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Matrix Biology 24 (2005) 3–13

MATRIX BIOLOGY

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Versican protects cells from oxidative stress-induced apoptosis ☆

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Received 13 April 2004; received in revised form 2 November 2004; accepted 29 November 2004

Abstract

Oxidant injury plays a critical role in the degenerative changes that are characterized by a decline in parenchymal cell numbers and viability, and occur with aging and in the etiology of many diseases. The extracellular proteoglycan versican is widely distributed in the extracellular matrix surrounding the cells. This study examines whether versican plays a role in protecting cells from free radical-induced apoptosis. Stable expression of versican or its C-terminal domain significantly decreased H_2O_2-induced cellular apoptosis. Cells in adherent monolayer were more resistant to H_2O_2-induced apoptosis than cells cultured in suspension. While vigorous trypsinization caused integrin cleavage and rendered the cells more susceptible to H_2O_2-induced damages, expression of versican or its C-terminal domain enhanced cell attachment and expression of β1 integrin and fibronectin. Enhanced cell–matrix interaction by addition of manganese ($MnCl_2$) to cultures also significantly diminished H_2O_2-induced apoptosis. The results suggest that versican plays an important role in reducing oxidant injury through an enhancement of cell–matrix interaction.
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Keywords: Free radical; G3 domain; Glycosaminoglycan; Interaction; Apoptosis; Adhesion

1. Introduction

Reactive oxygen species are among the most potent and ubiquitous threats to living organisms. The reactive oxygen species are generated in cells and tissues by toxic insults or normal metabolic processes. Oxidant injury is thought to play a critical role in the degenerative changes that are characterized by a decline in the parenchymal cell numbers and viability and occur with aging and in the etiology of many diseases including cancer, AIDS, Parkinson's disease, osteoarthritis, and atherosclerosis (Chandra et al., 2000).

Versican, an aggregating chondroitin sulfate proteoglycan, is widely distributed in the extracellular matrix (ECM) of a variety of tissues (Cattaruzza et al., 2002; Kresse and Schonherr, 2001; Wight, 2002). Versican tends to be expressed in tissues where cells are metabolically active and proliferating, such as in the early stages of tissue development. In epidermis, versican is detected only in the proliferating zone (Zimmermann et al., 1994). Versican is also highly expressed in many malignancies, including brain tumors (Paulus et al., 1996), breast cancer (Brown et al., 1999; Nara et al., 1997), melanoma (Touab et al., 2002), and prostate cancers (Ricciardelli et al., 1998). In previous studies, we demonstrated that versican promotes cell proliferation in NIH3T3 fibroblasts (Zhang et al., 1998b)

Abbreviations: EGF, epidermal growth factor; EGFR, EGF receptor; CRD, carbohydrate recognition domain; CBP, complement binding protein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HBSS, Hank's balanced salt solution; PAGE, polyacrylamide gel electrophoresis; G3, selectin-like domain; G3ΔEGF, versican G3 domain lacking two EGF-like motifs.

☆ This work was supported by grants from Heart and Stroke Foundation of Ontario (NA-5127), and National Sciences and Engineering Research Council of Canada (227937-01) to Burton B Yang.

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doi:10.1016/j.matbio.2004.11.007

and chicken chondrocytes (Zhang et al., 1999). Free radical damage and reduction of synthesis of proteoglycans are two factors realized to be important for the development of osteoarthritis (Ghosh and Smith, 2002). However, the role of proteoglycans in oxidative stress-induced cell damage has not yet been studied in depth. This study examined that role of an extracellular chondroitin sulfate proteoglycan versican in modulating cell death induced by oxidative stress.

2. Materials and methods

2.1. Materials and cell cultures

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin/EDTA, and Geneticin (G418) were from Invitrogen. ECL Western blot detection kit was from Amersham Life Science. Tissue culture plates were from Nunc Inc. Polyclonal antibodies against fibronectin and all chemicals (including H_2O_2 and $MnCl_2$) were from Sigma. Anti-β1 integrin monoclonal antibody was from Chemicon. The U87 cell line was obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in DMEM supplemented with 10% FBS at 37 °C in a humidified incubator containing 5% CO_2.

