SHORT TAKE

Impaired IGF1R signaling in cells expressing longevity-associated human IGF1R alleles

Cagdas Tazearslan, 1,* Jing Huang, 1,* Nir Barzilai1,2 and Yousin Suh1,2
1Departments of Medicine and Genetics, Albert Einstein College of Medicine, Bronx, NY 10461, USA
2Institute for Aging Research, Diabetes Research and Training Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Summary

Dampening of insulin/insulin-like growth factor-1 (IGF1) signaling results in the extension of lifespan in invertebrate as well as murine models. The impact of this evolutionarily conserved pathway on the modulation of human lifespan remains unclear. We previously identified two IGF1R mutations (Ala-37-Thr and Arg-407-His) that are enriched in Ashkenazi Jewish centenarians as compared to younger controls and are associated with the reduced activity of the IGF1 receptor as measured in immortalized lymphocytes. To determine whether these human longevity-associated IGF1R mutations affect IGF1 signaling, we engineered mouse embryonic fibroblasts (MEFs) expressing the different human IGF1R variants in a mouse IGF1 null background. The results indicate that MEFs expressing the human longevity-associated IGF1R mutations attenuated IGF1 signaling, as demonstrated by significant reduction in phosphorylation of both IGF1R and AKT after IGF1 treatment, in comparison with MEFs expressing the wild-type IGF1R. The impaired IGF1 signaling caused by the IGF1R mutations resulted in the reduced induction of the major IGF1-activated genes in MEFs, including EGR1, mCSF, IL3Rα, and TDAG51. Furthermore, the IGF1R mutations caused a delay in cell cycle progression after IGF1 treatment, indicating a dysfunctional physiological response to a cell proliferation signal. These results demonstrate that the human longevity-associated IGF1R variants are reduced-function mutations, implying that dampening of IGF1 signaling may be a longevity mechanism in humans.

Key words: human longevity; insulin/insulin-like growth factor-1 signaling; genetic variation; gene expression.

In recent years, there have been significant advances in our understanding of the pathways that modulate lifespan. The best characterized of all is the insulin/insulin-like growth factor-1 signaling (IIS) pathway. Complete or partial loss-of-function mutations in genes encoding components of the IIS pathway result in the extension of lifespan in yeast, worms, flies, and mice (Kenyon, 2010). This remarkable conservation throughout evolution suggests that altered IIS may also influence human lifespan. Recently, common genetic variations in the IIS candidate loci, including FOXO3A (Willcox et al., 2008; Flachsbart et al., 2009; Pawlikowska et al., 2009; Soerensen et al., 2010) and AKT1 (Pawlikowska et al., 2009), have been associated with human longevity. However, association studies typically leave open the question of whether an associated genetic variant is functionally important or can serve only as a genetic marker with the functional locus co-inherited on the polymorphic allele.

We previously identified two functionally significant IGF1R mutations (A37T and R407H) that are rare but enriched in Ashkenazi Jewish centenarians as compared to younger controls and are associated with the reduced activity of the insulin-like growth factor-1 (IGF1) receptor as measured in immortalized lymphocytes (Suh et al., 2008). As we do not have complete genotype information of the IGF1R mutation carriers, functional information obtained from immortalized lymphocytes from these subjects is correlational and cannot establish cause–effect relationship between the mutations and the associated phenotypes, e.g., longevity. Indeed, it is formally possible that the association of these IGF1R mutants and centenarian status might reflect variations of linked alleles, in this or another locus, that were not evaluated in our study.

To determine whether these human longevity-associated IGF1R mutations affect IGF1 signaling, we engineered mouse embryonic fibroblasts (MEFs) expressing the different human IGF1R variants in a mouse IGF1 null background (S1). After lentiviral transfection of IGF1−/− MEFS (Sell et al., 1994), we tested the differences in IGF1 signaling. The results show that, compared to MEFs expressing the wild-type (WT) IGF1R, MEFs expressing the human longevity-associated IGF1R variants, A37T (M1) and R407H (M2), showed attenuation of IGF1 signaling, as

*These authors contributed equally to this work.
1Present address: Aaron Diamond AIDS Research Center, New York, NY 10016, USA.

Accepted for publication 15 February 2011
demonstrated by significant reduction in the phosphorylation of both IGF1R and AKT after IGF1 treatment (Fig. 1). Of note, IGF1R expression at the protein (Fig. 1A) and RNA (data not shown) levels was similar in the engineered MEFs for all variants.

Once activated, IGF1R elicits the activation of a cascade of intracellular proteins leading to the regulation of gene expression, cell proliferation, or cell death (Kenyon, 2010). To test whether the longevity-associated IGF1R mutations affect gene expression, we measured the transcript levels of eight genes involved in mitogenesis, apoptosis, and differentiation processes (Cao et al., 1990), which are known to be up-regulated by IGF1 treatment (Dupont et al., 2001). We found that as compared to the WT, both A37T (M1) and R407H (M2) IGF1R mutations significantly reduced the expression of early growth response 1 (EGR1), T cell death-associated gene 51 (TDAG51), interleukin 3 receptor alpha (IL3RA), and macrophage colony-stimulating factor 1 (mCSF) in response to IGF1 treatment (Fig. 1E). Similarly, the expression of glial cell-derived neurotropic factor (GDNF) is attenuated in A37T (M1) cells, and the expressions of glycerol phosphate dehydrogenase 2 (GPDH2), the death domain-associated protein (DAXX), and TWIST are all attenuated in cells expressing the R407H (M2) mutation (Fig. 1E).
To investigate the functional consequences of differential IGF1 signaling and downstream gene expression caused by the longevity-associated IGF1R mutations, we measured cell cycle profiles. Figure 2 shows the results of fluorescence-activated cell sorting analysis of MEFs expressing different human IGF1R alleles either in serum-free media (SFM) or in SFM supplemented with IGF1. The cells expressing the longevity-associated IGF1R mutations clearly accumulated in the G1 phase of the cell cycle in SFM; 64.9% for M1 and 67% for M2 as compared to 53.7% for WT (both P < 0.05, Fig. 2A). After the cells were stimulated to proliferate by IGF1 (Fig. 2B), 50.4% of WT cells were in S phase vs. 39.1% of M1 cells and 33.9% of M2 cells (both P < 0.05), showing that the longevity-associated IGF1R mutations cause a defect in cell cycle progression.

In summary, we have demonstrated that the human longevity-associated IGF1R mutations cause functional impairments in IGF1 signaling, regulated gene expression, and cell cycle progression. The mechanistic parallels between the effects of IIS mutations in model organisms and those produced by longevity-associated IGF1R mutations in humans suggest that evolutionary conservation of IIS reduction and unusual longevity includes humans.

**Acknowledgments**

We thank Dr Renato Baserga (Kimmel Cancer Center, PA, USA) for the full-length human IGF1R cDNA and mouse IGF1r-/- mouse embryonic fibroblasts. This work was funded by NIH grant AG024391, AG027734, and AG17242. CT is a recipient of Ellison/AFAR postdoctoral fellowship.

**References**


Supporting Information

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Data S1 Materials and methods.

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