation. We conclude that hyperinsulinemia represents an important risk factor for cancer initiation or progression as well as a target for cancer prevention in diabetic patients.

© 2013 Elsevier Inc. All rights reserved.

Diabetes mellitus (DM), a disease with almost 350 million people affected worldwide [1], will be the seventh leading cause of death by 2030 [2]. Diabetic patients develop various types of complications such as nephropathy, cardiovascular problems, and neuropathy. In addition, patients with type 2 diabetes are more likely to develop cancer and to die from it than the general population [3]. Epidemiological studies reported a strong correlation between type 2 DM and various types of cancer such as cancer of the kidney, colon, and liver [4–8]. Studies exploring the relationship between diabetes and cancer yielded the strong hypothesis of hyperinsulinemia, the high insulin blood level characteristic of certain stages and types of diabetes, being a major risk factor [7,9,10]. In previous studies we showed that 5 nM insulin can stimulate reactive oxygen species (ROS) production, causing DNA oxidation and DNA damage in colon and kidney cell lines. The normal morning fasting blood level of insulin in healthy individuals is more than 50-fold below this concentration, but insulin levels after eating and in the state of hyperinsulinemia can be higher than 1 nM and persist throughout the day.

Every day, cells in the human body suffer from thousands of DNA lesions. These lesions can interfere with DNA replication and transcription, and they can lead to gene mutations or wider-scale genome aberrations [11]. DNA damage is also produced by ROS arising as by-products from oxidative respiration or through redox-cycling events involving environmental toxic agents, metals, or endogenous hormones or cellular enzymes such as NADPH oxidases [12–18]. Reactive oxygen and nitrogen species are also produced by macrophages and neutrophils under different conditions [19]. The resulting DNA alterations can be adducts that impair base pairing and/or block DNA replication and transcription, base loss, or DNA single-strand breaks (SSBs). Cellular repair attempts of DNA lesions can also lead to strand breakage. When two SSBs arise close together or when the DNA replication apparatus detects a SSB or certain other lesions, double-strand breaks (DSBs) are formed. Although DSBs do not occur as frequently as the other lesions listed above, they are difficult to repair and extremely toxic [20]. The comet assay, which was used in this study, detects both single- and double-strand breaks. Primary DNA damage can have a variety of effects on the cell and/or the organism. When this damage leads to mutations, gene transcription can be altered or protein activity can be modified. If mutations occur in tumor suppressor genes or oncogenes, the transformation of a normal cell into a cancer cell is made possible, because

* Corresponding author. Fax: +49 931 201 48446.
E-mail address: stopper@toxi.uni-wuerzburg.de (H. Stopper).

0891-5849/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.freeradbiomed.2013.12.010

mutations in these genes allow the cells to resist cell death, enable replicative immortality, and sustain proliferative signaling. Among other effects, these characteristics are considered major hallmarks of cancer [21].

It is known that ROS are important mediators during insulin-dependent cellular signaling [22–24]. One of the suggested signaling mechanisms for insulin is the activation of NADPH-dependent H_2O_2 generation [22]. This seems to be involved in the insulin-mediated ROS production in colon cells [25]. Another source for insulin-dependent ROS formation seem to be mitochondria, which were observed to play a role in ROS production in hepatocytes [26].

We showed before that insulin can induce oxidative stress, which can result in DNA damage in colon and kidney in vitro [17,18], and we used the ZDF rat to study the effects of hyperinsulinemia in vivo; we found that a pathophysiological level of insulin can cause oxidative stress and genomic damage [17]. In this study, we present the mechanistic pathway by which insulin can cause oxidative stress leading to genomic damage in colon and kidney, which in turn may initiate or support progression of carcinogenesis in diabetic patients.

Materials and methods

Chemicals and reagents

Wortmannin and Bisbenzimid H (33258) were purchased from Sigma–Aldrich (Munich, Germany). Human insulin, PPP ((5R,5aS, 8aR,9R)-9-hydroxy-5,8,8a,9-tetrahydro-5-(3,4,5-trimethoxyphenyl) furo[3′,4′:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one) (sc-204008), and Mito-TEMPO (sc-221945) were purchased from Santa Cruz Biotechnology (Heidelberg, Germany); $HNMPA(AM)_3$ (hydroxy-2-naphthalenylmethyl phosphonic acid acetoxymethyl ester) and JC-1 dye were purchased from Enzo Life Sciences (Loerrach, Germany); Fpg enzyme and buffer were purchased from New England Biolabs; dihydroethidium (DHE) was purchased from Merck Biosciences (Schwalbach, Germany). Gel red was purchased from Biotrend (Cologne, Germany). Cell culture media and reagents were obtained from PAA Laboratories (Pasching, Austria) and Invitrogen Life Technologies (Darmstadt, Germany).

