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Selenoprotein W during development and oxidative stress

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Abstract

Selenium is involved in prevention of cancer, heart and muscle diseases, is implicated in immune function, fertility and in delaying the aging process. Selenium deficiency is harmful to brain, heart and skeletal muscles. Selenoprotein W, a member of the selenoprotein family was expressed in developing nervous system, skeletal muscles and heart in mice. Selenoprotein W was highly expressed in proliferating myoblasts and less or not in differentiated myotubes. Selenoprotein W exhibited an immediate response to oxidative stress in proliferating myoblasts, after exposure to hydrogen peroxide, similar to gluteraldehyde-3-phosphate dehydrogenase. We suggest that Selenoprotein W is involved in muscle growth and differentiation by protecting the developing myoblasts from oxidative stress.

Keywords: Selenoprotein W; Mouse development; Myoblast differentiation; Oxidative stress

1. Introduction

Selenoproteins contain selenium (Se) in the form of the amino acid selenocysteine (Sec), which is encoded by the codon UGA in selenoprotein mRNA. There are 25 selenoprotein genes identified to date. Biosynthesis of selenocysteine from serine occurs on a distinct tRNA, i.e. selenocysteine-tRNA [1–4]. In addition to selenocysteine-tRNA, selenocysteine incorporation at UGA codons, rather than termination, requires a specialized mRNA secondary structure (selenocysteine insertion sequence, SECIS element), a novel mRNA binding protein (SBP2), and a selenocysteine-tRNA specific elongation factor (eEFsec) [5–7]. Limitation of the Se available for the synthesis of selenocysteine would result in reduced levels of proteins containing this amino acid. Most of the selenoproteins exhibit enzymatic function via selenocysteine, which determines their catalytic and anti-oxidant activities [3,8] and suggests that human diseases associated with Se deficiency may be attributed to increased oxidative stress and alterations in redox signaling.

Differences in SECIS, UGA context or UGA/SECIS separation are likely to be the reason selenoprotein mRNA competes with other selenoprotein mRNAs for translation when Se is limited. The antioxidant glutathione peroxidases (GPXs) and the redox-regulatory thierodoxin reductases (TRs) have genetic and epigenetic anti-carcinogenic impact. These pathways might remove DNA-damaging hydrogen peroxide (H_2O_2) and lipid hydroperoxides by blocking the production of reactive oxygen species (ROS) and malonyldialdehyde, or by regulating the redox signaling system that is critical for the growth of many cancers [9].

Se deficiency in humans has been recognized in Keshan cardiomyopathy and in deforming arthritis in Kashim-Beck disease [10]. Neuronal and neuromuscular disorders, including Alzheimer's, Parkinson, Amylotrophic Lateral Sclerosis, stroke and Duchenne Muscular Dystrophy are example diseases where oxidative stress and presence of ROS is induced [11–16]. Selenoprotein P provides Se to the brain and to the testis [17,18] by transporting Se to neuronal stem cells [19]. Absence of Selenoprotein P, in mice, in combination with low Se intake resulted to neurological dysfunctions [20].

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Selenoprotein W (SeW, Sepw1) spans 60 aminoacids, is highly conserved among mammalian species (83% homology) with the Sec residue at position 13 [21]. Se-deficient diet in mammals caused the absence of SeW in skeletal muscles, heart, intestine, prostate, esophagus and skin [22]. SeW binds glutathione [23] and has been suggested to act as an antioxidant in vivo [24]. Expression of mutant SeW in which selenocysteine 13 or cysteine 36 were replaced by serine did not confer resistance to H_2O_2, implicating the antioxidant activity of SeW [24,25]. Selenium deficiency in Caco-2 cells resulted in a 73% reduction in SeW mRNA [26]. SeW promoter studies indicated the presence of metal and glucocorticoid response elements (MRE and GRE) and its activity is higher than SV40 in glial and muscle cells [27]. SeW expression is Metal-transcription factor-1 (MTF-1) dependent, as SeW is down-regulated in $Mtf1^{Mx-Cre}$ mice both untreated and treated with cadmium [28].

