

Invited Mini Review

Growth signaling and longevity in mouse models

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Reduction of insulin/insulin-like growth factor 1 (IGF1) signaling (IIS) extends the lifespan of various species. So far, several longevity mouse models have been developed containing mutations related to growth signaling deficiency by targeting growth hormone (GH), IGF1, IGF1 receptor, insulin receptor, and insulin receptor substrate. In addition, p70 ribosomal protein S6 kinase 1 (S6K1) knockout leads to lifespan extension. S6K1 encodes an important kinase in the regulation of cell growth. S6K1 is regulated by mechanistic target of rapamycin (mTOR) complex 1. The v-myc myelocytomatosis viral oncogene homolog (MYC)-deficient mice also exhibits a longevity phenotype. The gene expression profiles of these mice models have been measured to identify their longevity mechanisms. Here, we summarize our knowledge of long-lived mouse models related to growth and discuss phenotypic characteristics, including organ-specific gene expression patterns. [BMB Reports 2019; 52(1): 70-85]

INTRODUCTION

Insulin/insulin-like growth factor 1 (IGF1) signaling (IIS) is evolutionarily conserved from worms to human (1). Decreased IIS has been related to longevity in an evolutionarily conserved manner (2), showing consistent results in major model organisms, including worms (1), flies (3), and mice (4). Human centenarian studies have revealed that variations are positioned at proteins involved in the IIS system (5). In rodents, IGF1 is mainly produced from the liver by stimulation of growth hormone (GH, also known as somatotropin) secreted from the somatotrophs in the anterior pituitary (6). Circulating IGF1 binds to insulin/IGF1 receptors on the cell membrane of organs and primarily promotes tissue growth at an early developmental stage. Insulin, the other binding substrate of the insulin/IGF1 receptors, is produced by pancreatic β cells in

response to postprandial nutrient influx (7). Insulin reduces high blood glucose levels after meals to maintain glucose homeostasis by suppressing glucose production in the liver, and stimulating glucose uptake in muscle and in fat by activation of their carbohydrate metabolism (8). This GH-IIS endocrine cascade axis is an excellent model for aging research to interpret how growth relates to metabolism. Insulin and IGF1 activate the IIS pathway through the insulin receptor (IR), IGF1 receptor (IGF1R), insulin receptor substrate 1 (IRS1), IRS2, phosphoinositide 3-kinase (PI3K), and protein kinase B (AKT) (7). Consequently, the IIS pathway changes gene expression through transcription factors, such as forkhead box O (FOXOs) and the v-myc myelocytomatosis viral oncogene homolog (MYC) (Fig. 1). In this review, we discuss physiological (Table 1) and global gene expression (Table 2) analyses in mice genetically modified to reduce GH-IIS axis in an effort to elucidate the longevity mechanism.

GH SIGNAL-DEFICIENT MICE

Several dwarf mouse models have been introduced in the aging research field, including Snell (disruption of the POU-domain transcription factor [Pit^{-/-}]), Ames (defect of the homeobox protein prophet of PIT-1 [Prop 1^{-/-}]), and Little (a missense mutation at growth hormone-releasing hormone receptor [Ghrhr^{-/-}]). The Snell and Ames mice are hypopituitary dwarf deficient in GH, prolactin (PRL), and thyroid-stimulating hormone (TSH). These dwarf mice have been shown to live 37-68% longer than their wild-type (WT) littermates (9-11). The Little mice are another GH-deficient model. It was generated by deletion of the Ghrhr gene and the mice have been shown to live 23-25% longer than their WT littermates (9). The Snell, Ames, and Little GH deficient mice commonly have small body sizes and high adiposity, with low IGF1 levels.

The mouse GH receptor (Ghr) gene was disrupted (GHR^{-/-}) to create a mouse model for human Laron syndrome (12). The GHR^{-/-} mouse has been shown to have very low (less than 10% of normal) plasma IGF1 level and severely reduced IGF binding protein 3 (IGFBP3) level (13). A GH receptor antagonist (GHA) transgenic (Tg) mouse was generated by a single amino acid substitution in GH (14). A 50% reduction in plasma IGF1 has been reported in GHA tg mice, compared to their WT littermates. Both GHR^{-/-} and GHA Tg mice have been shown

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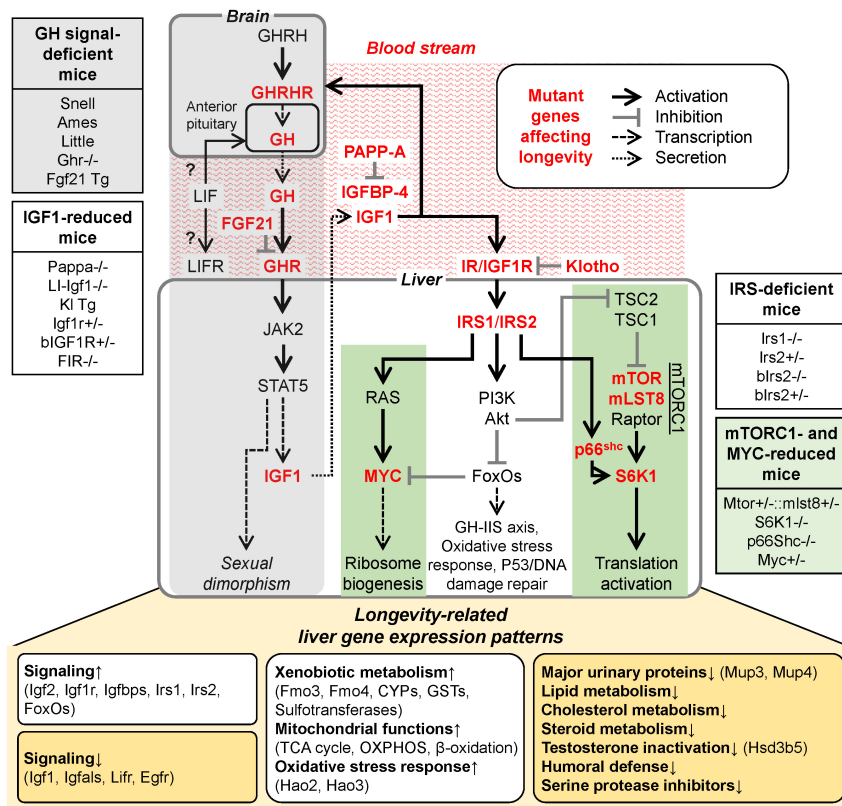


