Original Article

Decorin protects human hepatoma HepG2 cells against oxygen-glucose deprivation via modulating autophagy

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Abstract: This study is to investigate the effects of decorin (DCN) on human hepatoma HepG2 cells under oxygen-glucose deprivation (OGD) condition. HepG2 cells were cultured under OGD condition. CCK-8 assay was used to assess the cell survival, and flow cytometry was performed to detect the apoptosis. Protein expression levels were detected with Western blot analysis. Transfection was performed with liposome, and cells were screened with G418. The cell survival rates were significantly decreased in the OGD groups. When treated with autophagy inhibitor 3-MA, the survival rates were further declined in these cells. Moreover, flow cytometry indicated that apoptosis occurred in the HepG2 cells under OGD condition, and the apoptosis rates were significantly increased by the 3-MA treatment. Western blot analysis showed that, the expression levels of DCN were significantly elevated in OGD-preconditioned HepG2 cells. Meanwhile, the expression level of Beclin1 and the LC3BII/LC3BI ratio were significantly increased, while the expression level of P62 was significantly decreased, in HepG2 cells under OGD condition. Over-expression of DCN significantly increased the expression level of Beclin1 and the LC3BII/LC3BI ratio, while no significant changes were observed in the P62 expression level, in HepG2 cells. Under the OGD condition, the apoptosis rate was also significantly decreased in DCN-transfected HepG2 cells. DCN protects HepG2 cells against OGD-induced injury, via regulating autophagy. These results might contribute to a better understanding of the roles of DCN and autophagy in hepatocellular carcinoma, and the potential treatment for the disease.

Keywords: HepG2 cells, decorin (DCN), oxygen-glucose deprivation (OGD), autophagy

Introduction

Hepatocellular carcinoma (HCC) is one of the common malignant tumors in clinic, and the disease mortality ranks in the third place worldwide [1]. At present, surgical resection is still the major clinical treatment option for HCC. However, the postoperative survival rate for HCC is not satisfactory, due to the subtle nature of the disease and the invasion and metastasis [2]. In the rapid proliferation of HCC, the consumption of the environmental glucose and oxygen will by readily increased, leading to local oxygen-glucose deprivation (OGD) and finally inducing invasion and metastasis [3]. In recent years, autophagy is related to the pathogenesis of various tumors. Autophagy activity has been shown to be decreased in esophageal, breast, and colon cancers [4-6]. Moreover, autophagy is involved in tumor resistance to OGD and chemotherapy [7, 8].

Decorin (DCN) is one of the members of the leucine-rich glycoprotein family [9], and it is a small secreted protein with important functions. It has been shown that DCN could inhibit the metabolism and proliferation of breast cancer cells and block the colony formation [10, 11]. In addition, DCN has also been found to regulate the differentiation and chemotaxis of tumor cells [12]. However, the association of DCN with autophagy, especially concerning their roles in the pathogenesis, invasion, and metastasis of HCC, has been rarely reported. In this study, the effects of DCN on human hepatoma HepG2 cells under OGD condition were investigated, especially concerning the relationship between DCN and autophagy.

Materials and methods

Cell culture and modeling

Human hepatoma HepG2 cells were from American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in RPMI1640 medium (HyClone, Beijing, China),
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Figure 1. Autophagy protected HepG2 cells against OGD-induced injury. HepG2 cells were cultured under OGD condition for 2 h, 4 h, 6 h, 8 h, and 24 h, respectively. The HepG2 cells in the treatment group were then treated with 3-MA. A. The cell survival of OGD-preconditioned HepG2 cells was detected by the MTT assay. B. The apoptosis was detected by flow cytometry in HepG2 cells under OGD condition. Compared with the corresponding OGD group, *P < 0.05.

containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), in a 37°C, 5% CO₂ incubator. These cells were passaged when 90% confluence was reached, and the 3-5 passages were used in following experiments.

For the induction of OGD, HepG2 cells with 70% confluence were incubated with 5% CO₂, 1% O₂, and 94% N₂, in a humidified atmosphere at 37°C, for 2 h, 6 h, 8 h, 12 h, and 24 h, respectively. These cells were divided into (1) the OGD groups and (2) the treatment group, in which OGD-preconditioned cells were treated with 3-MA (Sigma, St. Louis, MO, USA).

