Effects of insulin-like growth factor 1 on glutathione S-transferases and thioredoxin in growth hormone receptor knockout mice

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Abstract Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) have been shown to affect processes involved in cellular stress defense, aging, and longevity. This study was designed to identify possible mechanisms of a disrupted GH signaling pathway on stress resistance using growth hormone receptor knockout (GHRKO) mice. GHRKO mice are GH resistant due to the targeted disruption of the GH receptor/binding protein gene, thus preventing GH from binding and exerting its downstream effects. These mice have very low circulating IGF-1 levels and high GH levels, are obese yet insulin sensitive, and live longer than their wild-type controls. Wild-type or GHRKO mice were treated with saline or IGF-1 (WT saline, GHRKO saline, GHRKO IGF-1) two times daily for 7 days. Glutathione S-transferase (GST) activities, proteins, and gene expression were determined. Liver mitochondrial

GSTA1, GSTA3, and GSTZ1 proteins were significantly higher in GHRKO when compared to those of WT mice. The 4-hydroxynonenal (4-HNE) GST activity was upregulated in GHRKO mice and was suppressed after IGF-1 administration. Interestingly, thioredoxin (Trx)1, Trx2, thioredoxin reductase (TrxR)1, and TrxR2 messenger RNA (mRNA) levels were significantly higher in the GHRKO as compared to WT mice, and IGF-1 treatment suppressed the expression of each. We also found that glutaredoxin (Grx)2 mRNA and cytosolic Grx activity were higher in GHRKO mice. These results suggest that the detoxification and stress response mechanisms in GHRKO mice are contributed in part by the circulating level of IGF-1.

Keywords Growth hormone · Glutathione S-transferase · Growth hormone receptor knockout mice · Thioredoxin · Glutaredoxin

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Introduction

Growth hormone receptor knockout (GHRKO) mice are growth hormone (GH) resistant due to the targeted disruption of the GH receptor/binding protein gene, thus preventing GH from binding and exerting its downstream effects (Zhou et al. 1997). Consistent with GH resistance, plasma levels of insulin-like growth factor 1 (IGF-1) are dramatically lower in GHRKO mice (Coschigano et al. 2000; Zhou et al. 1997). GHRKO mice live on average 21–40 % longer than wild-type (WT) mice (Coschigano et al. 2000). In addition, these

mice are smaller (50 %), obese, and exhibit enhanced insulin sensitivity and delayed reproductive capacity (Bartke and Brown-Borg 2004).

The GH/IGF-1 signaling pathway has been identified as a major regulator of longevity from nematodes to humans (Russell and Kahn 2007). Reduced GH/IGF-1 and insulin signaling is associated with enhanced antioxidative capacity and stress resistance, both of which are thought to contribute to life span extension (Brown-Borg 2007). We have shown that components of the glutathione (GSH) and methionine metabolic pathways are altered in GHRKO mice compared to those in WT controls (Brown-Borg et al. 2009). We previously demonstrated that GH administration in GH-deficient Ames dwarf mice altered the expression and activities of several glutathione S-transferases (GSTs) (Rojanathammanee et al. 2013). The GSTs are a complex group of phase II detoxification enzymes that use the nucleophilic thiol of GSH to detoxify a variety of electrophilic substances including carcinogenic, mutagenic, and toxic compounds (Cheng et al. 2001; Hayes et al. 2005; Toba and Aigaki 2000). These enzymes play an important role in cellular protection systems that counter oxidative stress damage (Gardner and Gallagher 2001; Hayes and Strange 1995; Yoshitake et al. 1994) and also serve as peroxidases, isomerases, and thiol transferases (Jowsey et al. 2003). Most of mammalian GST isozymes are found in the cytoplasm (Singh et al. 2002). However, it is known that mouse and human alpha classes easily translocate into the mitochondria (Gardner and Gallagher 2001; Prabhu et al. 2001; Raza et al. 2002).

