SHORT TAKE

Fibroblasts derived from long-lived insulin receptor substrate 1 null mice are not resistant to multiple forms of stress

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Summary

Reduced signalling through the insulin/insulin-like growth factor-1 signalling (IIS) pathway is a highly conserved lifespan determinant in model organisms. The precise mechanism underlying the effects of the IIS on lifespan and health is currently unclear, although cellular stress resistance may be important. We have previously demonstrated that mice globally lacking insulin receptor substrate 1 (Irs1−/−) are long-lived and enjoy a greater period of their life free from age-related pathology compared with wild-type (WT) controls. In this study, we show that primary dermal fibroblasts and primary myoblasts derived from Irs1−/− mice are no more resistant to a range of oxidant and nonoxidant chemical stressors than cells derived from WT mice.

Key words: aging; IRS1; NRF2; oxidative stress; stress resistance.

Introduction

It is evident that the insulin/insulin-like growth factor-1 signalling (IIS) pathway plays a conserved role in lifespan determination (Gems & Partridge, 2013). Precisely how the IIS elicits its effects is unclear, although stress resistance in longevity may be important. Several long-lived IIS mutant worms and flies are stress resistant compared with wild-type (WT) controls (Miller, 2009), and stress resistance correlates with longevity across species of mammals (Kapahi et al., 1999; Harper et al., 2007) and birds (Harper et al., 2011). An increased survival following paraquat or diquat injection has been reported in long-lived growth hormone (GH)-deficient Ames mice (Bokov et al., 2009), which have a secondary defect in IIS, and in female mice lacking a single copy of the IGF-1 receptor (Igf1R−/−) (Holzenberger et al., 2003; Bokov et al., 2011). A significant body of work has described broad-spectrum cellular resistance to various oxidative and nonoxidative stressors in GH dwarfs (Murakami et al., 2003; Salmon et al., 2005; Miller, 2009). While the precise mechanism underlying cellular stress resistance in GH dwarfs is unclear, it is associated with increased basal and arsenite-induced Nrf2 levels (Leiser & Miller, 2010), increased autophagy and reduced mTOR activity (Wang & Miller, 2012).

We previously reported that Irs1−/− mice were long-lived and had improved late-life health (Selman et al., 2008, 2011). Consequently, we predicted that Irs1−/− mice would demonstrate increased cellular stress resistance. To address this, we isolated and cultured dermal fibroblasts from young Irs1−/− and WT mice using published protocols (Murakami et al., 2003; Salmon et al., 2005; Leiser & Miller, 2010). Cells were exposed to hydrogen peroxide (H2O2), paraquat, cadmium chloride, arsenite and the DNA-alkylating agent methyl methanesulfonate (MMS) under both standard (21% oxygen) and physiological (3% oxygen) conditions in parallel experiments. Cytotoxicity was determined by calculating LD50 using probit analysis (Leiser & Miller, 2010). In contrast to our prediction, fibroblasts from Irs1−/− mice were not more resistant to cytotoxic stress at 21% oxygen (Fig. 1A–E) or 3% oxygen (Fig. S1A–E). Indeed, Irs1−/− cells were significantly more sensitive when treated with arsenite at 21% O2 (Fig. 1D). We also exposed primary myoblasts cultured at 3% oxygen (21% induced differentiation to myotubes) to H2O2 and paraquat and observed no genotypic effect on cellular resistance (Fig. 2A,B). Additionally, no differences in basal NFH2 or Keap1 protein levels (Fig. 2C,D), or in basal levels of Nrf1, Nrf2 and various antioxidant response element-dependent mRNA transcripts (Fig. 2E) were detected between WT and Irs1−/− fibroblasts. Cell size, proliferation rates and cell cycle distribution in cultured fibroblasts were similarly unaffected by genotype (Fig. S2A–C; P > 0.05 in all cases).