Cell transfection

Cell transfection was performed with liposome. The pcDNA3.1-DCN vector was constructed by RiboBio (Guangzhou, Guangdong, China). HepG2 cells in logarithmic growth phase were planted onto 24-well plates at the density of 2 × 10⁵ cells/well. The cells were incubated with antibiotic-free RIPA 1640 medium with 10% FBS. When 70% confluence was reached, 2 µg pcDNA3.1-DCN or pcDNA3.1 vector, together with 1 mL Lipo2000, was mixed with 50 µL Opti-Mem in an EP tube. 20 min later, the mixture was added into the wells to incubate cells for 6 h. The cells were incubated with RIPA 1640 medium containing 10% FBS for 48 h, and then screened with medium containing 400 µg/mL G418 for 21 d. The monoclonal cell culture was selected and expanded.

Cell count kit-8 (CCK-8) assay

Cell survival was assessed by the CCK-8 assay (Beyotime, Haiman, Jiangsu, China). After modulating and drug administration, HepG2 cells were collected and washed with PBS twice. Fresh medium containing 10% CCK-8 solution was added to incubate the cells at 37°C for 1 h, and the absorbance at 450 nm was read by a microplate reader. Experiments were performed in triplicates.

Flow cytometry

The apoptotic process was detected by flow cytometry. Cells were collected, and stained with the ANXN V FITC APOPTOSIS DTEC KIT I (BD, New Jersey, NY, USA), according to the manufacturer’s instructions. Cells positive for ANNEXIN V were recognized as early apoptosis, positive for PI were recognized as necrosis, and positive for both ANNEXIN V and PI were recognized as late apoptosis.

Western blot analysis

Cells were lysed with the RIPA lysis buffer containing 1% PMSF. The protein concentration was determined, and 6 mL protein sample was separated with the SDS-PAGE. Protein samples were electronically transferred onto a PVDF membrane, and then blocked with 5% fat-free milk at room temperature for 1 h. The blot was then incubated with rabbit anti-human anti-DCN (1:1000 dilution; Bioworld, Louis Park, MN, USA), anti-LC3B (LC3B1 and LC3BII; 1:1000 dilution; Beyotime), anti-Beclin1 (1:1000 dilution; Bioworld), anti-P62 (1:1000 dilution; CST, Boston, MA, USA), and mouse anti-human anti-GAPDH (1:10000 dilution; Bioworld), respectively, at 4°C overnight. After washed with PBST, the membrane was then incubated with HRP-
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**Results**

*Autophagy protects HepG2 cells against OGD-induced injury*

To investigate the role of autophagy in OGD-induced injury in HepG2 cells, the cell survival and apoptosis were detected with the MTT assay and flow cytometry, respectively. HepG2 cells were first cultured under OGD condition for 2 h, 4 h, 6 h, 8 h, and 24 h, respectively. The
results from the MTT assay showed that, compared with the control group, the cell survival rates were significantly decreased in the OGD groups ($P < 0.05$) (Figure 1A). Moreover, when treated with 3-MA, an inhibitor of autophagy, the survival rates were further declined in the HepG2 cells treated with OGD ($P < 0.05$) (Figure 1A). These results suggest that autophagy might protect the HepG2 cells against OGD-induced injury. In addition, the flow cytometry indicated that apoptosis occurred in HepG2 cells under OGD condition (Figure 1B). However, the apoptosis rates were significantly increased after the treatment of 3-MA ($P < 0.05$) (Figure 1B). These results suggest that the inhibition of autophagy could facilitate the apoptotic process in HepG2 cells treated with OGD.

Expression of DCN is elevated in HepG2 under OGD condition

To investigate the expression levels of DCN and autophagy-related proteins, the Western blot analysis was performed. Our results showed that, starting from 2 h, the expression levels of DCN were significantly elevated in OGD-preconditioned HepG2 cells ($P < 0.05$) (Figure 2). Meanwhile, for the autophagy-related proteins, the expression level of Beclin1 and the LC3BI/LC3BII ratio were significantly increased, while the expression level of P62 was significantly decreased, in HepG2 cells under OGD condition ($P < 0.05$) (Figure 2). These results confirmed the occurrence of autophagy in HepG2 cells under OGD condition, which might also be related to the altered expression level of DCN.