Growth hormone treatment of long-lived Ames mice also attenuates the activities of thioredoxin (Trx) and glutaredoxin (Grx) in liver mitochondria (Rojanathammanee et al. 2013). The thioredoxins are important regulators of oxidative stress defense, redox balance, and several metabolic pathways (Holmgren 1985; Lillig and Holmgren 2007; Powis and Montfort 2001). The thioredoxin system consists of Trx, thioredoxin reductase (TrxR), and nicotinamide adenine dinucleotide phosphate (NADPH; Holmgren 1989; Holmgren and Lu 2010). The function of Trx is dependent on the activity of TrxR and NADPH to maintain its reducing activity (Holmgren and Lu 2010; Lee et al. 2013). The Grx system consists of NADPH, GSH, GSH reductase, and glutaredoxin (Holmgren 1989). Glutaredoxin is a GSH-dependent hydrogen donor for

ribonucleotide reductase (Aslund and Beckwith 1999; Holmgren 1989; Lundberg et al. 2001). The function of Grx is to maintain a reduced state of cysteines in cellular protein (Gladyshev et al. 2001).

GHRKO mice are similar to Ames dwarf mice in many respects as they exhibit very low plasma IGF-1 levels, insulin sensitivity, and life span extension. However, the GHRKO mice differ in their antioxidative enzyme expression and functional capacity and other physiological parameters (Chandrashekar et al. 1999; Hauck et al. 2002). For example, kidney glutathione peroxidase (GPX) is higher in GHRKO mice compared to that in WT, but catalase does not differ and superoxide dismutase (SOD) activity was actually lower in these animals as opposed to Ames mice where GPX, catalase, and SOD are elevated (Hauck and Bartke 2000). It is unclear whether the differences in stress resistance result from the lack of an intact GH signaling pathway or the lack of IGF-1 alone. Therefore, the current study was designed to determine the impact of IGF-1 signaling on both mitochondrial and cytosolic GST isozymes, thioredoxin, and glutaredoxin systems in liver tissue of GHRKO mice.

Material and methods

Animals

Wild-type and GHRKO mice [generated by Zhou et al. (1997) and kindly provided by Andrzej Bartke] were bred and maintained at the University of North Dakota under controlled conditions of photoperiod 12:12-h light/dark cycle and temperature (22 ± 1 °C) with ad libitum access to food (Teklad #8640) and water. These animals were developed by crossing 129Ola/BALB/c normal (GHR+/-) animals with mice derived from crosses of C57BL/6 J and C3H/J strains; these mice were then maintained as a closed colony with inbreeding minimized by avoiding brother $\times$ sister mating (Panici et al. 2009). All procedures were reviewed and approved by the UND Institutional Animal Care and Use Committee. Six-month-old male animals were divided into three groups: group 1—WT treated with saline (WT saline, $n=13$); group 2—GHRKO treated with saline (GHRKO saline, $n=9$); and group 3—GHRKO treated with IGF-1 (GHRKO IGF-1, $n=9$). IGF-1 (ProSpec-Tany Technogene, East Brunswick, NJ) was injected s.c. (13 μ g/mouse/day) into the

GHRKO IGF-1 group. The same volume of saline was injected into WT saline and GHRKO saline groups. Mice were injected two times daily (8.00 a.m. and 5:00 p.m.) for 7 days. In the morning of day 7, 1 h following the last injection, nonfasted mice were sacrificed and tissues removed. Body and liver weights were recorded.

Enzyme-linked immunoabsorbent assay

An enzyme-linked immunoabsorbent assay (ELISA; mouse/rat IGF-I Quantikine ELISA kit; R&D Systems, Minneapolis, MN) was used to determine the concentration of plasma IGF-1 according to the manufacturer's instructions.