Fig. 1. GH-IIS axis and hepatic gene expression patterns in long-lived mice. Components of the GH-IIS axis impact on hepatic gene expression and affect longevity. We divided GH-IIS axis genetically-modified mouse models into four groups: 1) GH signal-deficient mice with mutation at genes affecting GH production and GH signaling, 2) IGF1-reduced mice with homozygous and heterozygous mutation at genes involved in IGF1 production, including IGF1R and IR, 3) IRS-deficient mice including homozygous and heterozygous mutations of *Irs1* and *Irs2*, and 4) mTORC1- and MYC-reduced mice with homozygous and heterozygous mutations for reduction of mTORC1 levels, lack of S6K1, p66Shc deficiency, and lower MYC levels. The target genes for long-lived mouse models are written in bolded red. Downstream liver gene expression patterns for longevity are displayed on the bottom panel.

to express obese and slow growth phenotypes (13, 15, 16), as well as small organs, including liver and kidney (17). *GHR^{-/-}* mice have been reported to live longer (30-55%) than their WT littermates (13), but *GHA Tg* mice lived as long as their WT littermates (17).

Fibroblast growth factor-21 (FGF21) is an endocrine hormone related to energy metabolism and stress response (18). FGF21 binds to the transmembrane protein β Klotho, which has a protein sequence similar to Klotho (Kl). While studies have shown that *Kl Tg* mice overexpressing Kl lived longer compared to WT mice, β Klotho^{-/-} mice lived as long as WT mice (19). Fasting has been shown to increase FGF21 levels, causing GH resistance by inactivation of downstream signal transducer and activator of transcription 5 (STAT5), and down-regulation of *Igf1* gene (20). Indeed, FGF21-overexpressing *Tg* mice were shown to have small body sizes (60% of WT), decreased serum insulin and IGF1 levels, and a 36% extended lifespan (21). These results suggest that FGF21 is an upstream regulator of the IIS pathway.

The effect of overexpression of GH in *Tg* mice has been studied by insertion of the GH gene from other species, including human, rat, bovine, and ovine. Most GH *Tg* mice showed a high plasma GH level and a giant body size (6, 22). Interestingly, bovine GH (bGH) *Tg* mice exhibited lean bodies

(16) and a 45% shorter lifespan than WT mice (22). In addition, bGH *Tg* mice had kidney damage, namely nephropathy with glomerular enlargement (23, 24). Human patients having an excess of GH show large hands, feet, face and internal organs, in a condition called acromegaly (25). Acromegaly patients also have lean bodies, heart problems, a high risk of thyroid cancer and colorectal cancer, and often have insulin-resistance or some diabetes (25, 26).

GLOBAL HEPATIC GENE EXPRESSION ALTERATION IN GH-RELATED MODEL MICE

GH stimulates its primary target, the liver, causing it to produce IGF1. A number of microarray analyses have identified genes for longevity mechanism using liver tissues taken from Ames and Snell dwarf mice (27-34). Although early studies identified only a few genes (27, 28), later studies using high-density oligonucleotide microarrays that can detect > 12,000 RNA transcripts identified hundreds of differentially expressed genes (DEGs) as candidates in the aging mechanism. Papaconstantinou *et al.* reported hepatic DEGs using young (3-6 months of age) Snell male mice. Major down-regulated DEGs included *Igf1*, Insulin like growth factor binding protein acid labile subunit (*Igfals*), and Forkhead box C1 (*Foxc1*).

Table 1. Phenotypic characteristics of mice with mutations affecting the GH-IGS axis and lifespan

Mice models	Key Physiology	Average Lifespan (%)	Body weight (%)	Body fat	Insulin sensitivity	Tumor incidence	Stress resistance	Plasma GH	Plasma IGF1	Plasma glucose	References
Long-lived models											
Snell (<i>Pou1f1</i> ^{-/-})	GH, PRL and TSH deficiency	F + 51 M + 29 (11718806) F and M + 38 (15699523)	↓ (25-33)	↑	↑	↓	↑	↓	↓	↓	16577229 1371619 11718806 15699523
Ames (<i>Prop1</i> ^{-/-})	GH, PRL and TSH deficiency	F + 68 M + 49 (89000272) F and M + 37 (11719795)	↓ (33)	↑	↑	↓	↑	↓	↓	↓	89000272 11719795
Little (<i>Ghrhr</i> ^{-/-})	GH deficiency	F + 25 M + 23 (11371619)	↓ (50-67)	↑	↑	↓	↑	↓	↓	↓	1270792 11371619
<i>Ghr</i> ^{-/-}	GH resistance	F + 38 M + 55 (10875265) F + 25 M + 16 (12933651) F + 30 M + 30 (16682650)	↓ (41-50)	↑	↑	↓	↑	↑	↓	↓ or ↔	10875265 12933651 16682650
<i>Fgf21</i> Tg*	GH resistance	F + 40 M + 30 (23066506)	↓ (60)	↔	↑	?	?	↑	↓	↓	18585098 23066506
<i>Ll-Igf1</i> ^{-/-}	IGF1 reduction 75-85%	F + 16 (21799924)	↓ (75-100)	↓	↓	?	?	↑	↓	↔	10359843 11423474 21799924
Heart <i>Igf1</i> Tg*	IGF1 excessed	M + 23 ^a (17973971)	↔ (100)	?	?	?	?	?	↑	↔	17973971
<i>Pappa</i> ^{-/-}	IGF1 reduced	F and M + 38 (17681037) F + 29 M + 24 (20351075)	↓ (40)	?	↔	↓	?	↔	↔	↔	14973274 17681037 20351075
<i>Klotho</i> Tg	IIS inhibition	F + 19 M + 31 (16123266) F + 19 M + 20 (16123266)	↔ (100)	?	F ↔ M ↓	?	↑	?	?	↔	16123266
<i>Igf1r</i> ^{+/-}	IGF1 resistance	F + 33 M NS (12483226) F and M NS (22132081)	↓ (90)	?	↓	↔	↑, F ↑ M ↔	?	↔	↔	12483226 22132081
Brain <i>IGF1R</i> ^{+/-} *	Brain IIS reduction	F + 8 M + 13 ^b (18959478)	↓ (90-92)	↑	↓	↓	↑	↓	↓	↔	18959478
<i>FIR</i> ^{-/-}	Fat-specific insulin resistance	F and M + 11 (12543978)	↓ (70)	↓	↑	?	?	?	?	↔	12110165 12543978
<i>Irs1</i> ^{+/-} *	Insulin resistance or normal	F + 16 M + 14 (21283571)	↓ (70)	↓	↓	?	?	↔	↔	↔	7969452 17928362 21283571 29779018
<i>Irs2</i> ^{+/-}	IRS2 reduced	F and M + 17 (17641201) F and M NS (17928362)	↔ (100)	?	↑ or ↔	?	?	↔	↔	↓	17641201 17928362
Brain <i>Irs2</i> ^{+/-}	Brain IRS2 reduced	F and M (+/-) + 18 (17641201)	↔ (100)	↑	↓	?	?	↔	↔	?	17641201
Brain <i>Irs2</i> ^{+/-} <i>mtor</i> ^{+/-} :: <i>mlst8</i> ^{+/-} *	Brain IRS2 reduced mTORC1 reduction 50%	F and M (-/-) + 14 (17641201) F + 14 M NS (22461615)	↔ (100)	?	↔	?	?	?	?	↔	22461615
<i>S6K1</i> ^{-/-} *	S6K1 deficient	F + 20 M NS (19797661)	↓ (48)	↓	↑	?	?	↔	↔	↔	19797661
<i>p66Shc</i> ^{+/-}	IRS inhibition	F and M (+/-) + 7 ^b (10580504)	↔ (100)	↓	↔	?	↑	?	?	↔	10580504
<i>p66Shc</i> ^{-/-}	S6K1 inhibition	F and M (-/-) + 28 ^b (10580504)	↔ (100)	↓	↔	?	?	?	?	↔	20624962
<i>Myc</i> ^{+/-} *	IGF1 reduction and MYC-regulated genes	F + 21 M + 11 ^a (25619689)	↓ (81)	↔	?	↔	?	?	↓	?	25619689