DCN protects HepG2 cells against OGD-induced injury

To further investigate the relationship between DCN and autophagy and the effects of DCN on HepG2 cells under OGD condition, these cells were over-expressed with DCN. After screening with G418 for 21 d, bright green fluorescence was observed in the HepG2 cells transfected with pcDNA3.1-DCN (Figure 3A), suggesting the stable expression of the vectors in these cells. Our results from the Western blot analysis showed that, the expression level of DCN was dramatically elevated in the HepG2 cells transfected with pcDNA3.1-DCN ($P < 0.05$) (Figure 3B). Moreover, in DCN-over-expressed HepG2 cells, the expression level of Beclin1 and the LC3BI/LC3BII ratio were drastically increased ($P < 0.05$), while no significant changes were observed in the expression level of P62 ($P > 0.05$) (Figure 3C). These results further indicate that DCN over-expression could induce autophagy in HepG2 cells.

To further investigate the protective effects of DCN, HepG2 cells transfected with pcDNA3.1-DCN were cultured under OGD condition, and then the apoptosis was detected with flow cytometry. Our results showed that, compared with the OGD group, the apoptosis rate was significantly decreased in the DCN-over-expressed HepG2 cells ($P < 0.05$) (Figure 3D). These results suggest that DCN could protect HepG2 cells against OGD-induced injury.

Discussion

In recent years, the incidence and mortality of hepatocellular carcinoma (HCC) have been continuously increasing in China, endangering the public health [13]. Numerous studies have been trying to investigate the molecular mechanisms for HCC, especially for the invasion and metastasis. Autophagy is an important cellular mechanism to achieve self-renewal and maintain homeostasis, which could phagocytose and degrade aging organelles, damaged DNAs, and misfolded proteins [14]. It has been shown that autophagy may have inhibitory effects on tumors, and the role of autophagy might depend on the tumor cell status as well as environment [4-7]. Decorin (DCN) is an important component of the extracellular matrix. Studies show that DCN can bind with growth factors and cytokines, to regulate the cell proliferation, apoptosis, and fibrosis [15]. DCN has been found to be abnormally expressed in various tumors, modulating the tumor biological function. Autophagy is attracting more attention in cancer research, which is related to a variety of cells and signaling pathways. Up to date, there have been few studies concerning the relationship between DCN and autophagy in HCC.

In the present study, HepG2 cells were cultured under OGD condition, and treated with the autophagy inhibitor 3-MA. Our results showed that the inhibition of autophagy could significantly decrease the survival rates and increase the apoptosis rates of HepG2 cells. Western blot analysis showed that the expression levels of DCN were gradually increased along with the
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increasing duration of OGD exposure. Moreover, the expression level of autophagy-related protein Beclin1 and the LC3BI/LC3BII ratio, were elevated, while the expression level of P62 was declined, in HepG2 cells under OGD condition, indicating the occurrence of autophagy in these cells. These results suggest a possible relationship between DCN expression level and autophagy in HepG2 cells under OGD condition. In addition, HepG2 cells were then transfected with vectors containing DCN and screened by G418. Our results from the Western blot analysis showed that DCN was stably over-expressed in the HepG2 cells following transfection. Moreover, the expression level of Beclin1 and the LC3BI/LC3BII ratio were increased, while the expression level of P62 was declined, in HepG2 cells under OGD condition, indicating the occurrence of autophagy in these cells. These results suggest a possible relationship between DCN expression level and autophagy in HepG2 cells under OGD condition. In addition, HepG2 cells were then transfected with vectors containing DCN and screened by G418. Our results from the Western blot analysis showed that DCN was stably over-expressed in the HepG2 cells following transfection. Moreover, the expression level of Beclin1 and the LC3BI/LC3BII ratio were increased, while the expression level of P62 was declined, in HepG2 cells under OGD condition, indicating the occurrence of autophagy in these cells. These results suggest a possible relationship between DCN expression level and autophagy in HepG2 cells under OGD condition.

In conclusion, our results showed that autophagy protected the HepG2 cells against OGD-induced injury. The inhibition of autophagy facilitated the cellular apoptosis in HepG2 cells under OGD condition. Moreover, the expression levels of DCN were elevated in OGD-preconditioned HepG2 cells, which might be related to the occurrence of autophagy. Furthermore, DCN over-expression could induce autophagy in HepG2 cells, and protect HepG2 cells against OGD-induced injury. These results might help to better understand the roles of DCN and autophagy in HCC, and the potential treatment for the disease.

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Disclosure of conflict of interest

None.

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