In this report we show that SeW RNA was detected as early as in implanted and gastrulating mouse embryos. SeW was also detected in early stages of development within the brain, somites, neural tube, skeletal muscles and heart and its levels increased by the end of the gestation period. SeW was expressed in proliferating skeletal myoblasts and its levels were increased 2 h after treatment with L-buthionine [*S*,*R*] sulfoxide (BSO) and H_2O_2. These data support the hypothesis that SeW acts as protectant against oxidative stress during myoblast growth and differentiation to a functional muscle group.

2. Materials and methods

2.1. Whole mount RNA-digoxigenin in situ hybridization

Mouse embryos were isolated at various gestational stages, fixed with 4% paraformaldehyde and dehydrated with methanol solutions (30–100%). Embryos were bleached with H_2O_2 and proteins were digested with proteinase K. Embryos were prehybridized and hybridized in the presence of 50% formamide and 3-[(3cholamidopropyl)-dimethylammino]-1propanesulfonate (CHAPS) at 55 °C for 16 h. Antisense RNA probes were labeled with digoxigenin (DIG). Embryos were washed and RNA was detected with anti-DIG antibody against conjugated with alkaline phosphatase (AP) (Roche). Nitro blue tetrazolium/bromochloro-indoryl phosphate (NBT/BCIP) substrate was used for visualization. Color reaction was stopped at the same time for all experimental embryos. Sense DIG-labeled probes and addition of no RNA probe were used as control and resulted in no color reaction. 3–5 embryos have been used for each developmental stage for each RNA probe.

2.2. Northern blot assays and RNA quantitation

Mouse embryos multiple tissue northern (MTN) blot (BD Biosciences) was probed using ExpressHyb hybridization solution (BD Biosciences) following the recommended protocol for cDNA probes. Plasmids containing gluteraldehyde-3-phosphate dehygrogenase (GADPH), SeW, and human β-actin coding regions were excised, gel-purified, and labeled with RediPrimeII Random Labeling Kit (Amersham). NorthernMax (Ambion) kit was utilized with approximately 25 μg of RNA per lane on a 1.2% agarose formaldehyde gel. Membranes were exposed for 1–7 days and films were scanned using a Personal Densitometer SI (Molecular Dynamics). Ethidium bromide (EtBr) staining of the 18S RNA was used for quantification. ImageQuant 5.2 (Molecular Dynamics) was used to quantify the relative intensities of each band. A thick line was drawn over the entire row of bands, and the "create graph" feature of ImageQuant was used to create a plot of relative intensity versus the longitudinal axis at the line. For example, a graph with three peaks was created for an area that contained three bands. The "peak finder" feature was used to identify each peak, and quantify the area beneath the peak. This process was repeated for each band that was quantified. Normalization of the experimental bands to the controls was carried out in the following manner: the most intense control band in each blot was considered to be 100%. Every control band was multiplied to bring the value to 100%, thus normalizing every control band to 100%. The same number used to bring each control band to 100% was also used to bring the respective experimental band to the normalized value. Percent (%) increase or decrease for experimental values were calculated based upon the change relative to the cells with no treatment.

2.3. Tissue culture and cytotoxicity assay

C2C12 murine myoblasts were cultured in 10 cm plates with Dulbecco's modified eagle's medium (DMEM), 10% bovine calf serum, in presence of 100 U/ml penicillin/streptomycin (GIBCO-BRL), and 2 mM L-glutamine (GIBCO-BRL) at 37 °C with 5% CO_2. Cells were grown to 80% confluency then treated with 300 μM L-buthionine-[*S*,*R*]-sulfoximine (BSO) (Sigma), a drug that inhibits glutathione (GSH) synthesis [29]. After 16 h BSO treated and untreated cells were exposed to 125 μM H_2O_2 for 0, 0.5, 1, 2, 12, 18 and 24 h. Cells were washed twice with ice cold phosphate buffered saline (PBS), harvested on ice by scraping, centrifuged and pellets were stored at −80 °C for a period not more than one week before total RNA preparation. Total RNA was prepared using RNeasy Midi or Mini Kit (Qiagen).

3. Results

3.1. SeW in embryonic development

SeW expression profile during mouse development was determined by RNA whole mount in situ hybridization assays (Fig. 1A). SeW was first detected in the decidua and the newly implanted embryo. Ectodermal cells of the egg cylinder stage, at embryonic day 6 (e6) were expressing SeW. At embryonic day 8 (e8), SeW expression was maintained in the ectoderm and ectoderm-derived tissues with