EXPERIMENTAL CELL RESEARCH 337 (2015) 146–159

Available online at www.sciencedirect.com

ScienceDirect

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

Research Article

Heme oxygenase-1 enhances autophagy in podocytes as a protective mechanism against high glucose-induced apoptosis

Chenglong Dong[a,1], *Haining Zheng*[b,1], *Shanshan Huang*[a], *Na You*[a], *Jiarong Xu*[a], *Xiaolong Ye*[a], *Qun Zhu*[a], *Yamin Feng*[a], *Qiang You*[a], *Heng Miao*[a], *Dafa Ding*[a,*], *Yibing Lu*[a,*]

[a]*Department of Endocrinology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing, China*
[b]*Department of Hyperbaric Oxygen, Nanjing General Hospital of Nanjing Military Command, Nanjing, China*

ARTICLE INFORMATION

Article Chronology:
Received 11 October 2014
Received in revised form
2 April 2015
Accepted 4 April 2015
Available online 13 April 2015

Keywords:
Autophagy
Heme oxygenase-1
Podocyte
Apoptosis
High glucose

ABSTRACT

Injury and loss of podocytes play vital roles in diabetic nephropathy progression. Emerging evidence suggests autophagy, which is induced by multiple stressors including hyperglycemia, plays a protective role. Meanwhile, heme oxygenase-1 (HO-1) possesses powerful anti-apoptotic properties. Therefore, we investigated the impact of autophagy on podocyte apoptosis under diabetic conditions and its association with HO-1. Mouse podocytes were cultured in vitro; apoptosis was detected by flow cytometry. Transmission electron microscopy and biochemical autophagic flux assays were used to measure the autophagy markers microtubule-associated protein 1 light chain 3-II (LC3-II) and beclin-1. LC3-II and beclin-1 expression peaked 12–24 h after exposing podocytes to high glucose. Inhibition of autophagy with 3-methyladenine or Beclin-1 siRNAs or Atg 5 siRNAs sensitized cells to apoptosis, suggesting autophagy is a survival mechanism. HO-1 inactivation inhibited autophagy, which aggravated podocyte injury in vitro. Hemin-induced autophagy also protected podocytes from hyperglycemia in vitro and was abrogated by HO-1 siRNA. Adenosine monophosphate-activated protein kinase phosphorylation was higher in hemin-treated and lower in HO-1 siRNA-treated podocytes. Suppression of AMPK activity reversed HO-1-mediated Beclin-1 upregulation and autophagy, indicating HO-1-mediated autophagy is AMPK dependent. These findings suggest HO-1 induction and regulation of autophagy are potential therapeutic targets for diabetic nephropathy.

© 2015 Elsevier Inc. All rights reserved.

Abbreviations: 3-MA, 3-methyladenine; AMPK, adenosine monophosphate-activated protein kinase; CIMP, conditionally immortalized mouse podocyte; DN, diabetic nephropathy; HG, high glucose; HO-1, heme oxygenase-1; mTOR, mammalian target of rapamycin; NG, normal glucose

*Corresponding authors. Fax: +86 25 5850 9990.
E-mail addresses: dingdafa2004@aliyun.com (D. Ding), luyibing2004@126.com (Y. Lu).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.yexcr.2015.04.005
0014-4827/© 2015 Elsevier Inc. All rights reserved.

Introduction

In humans, diabetic nephropathy (DN) is characterized by increased urinary albumin excretion (microalbuminuria); this often progresses to proteinuria, which is one of the most important prognostic risk factors for kidney disease progression [1]. Studies in humans and DN animal models reveal podocyte injury, detachment, apoptosis, and loss [2–4]. Although some stimuli induce podocyte necrosis in vitro, more in vivo evidence suggests podocyte loss in DN is associated with apoptosis [5,6]. More specifically, podocyte apoptosis coincides with albuminuria onset and precedes podocytopenia in different mouse models of diabetes. Although these observations identify podocyte apoptosis as one of the earliest cellular features of diabetic kidney disease, the mechanisms underlying podocyte loss in DN remain poorly understood. Thus, investigating the mechanisms of podocyte apoptosis in diabetic conditions will be critical for the development of novel preventive and therapeutic approaches to DN.

Podocytes, derived from embryonic precursor mesenchymal cells, are terminally differentiated cells in the mature kidneys [7]. As differentiated neuron-like epithelial cells, podocytes have limited capacity for cell division and replenishment. Therefore, maintaining homeostasis under certain pathophysiological stresses is an important determinant of podocyte fate. Autophagy was recently implicated in the pathogenesis of podocyte injury and depletion [8]; this finding has generated substantial interest in the field of kidney research.