Antibodies

Anti-AKT (protein kinase B) pS473(22650) was purchased from Rockland Immunochemicals (Gilbertsville, PA, USA). Anti-p-insulin receptor (IR) (Tyr1150/1151) and anti-p-insulin-like growth factor receptor (IGF-1R) (Tyr1161/1165/1166) were purchased from MerckMillipore (Darmstadt, Germany); anti-β-actin (T6199) was purchased from Sigma–Aldrich; anti-p53 (Pab 240, sc-99) and anti-Nox4 (3187-1) were from Epitomics (Burlingame, CA, USA); anti-Nox1 (sc-25545), anti-Nox2 (sc-130543), and the secondary antibodies (sc-2004, sc-2005, sc-2020) were from Santa Cruz Biotechnology.

Small interfering RNA (siRNA) oligonucleotides

The siRNA oligonucleotides for Nox2 (sc-35503), Nox4 (sc-41586), Nox1 (sc-43939), AKT (sc-29195), and control siRNA (sc-37007) were purchased from Santa Cruz Biotechnology.

Polymerase chain reaction (PCR) primers

Primers for PCR (Table 1) were taken from the literature or designed using the program Primer3 (http://frodo.wi.mit.edu/primer3) and ordered from MWG Biotech (Ebersberg, Germany).

Cell lines and cell culture reagents

HT29, a human colon adenocarcinoma cell line, was cultured at 37 °C, 5% (v/v) CO_2 in Dulbecco's modified Eagle medium (DMEM) with high glucose (4.5 g/L) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (w/v) L-glutamine, and 0.4% (w/v) antibiotics (50 U/ml penicillin, 50 mg/ml streptomycin). They were subcultured twice per week.

HK-2, a human kidney cell line with many properties of proximal tubular cells, was cultured at 37 °C, 5% (v/v) CO_2 in Dulbecco's modified Eagle medium (DMEM) with high glucose (4.5 g/L) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (w/v) l-glutamine, and 0.4% (w/v) antibiotics (50 U/ml penicillin, 50 mg/ml streptomycin). They were subcultured twice per week.

Rho0 cells (cells depleted of mtDNA)

HT29 and HK2 rho0 cells were established by treating cells with low concentration of ethidium bromide (0.4 μg/ml) for 4 weeks, after which the absence of genes that are encoded in mtDNA was confirmed. Cells were cultured at 37 °C, 5% (v/v) CO_2 in DMEM high glucose (4.5 g/L) supplemented with 10% (v/v) FBS, 1% (w/v) L-glutamine, 1% sodium pyruvate, and 50 μg/ml uridine. They were subcultured twice per week.

Table 1
List of the sequences, annealing temperatures, and sizes of the primers used.

Primer	Sequence	Annealing temp. (°C)	Size (bp)
Human insulin receptor [46]	Forward: 5′-AACCAGAGTGAGTATGAGGAT-3′ Reverse: 5′-CCGTTCCAGAGCGAAGTGCTT-3′	58	125
Human insulin-like growth factor 1 receptor	Forward: 5′-AGGGCGTAGTTGTAGAAGAGTTTCC-3′ Reverse: 5′-TACTTGCTGCTGTTCCGAGTGG-3′	58	101
Nox1	Forward: 5′-GGATGATCGTGACTCCCACT-3′ Reverse: 5′-AGGTTGTGGTCTGCACACTG-3′	59.5	458
Nox2	Forward: 5′-TGCAGCCTGCCTGAATTTCAAC-3′ Reverse: 5′-GAGGCACAGCGTGATGACAAC-3′	56	391
Nox4	Forward: 5′-CTGGTGAATGCCCTCAACTT-3′ Reverse: 5′-CTGGCTTATTGCTCCGGATA-3′	52	556
Human small mitoDNA [47]	Forward: 5′-CCCCACAAACCCCATTACTAAACCCA-3′ Reverse: 5′-TTTCATCATGCGGAGATGTTGGATGG-3′	58	220
β-Actin	Forward: 5′-CTCTTCCAGCCTTCCTTCCT-3′ Reverse: 5′-AGCACTGTGTTGGCGTACAG-3′	56	610

For analysis of mitochondrial DNA, QPCR primers were used in a RT-PCR protocol according to the recommendation of Ref. [46].

Download English Version:

https://daneshyari.com/en/article/8270964

Download Persian Version:

https://daneshyari.com/article/8270964

Daneshyari.com