Biochimica et Biophysica Acta 1810 (2011) 284–291

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

A T/C polymorphism in the *GPX4* 3′UTR affects the selenoprotein expression pattern and cell viability in transfected Caco-2 cells

Hannah Gautrey [a,b], Fergus Nicol [c], Alan A. Sneddon [c], Judith Hall [a], John Hesketh [a,b,*]

[a] Institute for Cell and Molecular Biosciences, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK
[b] Human Nutrition Research Centre, The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK
[c] Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Rd, Bucksburn, Aberdeen, AB21 9SB, UK

ARTICLE INFO

Article history:
Received 9 November 2010
Received in revised form 21 March 2011
Accepted 24 March 2011
Available online 1 April 2011

Keywords:
Selenium
Glutathione peroxidase 4
Oxidative stress
3′untranslated region
Selenoprotein hierarchy
Transfection

ABSTRACT

Background: Synthesis of selenoproteins such as glutathione peroxidases (GPx) requires a specific tRNA and a stem-loop structure in the 3′untranslated region (3′UTR) of the mRNA. A common single nucleotide polymorphism occurs in the *GPX4* gene in a region corresponding to the 3′UTR.
Methods: The two variant 3′UTR sequences were linked to sequences from a selenoprotein reporter gene (iodothyronine deiodinase) and expressed in Caco-2 cells. Clones expressing comparable levels of deiodinase (assessed by real-time PCR) were selected and their response to tert-butyl hydroperoxide assessed by cell viability and measurement of reactive oxygen species. Selenoprotein expression was assessed by real-time PCR, enzyme activity and immunoassay.
Results: When selenium supply was low, cells overexpressing the C variant 3′UTR showed lower viability after oxidative challenge, increased levels of reactive oxygen species and lower GPx activity and SelH mRNA expression compared to cells overexpressing the T variant. After selenium supplementation, cell viability and GPx4 expression were higher in the cells overexpressing the C variant. Expression of transgenes incorporating the T/C variant *GPX4* (rs713041) sequences in Caco-2 cells leads to alterations in both cell viability after an oxidative challenge and selenoprotein expression. This suggests that the two variants compete differently in the selenoprotein hierarchy.
General Significance: The data provide evidence that the T/C variant *GPX4* (rs713041) alters the pattern of selenoprotein synthesis if selenium intake is low. Further work is required to assess the impact on disease susceptibility.

1. Introduction

There is increasing evidence that genetic variants such as single nucleotide polymorphisms (SNPs) can alter nutrient metabolism and responses to changes in nutrient supply [1,2]. The interaction between such functional genetic variants and diet is likely to underlie many multifactorial diseases [e.g. 3]. Sub-optimal intake of the micronutrient selenium (Se) has been associated with increased risk of cardiovascular disease and prostate and colorectal cancers [4,5]. The metabolic functions of Se are thought to be due to its presence as selenocysteine in ~25 selenoproteins in mammals. Many of these proteins, including the glutathione peroxidases (GPx) and thioredoxin reductases (TR), are thought to have antioxidant and redox functions and protect cells from oxidative stress and cell damaging agents [6–8]. As a result, SNPs in selenoprotein genes may be expected to affect antioxidant functions.

Se is incorporated into selenoproteins during protein synthesis using UGA as the codon for selenocysteinyl-tRNA; this requires a selenocysteine insertion sequence (SECIS) in the 3′untranslated region (3′UTR) of the mRNAs and specific proteins that bind to these RNA structures [6]. When Se supply is limiting there is a tissue-specific prioritisation of selenoprotein synthesis so that synthesis of certain proteins is maintained at the expense of synthesis of others [6,9] and this leads to differential effects of Se supply on the levels of both the selenoproteins and their corresponding mRNAs. This prioritisation, often referred to as the selenoprotein hierarchy, is partly dependent on differences in the ability of the different 3′UTR sequences to interact with the selenocysteine incorporation machinery [6,9–11]. As a result, SNPs in the selenoprotein gene regions corresponding to the 3′UTR could have functional effects, potentially affecting not only the synthesis of the selenoprotein coded by the gene containing the SNP but also the synthesis of other selenoproteins.

A T/C variation (rs713041) in the region of the *GPX4* gene that corresponds to the 3′untranslated region (3′UTR) of the mRNA has been found in Caucasian and Asian populations [12,13]. Using a transfected cell model expressing a selenoprotein iodothyronine deiodinase (IDI) reporter gene the variants have been shown to drive

* Corresponding author at: Human Nutrition Research Centre, The Medical School, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK. Tel.: +44 191 222 8744; fax: +44 191 222 7424.
E-mail address: j.e.hesketh@ncl.ac.uk (J. Hesketh).