Autophagy is a normal cellular process involving the removal and recycling of bulk cytoplasmic constituents, misfolded proteins, and damaged intracellular organelles to maintain cellular homeostasis [9–11]. Cells generally exhibit a low basal rate of autophagy; however, the rate can increase in response to nutrient or growth factor deprivation in order to replenish amino acids and glucose for cellular functions. Therefore, autophagy has a pro-survival function; however, this “rescue” function can prove deleterious under certain circumstances. The effects of autophagy in podocytes have recently garnered substantial attention. Studies suggest autophagy is a key mechanism in the maintenance of homeostasis and integrity in podocytes. Hartleben et al. [8] report autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. However, although several recent papers suggest autophagy is involved in the pathogenesis of DN [12–14], the exact roles of autophagy in podocyte apoptosis and the mechanisms underlying diabetic conditions remain to be elucidated.

Heme oxygenase-1 (HO-1) is a prominent stress protein and multifunctional microsomal enzyme that regulates several biological responses including chemotaxis, cell cycle progression, differentiation, and apoptosis in target cells in a context- and cell-specific manner [15]. HO-1 inhibits apoptosis through several distinct mechanisms [16]: (1) through the adenosine monophosphate-activated protein kinase (AMPK) and the p38 mitogen-activated protein kinase signal transduction pathways; (2) by inducing the expressions of anti-apoptotic genes such as *Bcl-x_L* and *Bcl-2*, and by inhibiting the expressions of pro-apoptotic genes such as *Bax* and *Bak*; and (3) through its enzymatic activity. While most studies have focused on the anti-apoptotic role of HO-1, the regulation of autophagy by this enzyme is also of interest. Recent studies show that in addition to apoptosis, autophagy plays an essential protective role in kidney disease [17,18]. Furthermore, induction of HO-1 expression in podocytes may protect against apoptosis under diabetes conditions [19]. However, it is unclear whether autophagy is the mechanism by which HO-1 protection against apoptosis is mediated in podocytes under diabetes conditions.

This study examined the role of autophagy in high glucose-induced apoptosis in primary cultures of podocytes. We also attempted to determine whether HO-1 can inhibit high glucose-induced apoptosis and promote cell survival via autophagy activation. Finally, we investigated the molecular mechanisms by which HO-1 regulates the autophagic pathway.

Materials and methods

Cell culture and treatment

The conditionally immortalized mouse podocyte (CIMP) cell line was kindly provided by Dr. Zhihong Liu (Research Institute of Nephrology, Nanjing General Hospital of Nanjing Military Command, Nanjing, Jiangsu, China), while their cell lines were kindly provided by Dr. Peter Mundel (Mount Sinai School of Medicine, New York, NY, USA) [20]. Cells were cultured at the permissive temperature (33 °C) in RPMI-1640 medium supplemented with 10% fetal bovine serum (Invitrogen, Grand Island, NY, USA) and recombinant interferon-γ (Invitrogen, Carlsbad, CA, USA). To induce differentiation, podocytes were grown under non-permissive conditions at 37 °C for 10–14 days in the absence of interferon-γ. After serum starvation for 16 h, the cells were exposed to the indicated high-glucose conditions, i.e., concentration or time.

Induction of autophagy/apoptosis in vitro

To induce autophagy/apoptosis, cells were incubated with high glucose at concentrations ranging from 5.6–30 mmol/L for 0–48 h. In initial studies, the ideal exposure time and concentration of glucose was determined by podocyte viability and western blotting. Autophagy inhibitors 3-methyladenine (3-MA, 10 mmol/L, Sigma, St. Louis, MO, USA) or Beclin-1 siRNA (100 nmol/L, Shanghai, China) were added directly to cultures before cells were exposed to high glucose. Atg5 siRNA and the negative control siRNA were purchased from GenePharma (Shanghai, China). Cells were transfected with Atg5 siRNA or the negative control siRNA using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). After transfection for 6 h, fresh RPMI-1640 medium containing 10% fetal bovine serum was added. The cells were cultured for 24 h and subsequently treated with high glucose for another 24 h. The siRNA efficiency was then verified by western blotting.

Evaluation of autophagy by electron microscopy

CIMPs cultured at normal glucose (NG, 5.6 mM) and high glucose (HG, 30 mM) were washed and fixed with 2% glutaraldehyde buffered with 0.05 M Na cacodylate (pH 7.3). After fixation, the cells were prepared for electron microscopy (JEM-1010, Japan), and photographs were taken with a TEM CCD Camera (Tokyo, Japan).

Download English Version:

https://daneshyari.com/en/article/2130128

Download Persian Version:

https://daneshyari.com/article/2130128

Daneshyari.com