Table 1. Continued

Mice models	Key Physiology	Average Lifespan (%)	Body weight (%)	Body fat	Insulin sensitivity	Tumor incidence	Stress resistance	Plasma GH	Plasma IGF1	Plasma glucose	References
Normal or short-lived models											
GHA Tg	GH resistance	NS (12933651)	↓ (55-70)	↑	↑	↓	?	?	↓	↓ or ↔	1791834 11487276 12933651 15231300
Bovine GH Tg	GH excessed	M-45 (14583653)	↑ (200)	↓	↓	↑	?	↑	↑	↓	1791834 11701438 14583653 15231300 18948397
LID	75% reduced IGF1	M-? (22421704)	↔ (100)	↑	↓	↓	?	↑	↓	↔	10377413 11334415 22421704
KI ^{-/-} *	IIS activation, growth retardation	60.7 days ^c (9363890)	↓ (44-47)	?	↓	?	?	?	?	?	9363890
Irs2 ^{-/-}	Irs2 deletion	F-26 M+84 (9495343)	↓ (90)	?	↓	?	?	?	?	↑	9495343

* New added or updated mice models in this review.

↑ up-regulated; ↓ down-regulated; ↔ no difference; ?, no data available.

^aMedian lifespan. ^bSignificant at censored lifespan data before week 130-135. ^cNo lifespan data for control. NS, not significant.

Table 2. Transcriptome studies from long-lived mouse models for the GH-IGS axis

Genotype	Publication	Design (age; month)	Study Focus	Sex	Back-ground	Tissue	Platform (probesets)	DEG number (statistical criteria)
GH-deficient mice								
Snell	Dozmorov et al. 2002 11867646	WT vs Snell (6)	Systemic GH effects	Male	(DW × C3H)/F1	Liver	(2,352) Clontech Mouse Atlas 1.2 array	17 (t-test FDR < 0.05)
Snell	Boylston et al. 2004 15379852	WT vs Snell (3-5, 24-28)	Systemic GH effects	Male	C3H/HeJ × DW/J F1 or C3DW	Liver	(12,422) Affymetrix MG U74Av2 array	> 50 (t-test, P < 0.05 and FC > 2)
Snell	Papaconstantinou et al. 2005 15888324	Snell ctrl(+/?) vs Snell (3-6, 20-28)	IGF-1 axis protein levels	Male	C3H/HeJ × DW/J F1 or C3DW	Liver	(12,422) Affymetrix MG U74Av2 array	17 (t-test P < 0.05)
Snell	Boylston et al. 2006 19943135	Snell ctrl(+/?) vs Snell (4-6, 24-26)	Systemic GH effects	Male	C3H/HeJ × DW/J F1 or C3DW	Liver	(12,422) Affymetrix MG U74Av2 array	205 (t-test FDR < 0.05)
Ames	Dozmorov et al. 2001 11213270	WT vs Ames (5, 13, 22)	Systemic GH effects	?	?	Liver	(265) early version of spotting array	13 (t-test P < 0.01)
Ames	Tsuchiya et al. 2004 15039484	WT vs Ames (2)	Systemic GH effects	Female	?	Liver	(12,422) Affymetrix MG U74Av2 array	212 (two-way ANOVA FDR < 0.05)
Ames	Amador-Noguez et al. 2004 15569359	WT littermate vs Ames (3, 6, 12, 24)	Systemic GH effects against wt aging patterns	Male	C57Bl/6	Liver	(> 14,000) Affymetrix MOE340A array	122 (t-test Bonferroni < 0.05 and FC > 2)
Ames	Boylston et al. 2006 19943135	Ames ctrl(+/?) vs Ames (4-6, 12-14, 24-27)	Systemic GH effects	Male	C57Bl/6	Liver	(> 34,000) Affymetrix MG 430 2.0 arrays	785 (t-test FDR < 0.05)
Little	Amador-Noguez et al. 2004 15569359	WT vs Little mice (3,6,12,24)	Systemic GH effects against wt aging patterns	Male	C57Bl/6	Liver	(> 14,000) Affymetrix MOE340A array	104 (t-test Bonferroni < 0.05 and FC > 2)
bGH Tg	Olsson et al. 2003 12736163	WT vs bGH-Tg (6)	Systemic GH effects	Male	?	Liver	(11,000) Affymetrix mouse 11ka and 11kb arrays	45 (difference call parameter 3-4)
Ghr	Miller et al. 2002 12403853	WT vs Ghr ^{-/-} (8-9)	Systemic GH effects	?	?	Liver	(2,352) Clontech Mouse Atlas 1.2 array	10 (t-test FDR < 0.05)
Ghr ^{-/-}	Rowland et al. 2005 15601831	WT vs Ghr ^{-/-} (1.4)	Systemic GH effects	Male	B6.C3H-6T	Liver	(12,422) Affymetrix MG U74Av2 array	398 (t-test P < 0.0005 and FC > 1.5)
GHR-569, GHR-391	Rowland et al. 2005 15601831	WT vs GHR-569, GHR-391 (1.4)	Systemic STAT5 effects	Male	129/SVJ	Liver	(12,422) Affymetrix MG U74Av2 array	20 (t-test P < 0.0005 and FC > 1.5)
Ghr ^{-/-} , GHR-391	Barclay et al. 2011 21084450	WT vs Ghr ^{-/-} , GHR-391 (4)	STAT5-mediated effects	Male	C57Bl/6j	Liver	(46,632) Illumina MouseWG-6 v1.1 array	55 common genes out of 466 (t-test P < 0.05, FC > 2)
Ghr ^{-/-}	Stout et al. 2015 26436954	WT vs Ghr ^{-/-} (6)	Systemic GH effects on WAT and BAT	?	?	WAT and BAT	(28,853) Affymetrix GeneChip Mouse Gene 1.0 ST Array	346 in SubQ WAT, 319 in PERI WAT, 192 in EPI WAT, 280 in BAT (t-test FDR < 0.05 and FC > 1.5)