Immunoblotting

Liver mitochondrial and cytosolic fractions were isolated and used to assess specific changes in GST, Trx, and Grx proteins. Mitochondrial or cytosolic lysates were resolved by standard SDS-PAGE and immunoblotting techniques using antibodies to GSTA1 (#14475-1-AP),

GSTA3 (#16703-1-AP), GSTK1 (#14535-1-AP), GSTM4 (#16766-1-AP), GSTO2 (#14562-1-AP), GSTP1 (#15902-1-AP), GSTT2B (#17622-1-AP), and GSTZ1 (14889-1-AP) (Proteintech Group, Chicago, IL) and Trx2 (#AF3254) and Grx1 (#AF3119) (R&D Systems, Minneapolis, MN; Brown-Borg and Rakoczy 2005). Ponceau-S staining was used to verify equal loading of samples on each gel. Densitometry was performed to determine the level of protein expression.

Enzyme activity assays

GST activity was determined spectrophotometrically (Habig and Jakoby 1981; Mannervik et al. 1987). The formation of conjugates of GSH to trans-4-phenyl-3-butene-2-one (tpBO), bromosulphophthalein (BSP), dinitrochlorobenzene (DCNB), 1-chloro-2,4-dinitrobenzene (CDNB), and 4-hydroxynonenal (4-HNE) were monitored at 340 nm. Grx activity was assayed

Table 1 Primer pairs used for real-time RT-PCR analysis of gene expression

Gene	Primers 5'–3'
Trx1	For: CGT GGT GGA CTT CTC TGC TAC GTG GTG Rev: GGT CGG CAT GCA TTT GAC TTC ACA GTC
Trx2	For: GCT AGA GAA GAT GGT CGC CAA GCA GCA Rev: TCC TCG TCC TTG ATC CCC ACA AAC TTG
TrxR1	For: GGC CAA CAA AAT CGG TGA ACA CAT GGA AG Rev: CGC CAG CAA CAC TGT GTT AAA TTC GCC CT
TrxR2	For: GTC CCC TCC CAC ATC AAA AAA CTC CCA AC Rev: GGC CCA CAG GAC AGT GTC AAA GGT GC
Grx1	For: TGC AGA AAG ACC CAA GAA ATC CTC AGT CA Rev: TGG AGA TTA GAT CAC TGC ATC CGC CTA TG
Grx2	For: CAT CCT GCT CTT ACT GTT CCA TGG CCA A Rev: TCA TCT TGT GAA GCG CAT CTT GAA ACT GG
β2M	For: ATG GGA AGC CGA ACA TAC TG Rev: CAG TCT CAG TGG GGG TGA AT

Fig. 1 IGF-1 administration did not alter the body and liver weights in GHRKO mice. Body (a) and liver (b) weights of wild-type or GHRKO mice treated with saline or IGF-1 (WT saline, GHRKO saline, GHRKO IGF-1) were recorded before and/or after 7 days of saline or IGF-1 injections. Values represent mean±SEM. *** $p<0.001$ compared to WT saline. $N=9-13$ for each treatment group

Fig. 2 Plasma IGF-1 level was increased after IGF-1 administration. Plasma was collected after 7 days of saline or IGF-1 injections. ELISA was performed to detect IGF-1 levels. Values represent mean $\pm$ SEM. * p <0.05, *** p <0.001 compared to WT saline, ### p <0.001 compared to GHRKO saline. N =9–13 for each treatment group

using the spectrophotometric method of Holmgren (1979) (Robin et al. 2003) at 340 nm using GSH reductase as the coupling enzyme. The change in absorbance

with time was recorded. Trx activity was measured by its ability to reduce insulin disulfide in the presence of NADPH and thioredoxin reductase (Holmgren and Bjornstedt 1995; Luthman and Holmgren 1982). The absorbance was read at 340 nm, appropriate blanks were subtracted from total absorbance, and the activity was calculated.