While both male and female Irs1−/− mice are long-lived (Selman et al., 2008, 2011), we did not observe a cellular stress resistance phenotype as predicted and as reported in long-lived GH-deficient mice (Murakami et al., 2003; Salmon et al., 2005; Leiser & Miller, 2010). However, a similar absence of cellular stress resistance has been reported in long-lived methionine-restricted and caloric-restricted (CR) mice (Harper et al., 2006). A potential explanation given for the different cellular stress phenotypes in GH dwarfs, methionine-restricted and CR mice was that epigenetic changes leading to cellular stress resistance may be manifested during development, rather than postnatally (Harper et al., 2006). However, we can discount this explanation in our study as the deletion event in Irs1−/− mice occurs during development. Long-lived homozygous Chico flies, which lack the single Drosophila IRS protein (Clancy et al., 2001), and long-lived brain-specific Igf1R−/− mice (Kappeler et al., 2008) are also not resistant to oxidative stress. It is currently unclear why different long-lived mouse models show such differences in cellular stress resistance. Irs1−/− mice, unlike GH dwarfs, appear to have...
Fig. 1 Fibroblasts from Irs1<sup>−/−</sup> mice are not more resistant to lethal stress than fibroblasts derived from WT mice at 21% O<sub>2</sub>. Each symbol represents fibroblasts derived from a single individual; the horizontal line indicates mean value for each group (n = 7–9). (A) H<sub>2</sub>O<sub>2</sub>, (B) paraquat, (C) cadmium, (D) arsenite and (E) MMS. *denotes P < 0.05. The test statistics and p values are reported in Table S1.

Fig. 2 Myoblasts from Irs1<sup>−/−</sup> mice are not more resistant to (A) H<sub>2</sub>O<sub>2</sub> and (B) paraquat lethal stress than myoblasts from WT mice at 3% O<sub>2</sub>, and basal protein levels of Keap1 and Nrf2 (C, D) and basal transcript levels of Nrf1, Nrf2 and associated targets in fibroblasts are unaltered in Irs1<sup>−/−</sup> mice relative to WT mice. Each symbol in (A) and (B) represents myoblasts derived from a single individual; the horizontal line indicates mean value for each group (n = 6). The test statistics and p values are reported in Table S2. (C) Representative western blots of Keap1 and Nrf2 from fibroblasts, (D) quantification of Keap1 and Nrf2 protein levels and (E) transcript levels of Nrf-1, Nrf-2, glutathione S-transferase alpha 1 (GSTA1), haem oxygenase-1 (HMOX), thioredoxin-1 (TXNRD), NAD(P)H quinone oxidoreductase 1 (NQO1) in fibroblasts from WT and Irs1<sup>−/−</sup> mice (n = 6) cultured at 21% O<sub>2</sub>, all target genes are corrected for the expression of Gapdh and Hprt. Data are normalized to WT values in all cases, P > 0.05. Mean ± SEM. Open bars = WT and filled bars = Irs1<sup>−/−</sup> mice.
Cells from Irs1<sup>−/−</sup> mice are not stress resistant, M. M. Page et al.

relatively normal somatotropic function and GH levels (Selman et al., 2008) and this may help explain the absence of a stress-resistant phenotype. In Ames mice, treatment with GH shortened lifespan, decreased cellular stress resistance (Panici et al., 2010) and attenuated components of the cellular detoxification pathway, including glutathione transferases (Rojanathammamee et al., 2013). As discussed elsewhere (Harper et al., 2006), it may be that the precise cell type demonstrating cellular stress resistance, and hence critical to longevity, may vary across different long-lived mouse models. For example, while fibroblasts from methionine-restricted and CR mice are not stress resistant, both models, unlike Snell and GHR-KO mice, are resistant to acetaminophen (Harper et al., 2006). In conclusion, our findings demonstrate that fibroblasts and myoblasts from Irs1<sup>−/−</sup> mice are no more stress resistant than those derived from WT mice, suggesting that cellular stress resistance does not underlie the extended healthy lifespan of Irs1<sup>−/−</sup> mice.

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**Conflict of interest**

There are no conflicts of interest to declare.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

**Table S1** LD<sub>50</sub> values of dermal fibroblasts isolated from WT and Irs1<sup>−/−</sup> mice exposed to lethal oxidative and non-oxidative stressors; cultured at 3% and 21% O<sub>2</sub>.

**Table S2** LD<sub>50</sub> values of myoblasts isolated from WT and Irs1<sup>−/−</sup> mice exposed to lethal oxidative stressors; cultured at 3% O<sub>2</sub>.

**Table S3** Primers used in the present study.

**Fig. S1** Fibroblasts from Irs1<sup>−/−</sup> mice are not more resistant to lethal stress than fibroblasts from WT mice at 3% O<sub>2</sub>.

**Fig. S2** Fibroblasts from Irs1<sup>−/−</sup> mice do not differ in terms of (A) proliferation rates, (B) cell cycle progression or (C) growth rates after 24 h or 48 h of culture (21% O<sub>2</sub>) compared with cells derived from WT mice (n = 4 per group).

**Data S1** Experimental Procedures.

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