0304-4165/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagen.2011.03.016

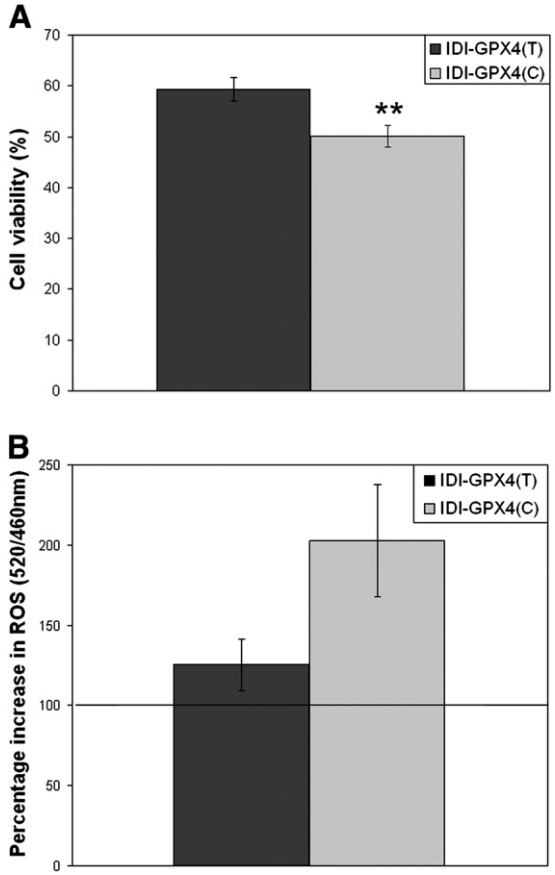

Fig. 1. Cell viability and ROS levels in response to the oxidant *tert*-butylhydroperoxide. (A) Percentage viability of the IDI-T and IDI-C clones after 4 hours of treatment with 3 mM *tert*-butylhydroperoxide compared to untreated IDI-T and IDI-C clones. (B) Percentage increase in ROS after 4 hours of treatment with 100 μM *tert*-butylhydroperoxide compared to untreated cells. All cells were grown in normal serum containing media. Values shown are means ± s.e.m.

selenoprotein synthesis to different extents [14]. Furthermore, results from a human supplementation trial suggest that this SNP affects expression of lymphocyte GPx1 and GPx4, and *in vitro* assays with Caco-2 cell extracts indicate that the T and C variants of the 3′UTR show different protein binding characteristics [15], suggesting that the variants differ in their ability to interact with the Se incorporation machinery. However, it is not known if the SNP affects either the cells' ability to respond to an oxidative challenge or the hierarchy of selenoprotein synthesis.

The aim of the present work was to investigate whether the T and C variants of this SNP differ in their ability to affect these parameters. To do this we produced stable clones of transfected Caco-2 cells over-expressing comparable amounts of transcripts encoding the selenoprotein iodothyronine deiodinase linked to the *GPX4* 3′UTR containing either T or C variant. These transfected cells were used to assess the impact of the presence of T and C variant transcripts on selenoprotein expression and response to a *tert*-butylhydroperoxide challenge. The results are consistent with over-expression of transcripts containing the C variant of rs713041 having a greater effect on the selenoprotein hierarchy than the T variant.

2. Methods

2.1. Cell culture

Caco-2 cells were maintained in Dulbecco's minimal Eagle's medium (DMEM) containing 10% foetal calf serum and penicillin and streptomycin. Cells were incubated at 37 °C with 5% CO_2. For growth of cells in a specific concentration of selenium, cells were grown in serum free DMEM with added insulin (5 μg/ml) and transferrin (5 μg/ml) and varying concentrations of sodium selenite, as described previously [16]. Caco-2 cells are heterozygous C/T for rs713041.

2.2. DNA transfection

The construction of the pCDNA3.1/Zeo (+) plasmids containing the coding region of rat type 1 iodothyronine deiodinase (IDI) with the 3′ UTR of *GPX4* containing either a T (IDI-GPX4(T)) or a C (IDI-GPX4(C)) at position 718 was described previously [14]. Caco-2 cells were transfected at 90–95% confluency with endotoxin-free IDI-GPX4(T) or IDI-GPX4(C) plasmids (μg) using Lipofectamine 2000 reagent (μl) (Invitrogen) in a 1:3.5 ratio according to the manufacturer's instructions. After 24 hours, the cells were split (1:5) and grown for an additional 24 hours in normal media. Cells were then grown in a selective media containing 200 μg/ml of the antibiotic zeocin and stably transfected colonies isolated for both IDI-GPX4(T) or IDI-GPX4(C) transfected cells. Two IDI-GPX4(T) and two IDI-GPX4(C) clones were selected for use in further experiments based on their IDI expression levels.

2.3. Cell viability assays

Ninety-six well plates were seeded with 6×10^4 cells/well, and after 24 hours half the cells were treated with varying concentrations of *tert*-butylhydroperoxide diluted in media. After 4 hours the medium containing *tert*-butylhydroperoxide was removed and fresh medium added to the cells. Cell viability was assessed by the addition of 20 μl of CellTiter-Blue® reagent (Promega) and after 4 hours absorbance was measured at 560 nm.

2.4. ROS measurement

Cells were seeded at a density of 3×10^4 cells/well in 96 well plates and grown for 2 days. To induce ROS production, cells were incubated for 4 hours with 100 μM *tert*-butylhydroperoxide, following instructions from the manufacturers of the detection kit. Cells were washed with Hanks' balanced salt solution without phenol red (HBSS; from Invitrogen) before incubation with 25 μM 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA (Invitrogen)) and 0.5 μM Hoechst 33342 dye diluted in HBSS . After 15 minutes incubation the cells were then washed twice with HBSS after which 50 μl of HBSS was added to each well. Florescence of the two dyes was measured by excitation at 485 nm and detection of emitted fluorescence at 520 nm for carboxy-H_2DCFDA, and by excitation at 355 nm and detection of emitted fluorescence at 460 nm for Hoechst 33342.

2.5. RT-PCR

Cells were washed with phosphate buffered saline before total RNA was extracted with Trizol (Invitrogen) and purified using PureLink™ columns (Invitrogen). cDNA was synthesised at 55 °C for

Fig. 2. Selenoprotein expression in clones expressing IDI-C and IDI-T. (A) Levels and activity of selenoproteins in the IDI-T and IDI-C clones grown in normal serum containing medium. TR1 and GPx4 protein levels were measured by ELISA and normalised by total protein. GPx activity was measured and normalised by total protein content. (B) Real time PCR analysis of the IDI-T and IDI-C clones grown in normal serum containing media. Gene expression was measured for the selenoproteins TR1, GPx4, GPx1 and SelH and normalised by GAPDH (ratio). Values shown are means ± s.e.m.