Reverse transcription polymerase chain reaction

Gene expression was evaluated in liver tissue using reverse transcription polymerase chain reaction (RT-PCR) techniques (Brown-Borg et al. 2009). Total RNA was extracted from tissue using Ultraspec RNA (Biotecx, Houston, TX). Two micrograms of total RNA was utilized to synthesize cDNA and perform real-time quantitative PCR using a QuantiTect SYBR Green RT-PCR kit and was assayed using a SmartCycler instrument (Cepheid, Sunnyvale, CA). Gene expression was quantified using the comparative C_T (threshold cycle)

Fig. 3 Mitochondrial protein level GSTA1 was decreased after IGF-1 administration in GHRKO mice. Liver mitochondrial and cytosolic protein levels were determined via immunoblotting using GSTA1 (a) and GSTA3 (d) antibodies. The optical densities (O.D.) of mitochondrial GSTA1 (b), GSTA3 (e), cytosolic GSTA1

(c), and GSTA3 (f) were analyzed. Values represent mean $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.001 compared to WT saline, ### p <0.001 compared to GHRKO saline. N =8 for each treatment group

method (Heid et al. 1996). The amount of target was normalized to an endogenous reference gene (β 2-microglobulin) and expressed relative to the control group (WT saline). The primer pairs utilized are listed in Table 1.

Statistical analysis

Body and liver weight comparisons were performed using Student's *t* tests. To compare the differences among the WT saline, GHRKO saline, and GHRKO IGF-1 groups, the data were analyzed using one-way analysis of variance (ANOVA) with a Tukey post hoc test. Data are presented as means $\pm$ SEM, and significance level considered $p<0.05$. All statistical analyses were performed using GraphPad Prism software (GraphPad, San Diego, CA).

Results

Daily IGF-1 subcutaneous injections for 7 days did not significantly affect body or liver weights of GHRKO mice (Fig. 1a, b). By contrast, plasma levels of IGF-1 in GHRKO mice were significantly increased after 7 days of IGF-1 administration (Fig. 2).

Mitochondrial and cytosolic fractions were isolated from liver tissues to investigate whether IGF-1 signaling affects translocation or compartmentalization of GST isozymes. The mitochondrial and cytosolic protein levels of GSTA1 (Fig. 3a–c) as well as GSTA3 (Fig. 3d–f) were significantly higher in GHRKO as compared to those in WT mice. Interestingly, mitochondrial GSTA1 (Fig. 3b) but not GSTA3 protein levels (Fig. 3e) were significantly decreased after 7 days of IGF-1 administration in GHRKO mice. No significant differences in cytosolic GSTA1 and GSTA3 (panels c and f of Fig. 3, respectively) were observed after IGF-1 administration in GHRKO mice.

We next examined the effect of IGF-1 on the protein level of GSTM4. There was no difference in mitochondrial GSTM4 compared among WT, GHRKO, and IGF-1-treated GHRKO mice (Fig. 4a, b). However, the GSTM4 protein expression in the cytosol was significantly lower in GHRKO as compared to that in WT (Fig. 4a, b) mice, and IGF-1 administration increased the levels of this protein (Fig. 4a, c). Mitochondrial protein levels of GSTZ1 were higher in GHRKO mice (Fig. 5a, b), and 7 days of IGF-1 treatment did not alter this protein. No differences in cytosolic GSTZ1 were observed among the WT, GHRKO, and GHRKO IGF-1 (Fig. 5a, c) groups. We further investigated GSTK1 in both cellular fractions. No

Fig. 4 Cytosolic protein level GSTM4 was increased after IGF-1 administration in GHRKO mice. Liver mitochondrial and cytosolic protein levels were determined via immunoblotting using GSTM4 (a) antibodies. The optical densities (O.D.) of mitochondrial (b) and

cytosolic GSTM4 (c) were analyzed. Values represent mean $\pm$ SEM. ** $p<0.01$ compared to WT saline, ### $p<0.001$ compared to GHRKO saline. $N=8$ for each treatment group

Fig. 5 The levels of mitochondrial and cytosolic GSTZ1 were not affected by IGF-1 injection in GHRKO mice. Liver mitochondrial and cytosolic protein levels were determined via immunoblotting using GSTZ1 (a) antibodies. The optical densities (O.D.) of

mitochondrial (b) and cytosolic GSTZ1 (c) were analyzed. Values represent mean $\pm$ SEM. ** $p < 0.01$ compared to WT saline. $N = 8$ for each treatment group

differences in mitochondrial GSTK1 levels in liver tissue were found between the treatment groups (Fig. 6a, b). However, cytosolic GSTK1 protein levels increased following IGF-1 administration in GHRKO mice (Fig. 6a, c).