Table 2. Continued

Genotype	Publication	Design (age; month)	Study Focus	Sex	Back-ground	Tissue	Platform (probesets)	DEG number (statistical criteria)
<i>Chr^{-/-}</i>	Chang et al. 2016 27064376	WT vs <i>Chr^{-/-}</i> (3.7-4.2)	Systemic GH effects on lncRNA and miRNA	Male and Female	C57Bl/6J	Liver	(25,376 transcript, 31,423 lncRNAs, 3,1000 miRNAs) Agilent Mouse LncRNA Array v2.0	3,442 lncRNAs, 1,750 miRNAs, 30 miRNAs in female, 2,642 lncRNAs, 1,489 miRNAs, 57 miRNAs in male (FC ≥ 2)
<i>Fgf21-Tg</i>	Zhang et al. 2012 23066506	WT vs <i>FGF21^{-/-}</i> (3)	Systemic FGF21 effects	Male	C57Bl/6J	Liver, WAT, Muscle	(> 25,600) Illumina mouseRef-8 v2.0 BeadChip	33 in liver, 8 in muscle, 22 in fat (t-test FDR < 0.1 and FC > 2)
Sex-specific liver gene expression								
Ames	Amador-Noguez et al. 2005 15925325	WT vs Ames (3-6)	Sexual dimorphism of GH effects	Male vs Female	C57Bl/6	Liver	(> 14,000) Affymetrix MOE430A array	381 sex-independent, 110 sex-dependent (two-way ANOVA P < 0.001 and FC > 1.5)
<i>STAT5b^{-/-}</i>	Clodfelter et al. 2006 16469768	WT vs <i>STAT5b^{-/-}</i> (1.8-2.1)	Sexual dimorphism of STAT5b effects	Male vs Female	?	Liver	(23,574) Agilent Sosepta/Merck Mouse TOE 75k array	85 in female (t-test P < 0.05, FC > 1.5)
<i>STAT5a^{-/-}</i>	Clodfelter et al. 2007 17536022	WT vs <i>STAT5a^{-/-}</i> (1.4-2.1)	Sexual dimorphism of STAT5a effects	Male vs Female	129 x Black/Swiss	Liver	(23,574) Agilent Sosepta/Merck Mouse TOE 75k array	769 in male, 461 in female (t-test P < 0.05, FC > 1.5)
IGF1- and IIS-reduced mice								
<i>FIR^{-/-}</i>	Bluhner et al. 2004 15131119	WT vs WAT (3)	Fat-specific IR & Adipocyte size effect	Male	C57Bl/6 x 129/Sv	WAT	(12,488) Affymetrix MG-U74A-v2 array	48 for adipocyte size, 63 for IR signaling (t-test P < 0.001 and $\mu 1+\mu 2 > 2SD$)
<i>FIR^{-/-}</i>	Katic et al. 2007 18001293	WT vs <i>FIR^{-/-}</i> (6, 18, 30-36)	Fat-specific IR effect	Male	129sv/C57Bl6J	WAT	(22,690) Affymetrix mouse 430A array	? (ANOVA P < 0.01)
<i>Irs1^{-/-}</i>	Selman et al. 2008 17928362	WT vs <i>Irs1^{-/-}</i> (2.6, 15, 22)	Systemic IIS effects	Female	C57Bl/6	Liver	(> 34,000) Affymetrix MG 430 2.0 array	? (t-test P < 0.05)
<i>Irs1^{-/-}</i>	Page et al. 2018 29779018	WT vs <i>Irs1^{-/-}</i> (15)	Systemic IIS effects	Female	C57Bl/6	Liver, Muscle, Brain, WAT	RNA-sequencing by Thermo Fisher Ion Proton system	124 (101, specific) in liver, 185 (137) in muscle, 111 (94) in brain, 344 (319) in WAT (t-test FDR < 0.1)
mTORC1- and MYC-reduced mice								
<i>S6K1^{-/-}</i>	Selman et al. 2009 19797661	WT vs <i>S6K1^{-/-}</i> (20)	Systemic S6K1 effect	Female	C57Bl/6	Liver, Muscle, WAT	(> 38,000) Affymetrix MG 430 2.0 array	1,843 in liver, 2,309 in muscle, 1,970 in WAT (q-value < 0.01)
<i>p66Shc^{-/-}</i>	Tomilov et al. 2010 19892704	WT vs <i>p66Shc^{-/-}</i> (3, 12)	Systemic p66Shc effect	Male and Female	?	Liver, Spleen, Lungs, WAT, peritoneal macrophage	(> 38,000) Affymetrix MG 430 2.0 array (12,422) Affymetrix MG U74Av2 array	top 250 genes (t-test P < 0.05)
<i>Myc^{+/-}</i>	Hofmann et al. 2015 25619689	WT vs <i>Myc^{+/-}</i> (5, 24)	Systemic MYC effects	Male	C57Bl/6NChI	Liver, Muscle, WAT	(28,853) Affymetrix Mouse 1.0 Gene ST arrays	307 in liver, 160 in muscle, 412 in WAT (t-test FDR < 0.05 and FC > 1.5)

Major up-regulated DEGs included Igf2, Igf1 receptor (Igf1r), Igf binding protein 1 (Igfbp1), Igfbp2, Igfbp3, Irs1, Irs2, and Foxp1 (33).