2.2. Isolation of astrocytoma cell lines expressing transgenes

We have previously described the construction of a mini-versican containing the versican G1 domain, G3 domain, and a fragment of CSα domain in pcDNA3 vector (Zhang et al., 1998a). To enhance secretion of the mini-versican product, the leading peptide of link protein was added to this construct. This leading peptide serves a dual role: it allows the versican mini-gene products to be secreted and it harbors an epitope recognized by the monoclonal antibody 4B6 (Binette et al., 1994). Versican C-terminal domain ("G3ΔEGF") containing CRD (carbohydrate recognition subdomain) and CBP (complement binding protein subdomain) in pcDNA3 has been generated previously (Yang et al., 2000; Zhang et al., 1998b). The leading peptide of link protein was also added to the 5′ end of G3ΔEGF. Astrocytoma cell line U87 was stably transfected with mini-versican, G3ΔEGF, or the vector pcDNA3 as previously described (Wu et al., 2001).

Astrocytoma cells were seeded on 100-mm tissue culture plates at 20% cell density and incubated overnight in DMEM supplemented with 10% FBS. The following day, plasmid DNA (5 μg) was incubated with 8 μl Lipofectin (Invitrogen) for 15 min in 200 μl DMEM. The DNA–Lipofectin mixture was applied to the washed cultures in DMEM and incubated for 5 h. The culture medium was then replaced with DMEM supplemented with 10% FBS. The next day, Geneticin (1.5 mg/ml) was introduced into the growth medium and the cells were maintained in this medium until individual colonies were large enough for cloning. The selected cell lines were stored in liquid nitrogen or maintained in growth medium containing 1.0 mg Geneticin/ml for subsequent gene expression assays and functional studies. Cell lines were monitored to ensure expression of the transgenes for the duration of functional studies.

2.3. Annexin V apoptosis assay quantified by FACScan

Annexin V Apoptosis Detection Kit was purchased from Santa Cruz Biotechnology. The assay was performed following the manufacturer's instructions. Briefly, 2×10^5 cells were harvested and pelleted by centrifugation at $1500\times g$ for 5 min, washed twice with cold PBS and resuspended in 300 μl 1×Assay Buffer. Annexin V FITC (8 μl) was added and incubated for 15 min in darkness on ice followed by cold PBS wash and was subjected to flow cytometry analysis immediately. Cells without staining were used as negative controls.

2.4. Induction of apoptosis by H_2O_2

Astrocytoma U87 cells, stably transfected with mini-versican, G3ΔEGF, or a control vector were cultured to reach confluence. In suspension cultures, 2×10^5 cells were seeded on 6-well tissue culture plates in DMEM containing 10% FBS in the presence of different concentrations of H_2O_2 and incubated at 37 °C for different time periods as indicated in the figures. H_2O_2 was also added to monolayer cultures, in which 2×10^5 cells were seeded on 6-well tissue culture plates in DMEM containing 10% FBS and incubated at 37 °C for 6 or 24 h as indicated to allow the cells to attach to the plates. After treatment with trypsin/EDTA, the cells became more sensitive to H_2O_2 treatment. H_2O_2 (1.2 or 1.5 mM) was added to the cultures and maintained for 6 or 24 h as indicated.

To examine the effects of manganese on H_2O_2-induced cell apoptosis, 2 mM $MnCl_2$ was added to the suspension cultures or to the monolayer cultures as indicated in the figures. After treatment, cells were harvested and pooled. Cells were then centrifuged and subjected to apoptosis assays and trypan blue staining.

2.5. Trypsinization and apoptosis

Confluent U87 cells cultured on 100 mm tissue culture plates were washed with PBS and trypsinized with 3 ml 0.05% trypsin/EDTA for 6 and 12 min at room temperature. The trypsin solutions were recovered and subjected to Western blot analysis using 7% gel for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) probed with anti-integrin β1 monoclonal antibody (Chemicon). The cells were harvested in DMEM supplemented with 10% FBS. Cells (2×10^5) were seeded on 6-well tissue culture plates in DMEM containing 10% FBS in the

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