The activities of GST were examined using different substrates. GHRKO mice exhibited lower activity levels to GST CDNB- (Fig. 7a) and tPBO-specific (Fig. 7c) substrates when compared to WT mice. However, we

Fig. 6 Mitochondrial protein levels of GSTK1 were increased after 7 days of IGF-1 injection in GHRKO mice. Liver mitochondrial and cytosolic protein levels were determined via immunoblotting using GSTK1 (a) antibodies. The optical densities (O.D.)

of mitochondrial (b) and cytosolic GSTK1 (c) were analyzed. Values represent mean $\pm$ SEM. # $p < 0.05$ compared to GHRKO saline. $N = 8$ for each treatment group

found that IGF-1 treatment did not affect GST activity with these substrates. In contrast, the specific GST activity to 4-HNE was increased in GHRKO mice as compared to their respective WT mice (Fig. 7e) and significantly reduced after IGF-1 administration when compared to saline-treated GHRKO mice (Fig. 7e). When the substrate DCNB was used, IGF-1 tended to decrease the GST activity level (ANOVA, $p=0.0591$). No significant differences in GST activities were observed among the groups of mice when BSP was utilized as a substrate (Fig. 7b, d).

GHRKO mice expressed higher levels of both Trx1 and Trx2 messenger RNA (mRNA) expression when compared to the WT saline group (Fig. 8a, b). Moreover, 7 days of IGF-1 administration in GHRKO mice reduced Trx1 and Trx2 mRNA expression (Fig. 8a, b). There were no differences in mitochondrial Trx2 protein (Fig. 8c, d) or Trx activity (Fig. 8e) between mice in the three treatment groups. Interestingly, gene expression of TrxR1 and TrxR2 was markedly elevated in the saline-treated GHRKO mice as compared to that in WT animals (Fig. 9a, b) and downregulated after 7 days of IGF-1 treatment in GHRKO mice (Fig. 9a, b).

Fig. 7 IGF-1 administration affected GST activity to specific substrates in GHRKO mice. Liver lysates were used to analyze GST activity using different substrates: **a** CDNB, **b** DCNB, **c** tPBO, **d** BSP, and **e** 4-HNE. Values represent mean±SEM.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to WT saline. † $p=0.0591$, # $p<0.05$ compared to GHRKO saline. $N=9-13$ for each treatment group

Fig. 8 The IGF-1 administration in GHRKO mice reduced the expression of Trx2 and Trx2 mRNA. RT-PCR was performed to analyze Trx1 (a) and Trx2 (b) mRNA expression. Liver mitochondrial lysates were used to determine protein levels using Trx2 antibodies (c). The optical densities (O.D.) of mitochondrial Trx2 (d)

were analyzed. Liver mitochondrial fractions were used to analyze Trx activity (e). Values represent mean $\pm$ SEM. * $p < 0.05$, *** $p < 0.001$ compared to WT saline. # $p < 0.05$ compared to GHRKO saline. $N = 8$ for each treatment group for gene and immunoblotting analyses, $n = 9-13$ for each treatment group for enzyme activity assay

While no differences in Grx1 mRNA were observed between groups (Fig. 10a), Grx2 mRNA in liver tissue was increased in GHRKO saline-treated mice as compared to that in the WT saline group (Fig. 10b). With IGF-1 treatment, Grx2 mRNA expression decreased significantly in GHRKO mice (Fig. 10b). We found no differences in mitochondrial protein expression of Grx1 among saline- or IGF-1-treated animals (Fig. 10c, d). However, liver cytosolic Grx1 protein increased in GHRKO mice treated with IGF-1 when compared to saline-treated WT and GHRKO animals (Fig. 10c, e). Mitochondrial Grx activity did not differ between treatment groups (Fig. 10f), whereas cytosolic Grx activity in GHRKO mice was elevated compared to that in WT

saline mice (Fig. 10g). IGF-1 administration had no effect on cytosolic Grx activity (Fig. 10g).