Tsuchiya *et al.* identified 212 DEGs (FDR < 0.05) in Ames dwarf mice livers, including two genes encoding Forkhead transcription factors (Foxa3 was up-regulated and Foxa2 was down-regulated), as well as up-regulated xenobiotic metabolism and glutathione synthesis genes (31). Another transcriptome study using Ames dwarf mice livers showed hundreds of genes affected by aging. Amador-Noguez *et al.* reported that 357 DEGs (P < 0.001) were associated with age progression (3, 6, 12, and 24 months old) in Ames dwarf male mice livers. In particular, they found up-regulated genes for cholesterol and steroid biosynthesis (29). They also found an increase in Igf1 transcript levels during aging, as well as progressive decreases of Igfbp1 and Igfbp2 transcript levels (29). Boylston *et al.* identified 49 DEGs (FDR < 0.05) in livers from both Snell and Ames dwarf male mice, at both young and old ages. They used 4-6 month old (young age) and 24-26 month old (old age) for Snell mice, and 4-6 month old, 12-14 month old (middle age) and 24-27 month old for Ames mice. Among the DEGs, they found up-regulated genes encoding cytochrome P450 (CYP), and Flavin containing monooxygenase 3 (Fmo3). Fmo3 encodes a flavin adenine dinucleotide (FAD)-dependent monooxygenase that metabolizes various sulfur- and nitrogen-containing metabolites (35). There was a down-regulation of Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 5 (Hsd3b5). The Hsd3b5 gene plays important roles in the biosynthesis of various steroid hormones (36). The Hydroxyacid oxidase 2 (Hao2) gene, which encodes a flavin mononucleotide (FMN)-dependent peroxisomal 2-hydroxy acid oxidase for long chain 2-hydroxy acid substrates was up-regulated (37).

Using Ames and Little male mice, 547 common DEGs (P < 0.001) were identified in liver during the aging process in 3, 6, 12, and 24 month old mice (29). Gene ontology (GO) analysis showed that the up-regulated DEGs are involved in the oxidative stress response, xenobiotic metabolism, mitochondrial respiration and β -oxidation, and the down-regulated DEGs are involved in humoral defense, including the complement system regulatory genes.

Using GHR^{-/-} male mice (1.5 months old), 330 DEGs were identified in the liver. Up-regulated DEGs were those for β -oxidation, oxidative phosphorylation (OXPHOS), tricarboxylic acid (TCA) cycle, and factors for transcription and translation. Serine protease inhibitors were down-regulated DEGs (38). Using two GHR site-specific truncated mutants, GHR-569 and GHR-391 mouse strains, phosphorylation status of STAT5 was either decreased or removed in the liver by bGH treatment, respectively (38). GHR-STAT5 signaling was severely weakened in these two mutant mice. Using these two mutant mice, 20 common DEGs (P < 0.0005 and FC > 1.5) were identified in the liver (38). Down-regulated DEGs included Igf1, Igfals, Epidermal growth factor receptor (Egfr), and Hsd3b5. Up-

regulated DEGs included Sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 2 (Sult2a2), Angiogenin, ribonuclease, RNase A family, 5 (Ang), and Hao2. Sult2a2 and Ang play roles in the sulfation of hydroxysteroid and host defense, respectively. Up-regulated DEGs also included several genes encoding CYPs and glutathione S-transferases (GSTs) (38). Additional studies of GHR-391 and GHR^{-/-} mice livers showed that the loss of GHR-STAT5 signaling caused intracellular lipid accumulation and steatosis by increasing phosphorylation of STAT1 and STAT3 proteins (39). A recent study using young GHR^{-/-} male and female mice (16-18 weeks old) showed a transcript regulatory network for steroid hormone biosynthesis in the liver, composed of several long-noncoding RNA and microRNAs (40).

Using 3 month old young Fgf21 Tg male mice, transcriptome analysis identified 33, 22, and 8 DEGs (FDR < 0.1 and FC > 2) from the liver, epididymal white adipose tissue (WAT), and gastrocnemius, respectively (21). The hepatic DEGs included up-regulation of Fgf21, Fmo3, Cytochrome P450, family 2, subfamily b, polypeptide 9 (Cyp2b9), and Metallothionein 1 (Mt1) genes; and down-regulation of Igfals, Hsd3b5, Major urinary protein 4 (Mup4), Glutathione S-transferase, pi 1 (Gstp1), and Sterol carrier protein 2 (Scp2) genes (21). The authors compared the hepatic DEGs they found to 43 common hepatic DEGs from the four long-lived dwarf strains, including Snell, Ames, Little, and GHR^{-/-} mice (41). They found eight overlapped genes, including down-regulated Igfals, Hairy and enhancer of split 6 (Hes6), Aminolevulinic acid synthase 2, erythroid (Alas2), Cyp4a12b, Mup4, Serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12 (Serpina12), and Hsd3b5; as well as up-regulated Fmo3 (21). Although only a small number of DEGs were found in the liver, white fat and skeletal muscle, the young long-lived Fgf21 Tg male mice showed dramatic phenotypical and physiological changes, including low body weight and low levels of plasma IGF-1, insulin, and glucose, as well as high levels of plasma ketone bodies (21).

META-ANALYSIS OF TRANSCRIPTOME FROM GH SIGNAL-DEFICIENT MICE

Swindell performed a meta-analysis using microarray data obtained from livers of Snell, Ames, Little, and GHR^{-/-} mice. DEG selection (mean difference significance test P < 0.05) revealed different DEG numbers among these mice models. The GHR^{-/-} mice showed the smallest number of DEGs (46 DEGs), and other mice strains showed hundreds of DEGs: 151-316 DEGs from Snell, 400-987 DEGs from Ames, and 475-650 DEGs from Little (41). The range in number of DEGs was due to different ages of the mice. The results revealed that these mice strains shared strong down-regulation of Igf1 (41). Comparison of gene expression across these four dwarf mice identified 13 common DEGs, including 3 up-regulated genes:

Hao3, Sult2a2, and Serine peptidase inhibitor, Kazal type 3 (Spink3). It also identified 10 down-regulated genes: Mup3, Mup4, Igf1, Igfals, Egfr, Socs2, Leukemia inhibitory factor receptor alpha (Lifr), Carboxylesterase 3A (Ces3a: also known as Es31), Hsd3b5, and Kidney expressed gene 1 (Keg1) (41). One down-regulated common DEG, Lifr, has not yet been studied for its role in aging and longevity. The Lifr gene encodes a receptor for the leukemia inhibitory factor (LIF) cytokine that affects various biological functions, including stem cell self-renewal, the reproductive process, and bone modeling (42). Interestingly, LIF can affect the anterior pituitary gland to decrease somatotroph, lactotroph and gonadotroph cells (43).

Malfunction of genes for DNA repair, such as Excision repair cross-complementing rodent repair deficiency, complementation group 1 (Ercc1), Ercc6 (also known as CSB), and Xeroderma pigmentosum, complementation group A (Xpa), caused progeroid syndromes in DNA repair-deficient mouse models. *Csbm/m::Xpa^{-/-}* and *Ercc1^{-/-}* mice showed a severe phenotype, *Ercc1^{-Δ7}* showed intermediate, *Csbm/m* showed mild, and *Xpa^{-/-}* showed no phenotype in mouse models (44, 45). Interestingly, *Csbm/m::Xpa^{-/-}* progeria mice showed reduction of serum IGF1 and glucose levels, and suppression of the GH-IIS axis (45). Schumacher *et al.* performed a meta-analysis to compare transcriptome data between progeria mice (*Csbm/m::Xpa^{-/-}*, *Ercc1^{-/-}*, *Ercc1^{-Δ7}*, *Csbm/m* and *Xpa^{-/-}*) and long-lived dwarf mice (Snell, Ames and Little) (46). Strikingly, the hepatic transcriptome data showed a high correlation ($r > 0.7$) in gene expression patterns. They found a trend for down-regulation of genes involved in the GH-IIS axis, oxidative metabolism, and energy metabolism, and an up-regulation trend in genes involved in the stress response (46).

SEXUAL DIMORPHISM OF TRANSCRIPTOME IN LIVER

At the GH signal-deficient models including Snell, Ames, *GHR^{-/-}*, and *Fgf21 Tg* mice, female models showed a greater difference of lifespan than male models (Table 1). GH secretion from the pituitary gland is stimulated by sex-steroids (47), and there is a different pattern of secretion between males and females (48). This indicates sexual dimorphism, including global gene expression in the liver (49-51).

Amador-Noguez *et al.* studied sexual dimorphism in the liver using 126 DEGs ($P < 0.001$ and $FC > 1.5$) between female and male WT mice (32). They found that genes involved in fatty acid biosynthesis and steroid hormone metabolism, especially *Hsd3b2*, *Hsd3b3*, *Hsd3b5*, and *Hsd3b6* encoding a hydroxysteroid dehydrogenase, were down-regulated in female WT mice. In contrast, genes for metabolic enzymes including *Fmo3*, metallothioneins, and peroxisomal acyl-CoA thioesterases were up-regulated in female WT mice. Particularly, xenobiotic metabolizing CYP genes were dominantly up-regulated in female WT mice. Interestingly, sex-specific expression of those 126 DEGs in WT were almost completely

lost in Ames dwarf mice (32). The complete loss of sexual dimorphism in the long-lived Ames dwarf mice might reflect a reduction of reproduction that needs a costly physiological investment. This may be a potentially important determinant of the extended longevity of these mice.

JAK2 phosphorylates GHR serving as a docking site for STAT transcription factors in GH signaling in the liver. There are seven mouse STAT proteins, and STAT5a and STAT5b proteins play key roles in sex-specific hepatic gene expression (52, 53). In young (1.4-1.8 month old) mice, STAT5a regulated 23% (89 out of 393 genes) of the sex-specific gene expression, including down-regulation of Y-linked *Ddx3y*, *Eif2s3y*, and *Jarid1d* genes, up-regulation of *Sult* (sulfotransferase) genes, and both up- and down-regulation of CYP genes in female livers (53). STAT5b regulated 90% (767 out of 950 genes) of down-regulated genes and 61% (461 out of 753) of up-regulated genes in females (52). STAT5b-dependent sex-specific genes were primarily involved in oxidative metabolism, tryptophan and fatty acid metabolism, steroid metabolism, signaling, xenobiotic metabolism, and serine protease inhibition (52).

IGF1 AND IIS-REDUCED MICE

The major proportion of circulating IGF1 is produced by the liver (54). In the liver, by the binding of GH, GHR signaling transmits through JAK-STAT, and consequently activates IGF1 transcription, production, and secretion into the blood stream (6). Circulating IGF1 bioavailability is mediated by IGF binding proteins (IGFBPs). Deletion of the *Pappalysin-1* that cleaves IGF1 bound to IGFBP4 decreased bioavailability of IGF1 (55). Indeed, *Pappalysin-1^{-/-}* mice showed 40% smaller body mass, lower cancer incidence, and a 24-38% longer lifespan than WT controls (56-58).

Igf1-deficient (*Igf1^{-/-}*) mice showed variable neonatal lethality in several different genetic backgrounds such as 129/Sv mice (10% survival), C57BL/6J x 129/Sv F1 hybrids (16% survival), and MF1 x 129/Sv F1 hybrids (68% survival) (59). The surviving *Igf1^{-/-}* mice showed 24-40% lower body weights and increased maximum lifespan, but no significant change in mean lifespan (60). Although their body mass was lower than WT (WT 39 g vs *Igf1^{-/-}* 30 g for male; WT 28 g vs *Igf1^{-/-}* 17 g for female), there was almost no impact on the size of major organs, including liver (*Igf1^{-/-}* 1.37 g vs WT 1.39 g), kidney (*Igf1^{-/-}* 0.21 g vs WT 0.21 g), brain (*Igf1^{-/-}* 0.44 g vs WT 0.47 g), and heart (*Igf1^{-/-}* 0.16 g vs WT 0.22 g) (60). The IGF1 receptor (IGF1R) deficient (*Igf1r^{-/-}*) mice show respiratory failure at birth that is 100% lethal (59). Similar to *Igf1r^{-/-}* mice, homozygous insulin receptor (*InsR*)-deficient mice suffered from hyperglycemia and died soon after birth (61). Heterozygous *InsR* knockout (*IR^{+/-}*) male mice showed heteroinsufficiency, including insulin insensitivity and a 20% extended maximum lifespan, but no significant change in average lifespan (62).