Discussion

Potential mechanisms of life span extension in mammals involve multiple systems including metabolism, growth, reproduction, stress resistance, and repair (Brown-Borg 2007). The reduction in plasma IGF-1 levels found in GHRKO mice results in reduced circulating insulin, glucose, and thyroid hormone, as well as altered reproductive capacity, hyperprolactinemia, and

Fig. 9 TrxR1 and TrxR2 mRNA expressions were downregulated after 7 days of IGF-1 administration in GHRKO mice. RT-PCR was performed to analyze TrxR1 (a) and TrxR2 (b) mRNA expression. Values represent mean $\pm$ SEM. ** p <0.05 compared to WT saline. # p <0.05, ## p <0.01 compared to GHRKO saline. N =8 for each treatment group

life span extension (Bartke et al. 1999; Coschigano et al. 2000).

The present study demonstrated that 7 days of IGF-1 administration increased plasma levels of IGF-1 in GHRKO mice without alterations in body or liver weights. GHRKO mice exhibited elevated mitochondrial and cytosolic protein levels of the alpha class GSTs. Interestingly though, only mitochondrial GSTA1 was suppressed by IGF-1 administration. The GSTs represent a family of major cellular detoxification enzymes that utilize large amounts of glutathione (Cheng et al. 2001; Hayes et al. 2005; Toba and Aigaki 2000). The level of GST expression represents an important factor in determining the sensitivity of organisms to endogenous and exogenous compounds and has been shown to decline with aging (Cho et al. 2003; Richie et al. 1992). Moreover, GH has been shown to regulate the level of cytosolic GSTs in certain target organs (Coecke et al. 2000; Srivastava and Waxman 1993; Staffas et al. 1992). In our study, although no differences of the M

class GSTs in mitochondria were observed, cytosolic GSTM4 was higher in WT mice and increased in IGF-1-treated GHRKO mice. These observations differ dramatically from that found in GH-deficient Ames dwarf mice where we found that mitochondrial GSTM4 was higher in dwarf mice and GH treatment decreased protein expression (Rojanathammanee et al. 2013). These results suggest that the lack of an intact GH signaling pathway may affect both GST expression and translocation.

IGF-1 treatment lowered mitochondrial GSTZ1 protein levels in GHRKO mice to that of WT animals. This finding also differs from that reported in Ames dwarf mice, showing lower levels of GSTZ1 expression thus suggesting that GSTZ1 maybe regulated in part by plasma IGF-1 levels (Rojanathammanee et al. 2013).

We previously reported that GHRKO mice exhibited elevated levels of general GST activity in liver, kidney, and heart tissues (Brown-Borg et al. 2009). In the present study, GHRKO mice exhibited elevated 4-HNE-specific GST activities that were suppressed in the presence of IGF-1. Oxidative processes such as lipid peroxidation increase the generation of 4-HNE. Our current work in the GHRKO corresponds with the data recently reported in Ames dwarf mice treated with GH (Rojanathammanee et al. 2013), demonstrating that GH specifically suppresses the elevated 4-HNE GST activity exhibited in these animals. Liver F2-isoprostanes, lipid peroxidation products, have previously been shown to be lower at all ages in Ames dwarf compared to age-matched wild-type mice but no data is available in GHRKO mice (Choksi et al. 2007). These results suggest that GH and/or IGF-1 signaling can modulate GST activity against a specific substrate. Although there is evidence that the Nrf2/ARE transcriptional network plays a role in controlling rodent GST gene expression, we found no information suggesting substrate-specific regulation by hormones other than that showing thyroid hormones (T3, T4) and estrogen modulation of GST activities to CDNB and DCNB via M class isozymes (Coecke et al. 2000).