Both inducible liver-specific Igf1-disruption mice (LI-Igf1^{-/-}) (54), and liver-specific Igf1-deficient mice (LID mice) (63) showed a 75% decrease IGF1 levels and high GH levels in serum. Igf1 expression was shut off after ~28 days for LI-Igf1^{-/-} mice (54) and after ~10 days in LID mice (63). LI-Igf1^{-/-} female mice, but not male mice, lived 16% longer than WT mice (64), whereas male LID mice exhibited a shortened lifespan (65).

Heterozygous Igf1r^{+/-} mice exhibited 8% smaller body sizes and ~40% higher serum IGF1 level than those of WT mice (4). Increased serum IGF1 level is a compensatory response to reduced IGF1 receptor levels. Igf1r^{+/-} mice also showed a low cellular IGF1 signaling activity because of glucose tolerance in the liver (4). Igf1r^{+/-} mice lived 26% longer than WT controls, 33% longer for female mice and 16% longer for male mice, though the male difference was not statistically significant (4). Another independent study using Igf1r^{+/-} mice showed negligible changes in lifespan (66). Ladiges *et al.* discussed that the different results were due to the unusually short lifespan of WT mice in the original study because of sub-optimal husbandry conditions, and may lead to incorrect conclusions about the longevity of Igf1r^{+/-} mice, in comparison (67).

Klotho (Kl) encodes a circulating hormone and functions as an antagonist against IR and IGF1R, increasing insulin resistance (68). Kl^{-/-} mice die early at < 100 days of age and show systemic aging phenotypes: growth retardation (small body size, and atrophy of genital organs and thymus), hypokinesia, arteriosclerosis, ectopic calcification in various organs and arterial walls, osteoporosis, and skin atrophy (69). In contrast, Kl overexpression significantly extended lifespan (19-31%) of Kl Tg mice through GH-independent suppression of the IIS pathway (68). Inhibition of IIS by additional genetic interventions, like Kl^{-/-}::IRS1^{+/-} ameliorated aging-like phenotypes of Kl^{-/-} mice and improved survival (68).

IRS-DEFICIENT MICE

IRS1 and IRS2 are required for the IIS pathway and are evolutionarily-conserved mammalian lifespan regulators (70). IRS1 binds to the IR, activating the PI3K-AKT signaling cascade to regulate glucose metabolism through down-regulation of gluconeogenic enzymes and up-regulation of lipogenic enzymes (71). Irs1^{-/-} mice showed insulin-resistance with defects in insulin signaling (72), and lived 14-16% longer than WT littermates (73). The Irs1^{-/-} mice showed small body size, reduced fat mass, and protection from aging-induced insulin resistance (70). Heterozygous Irs1^{+/-} mice had the same lifespan as WT (70). The lifespan extension seen in the Irs1^{-/-} mice was shorter than that of Snell (29-51%), Ames (37-68%), Little (23-25%), or GHR^{-/-} (16-55%) dwarf mice strains. These lifespan differences suggest that there are IRS1-independent, but GH-dependent mechanism for longevity.

IRS2 is known to play a central role in pancreatic β -cell

function (74). Irs2^{-/-} mice die early (maximum 3 months of age) due to their diabetic phenotypes that include defects in hepatic insulin signaling, glucose deregulation, and β -cell failure or apoptosis (70). Several studies have tried to restore the β -cell function of Irs2^{-/-} mice, through mechanisms that included overexpression of transcription factor, Pancreatic and duodenal homeobox 1 (PDX1) to enhance β -cell functions (75), deletion of Protein tyrosine phosphatase, non-receptor type 1 (PTPN1) to reduce insulin sensitivity (76), and reduction of Phosphatase and tensin homolog (PTEN) expression, a potent inhibitor of insulin action (77). These trials achieved some improvements in lifespan, but created additional problems. The lifespan of Irs2^{-/-}::Pdx1 Tg mice increased by 15 months, but they had severe spinal deformities. Irs2^{-/-}::Ptpn1^{-/-} mice had lifespan increases of 8-9 months, but β -cell function deterioration. And Irs2^{-/-}::Pten^{+/-} mice gained 10-12 months of life, but had lymph proliferative disease (78). Heterozygous Irs2^{+/-} mice showed conflicting lifespan results. Taguchi *et al.* showed 17% longer (79) and Selman *et al.* showed no significant change in the lifespan of Irs2^{+/-} mice (70). Selman *et al.* argued that the WT (C57BL/6J) lifespan profile in the study by Taguchi *et al.* had a significantly different shape compared to other independent WT (C57BL/6J) survival curves (80). KO of liver-specific Irs1 (Lirs1^{-/-}) and Irs2 (Lirs2^{-/-}) in mice exhibited insulin resistance with different meal-timing, after refeeding, and during fasting (81). Interestingly, fasting/fed-responsive genes in the liver were significantly down-regulated in Lirs1^{-/-} mice, but those genes were not changed in Lirs2^{-/-} mice (82). These results suggest that IRS1 is a principal mediator of the regulation of hepatic glucose homeostasis.

Selman *et al.* measured global hepatic gene expression in female WT and Irs1^{-/-} mice at young (2.6 months old), middle (15 months old), and old (22 months old) ages (70). The results showed up-regulation of several genes in different ages of Irs1^{-/-} mice, including catalase-encoding genes in old age, a glutathione S-transferase gene Gsta4 in young age, an excision repair gene Ercc8 at all ages, and a growth regulator Gadd45b at all ages (70). At both young and middle ages, genes for energy metabolism were up-regulated and they were involved in carbohydrate metabolism, acetyl-CoA metabolism, mitochondrial metabolism, including OXPHOS and TCA cycle; and coenzyme and cofactor catabolism. Down-regulated genes at young and middle ages were involved in immune response and antigen processing (70).