There are two thioredoxins that have been identified in mammals, Trx1 and Trx2, where Trx1 is localized in the cytoplasm/nucleus and Trx2 is localized in the mitochondria (Spyrou et al. 1997). Thioredoxin reductase is a selenoenzyme with three isoforms: TrxR1 (cytosolic), TrxR2 (mitochondrial), and TrxR3 (primarily localized to the testis; Amer 2009). In our study, gene expressions of the thioredoxin components, Trx1, Trx2, TrxR1, and TrxR2, were upregulated in

Fig. 10 The IGF-1 administration reduced the Grx2 mRNA expression in GHRKO mice. RT-PCR was performed to analyze Grx1 (a) and Grx2 (b) mRNA expression. Liver mitochondrial lysates were used to determine protein levels using Grx1 antibodies (c). The optical densities (O.D.) of mitochondrial (d) and cytosolic Grx1 (e) were analyzed. Liver mitochondrial (f) and

cytosolic fractions (g) were used to analyze Grx activity. Values represent mean $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.001 compared to WT saline. # p <0.05, ### p <0.001 compared to GHRKO saline. N =8 for each treatment group for gene and immunoblotting analyses, n =9–13 for each treatment group for enzyme activity assay

GHRKO mice and IGF-1 administration suppressed each gene examined. We observed similar effects of GH in Ames dwarf mice (Rojanathammanee et al. 2013). It has been reported that transgenic mice

overexpressing Trx are more resistant to a variety of oxidative stresses and exhibit extended median and maximum life spans compared to WT mice (Mitsui et al. 2002). The increased levels of Trx1 in transgenic

mice overexpressing this protein were associated with increased resistance to oxidative stress (Perez et al. 2011). In addition, oxidative damage to proteins and levels of lipid peroxidation were significantly lower in the livers of the Trx1 transgenic mice (Perez et al. 2011). It has also been shown that a Trx2 mutant in *Drosophila* is hypersusceptible to paraquat (Tsuda et al. 2010). Functional aspects of the defense capacity of the GHRKO compared to the Ames dwarf are observed in the resistance to various cellular stressors (UV light, hydrogen peroxide and paraquat; Salmon et al. 2005). Additionally, although catalase, GPX, and SOD levels in the GHRKO differ from that in the dwarf, the upregulated thioredoxin system in these mice may be the key factor in their antioxidative defense. Therefore, our findings suggest that GH/IGF-1 signaling may be involved in the regulation of the thioredoxin system and, thus, affect antioxidative defense, stress resistance, and potentially, life span extension.

In addition to the thioredoxin system, we also demonstrated that Grx2 was upregulated in GHRKO mice and that IGF-1 administration suppressed this expression. Grx2 was not elevated in Ames mice and GH had no additional effects. In the current study, cytosolic Grx activity was higher in GHRKO mice consistent with that found in Ames mice. The Grxs likely work in parallel and are able to compensate for the Trx systems by reducing both protein disulfides and GSH-mixed disulfides (Fernandes and Holmgren 2004). The antioxidative effects of the Trx and Grx pathways are associated with a conserved family of forkhead box subgroup “O” (FOXO) transcription factors, which freely bind to promoters of antioxidant enzymes and upregulate their expression in the absence of insulin/IGF-1 signaling pathway (Kops et al. 2002; Stone et al. 2012). Therefore, our findings support the suggestion that the Trx and Grx may be regulated in part by hormone status. Hormone status, in turn, may play an important role in protecting cells from oxidative stress and ultimately contribute to life span extension in GH-resistant GHRKO mice.

Conclusion

GHRKO mice are GH resistant due to a targeted disruption in the GH receptor resulting in a lack of circulating IGF-1. They live longer than their WT controls similar to Ames dwarf mice. However, the stress response

mechanisms such as GST, Grx, and Trx activities and protein expression in GHRKO mice are different from Ames mice. It is possible that the differences that GH and IGF-1 exert on glucose metabolism may affect these systems. Our data suggest that these stress resistance mechanisms (GSTs) are directly affected by circulating GH level and less so by IGF-1 in animals with an intact GH signaling system. Similarly, the components of Trx and Grx pathways may also be differentially affected by GH resistance versus GH deficiency if GH is the primary growth factor responsible for the alterations observed.

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