FOXO-MEDIATED LONGEVITY GENES

FOXO transcription factors (FoxOs) are inhibitory downstream targets of IIS through activation of AKT, TSC complex subunit 2 (TSC2) and Glycogen synthase kinase 3 (GSK3) (83). The FoxOs are known as evolutionarily conserved anti-aging effectors (84) with broad roles in glucose homeostasis, tumor suppression, autophagy, and resistance to oxidative stress (84,

85). Among the four mouse FoxO homologs (FoxO1, FoxO3, FoxO4 and FoxO6), FoxO3 was reported to be a longevity factor under dietary restriction condition (86). Lifespan extension by overexpression of FoxOs has not been reported in mice yet. In fresh water polyp Hydra, FoxO achieves immortality through maintaining self-renewal capacity of stem cells (87).

A meta-analysis by Webb *et al.* using FoxO ChIP-seq data from four mouse cell types (neural progenitor cells, memory CD8+ T-cells, pre-B cells, and T regulatory cells) showed that FoxO commonly binds to the promoter areas (from 5 kb upstream to 1 kb downstream of transcription start site) of both pro-longevity and anti-longevity genes belonging to the GH-IIS axis (e.g., Gh, Igf1r, Akt1, and Lrs2), the oxidative stress response (e.g., Cat, Txn1, and Prdx1), and p53/DNA damage repair (e.g., Ercc2, Atr, and Trp53) (88).

TISSUE-SPECIFIC EFFECTS OF GH-IIS AXIS

Recently, Page *et al.* measured liver, muscle, brain and WAT transcriptomes in middle age (15 months old) female *Lrs1*^{-/-} mice using an RNA-sequencing approach. They identified 124 DEGs (FDR < 0.1) in the liver, 185 DEGs in muscle, 111 DEGs in brain, and 344 DEGs in WAT (89). There was no common DEG across the four different tissues. DEGs from liver, muscle, and brain tissues shared 5 up-regulated genes encoding ribosomal proteins, including Ribosomal protein L37a (Rpl37a), Ribosomal protein S26 (Rps26), and Rpl31; and hemoglobin proteins, including Hemoglobin alpha, adult chain 1 (Hba-a1), and Hemoglobin beta, adult t chain (Hbb-bt). DEGs from muscle and WAT had the largest number of shared genes, including 7 up-regulated genes and 15 down-regulated genes (89). They also found some shared gene ontology terms across tissues. ECM term was shared in down-regulated DEGs across all 4 tissues. Organ development and receptor binding terms were shared from down-regulated DEGs across muscle, brain, and WAT. Ribosomal protein, haptoglobin complex, ECM, and antioxidant activity terms were shared from up-regulated DEGs across liver, muscle, and brain (89). Across both muscle and WAT, DEGs related to fatty acid metabolism were up-regulated, and DEGs related to inflammation were down-regulated. Especially, in liver, up-regulated DEGs were involved in B cell proliferation and biosynthesis of ribosomal small subunits, and included CYP genes for fatty acid metabolism. The down-regulated DEGs were involved in negative regulation of RNA metabolism by transcription factors. In muscle, up-regulated DEGs were involved in mitochondrial functions, including oxidoreductase activity, electron carrier activity, and cytochrome-c oxidase activity. Down-regulated DEGs were involved in inflammatory processes, including leukocyte proliferation, interferon- γ response, and serine-type endopeptidase inhibition. In brain, up-regulated DEGs were involved in regulation of adenylate cyclase and G-protein coupled receptor signaling, and

down-regulated DEGs were involved in morphogenesis and angiogenesis. These results indicate that *Lrs1*^{-/-} mice have tissue-specific longevity mechanisms.

Fat-specific reduction of GH-IIS axis

Ames, Snell, and *GHR*^{-/-} dwarf mice showed an obese phenotype due to their high capacity for lipid storage and activation of pre-adipocyte differentiation (90). Stout *et al.* measured transcriptomes from brown adipose tissue (BAT) and several WATs at different locations including inguinal, epididymal and perirenal fat from *GHR*^{-/-} mouse (91). They found opposite gene expression trends between WATs and BAT (91). In BAT, 252 DEGs (FDR < 0.05 and FC > 1.5) were identified. Up-regulated DEGs were involved in several metabolic processes including organic acid metabolism, oxidation-reduction process, cholesterol biosynthesis, and lipid and acetyl-CoA metabolism. Down-regulated DEGs were involved in immune and inflammation, including immune system regulation, innate immune response, inflammation response, and wound healing. In WATs, 346, 319, and 192 DEGs were identified in inguinal, perirenal, and epididymal fat tissues, respectively. Common DEGs of WATs from the three different locations showed up-regulation of dendritic cell-expressed genes; and down-regulation of cellular respiration-related genes, and mitochondrial inner envelope-related genes. The up-regulation of dendritic cell-expressed genes might represent an increase of infiltrated dendritic cells in WATs that correlates with the obese status. Masternak *et al.* performed surgical removal of visceral fat in both WT and *GHR*^{-/-} mice (92). Visceral fat removal (VFR) from *GHR*^{-/-} mice resulted in decreased adiponectin levels, glucose tolerance, and insulin sensitivity, accompanied by increased glucose (92). These results suggest that WAT actually has a beneficial effect on longevity of the *GHR*^{-/-} mouse. In contrast, WT mouse with VFR showed improved insulin sensitivity, reduced body temperature, and decreased respiration rates; opposite effects, compared to *GHR*^{-/-} mice with VFR (92).

Fat-specific insulin receptor knockout (*FIR*^{-/-}) mice showed adipocyte-specific insulin resistance, reduced body fat mass, protection from age- and hyperphagia-associated obesity, and protection from age-related insulin resistance (93). There was an interesting difference in adipocyte size in *FIR*^{-/-} mouse, compared to WT. Adipocytes isolated from epididymal WAT of *FIR*^{-/-} mice divided into two sizes, small (< 75 μ m) and large (> 100 μ m), compared to one medium size (75-100 μ m) in WT mice (93). Bluher *et al.* isolated the small and large adipocytes from 3 month-old young *FIR*^{-/-} male mice using 75 μ m pore size nylon mesh screen and identified 63 DEGs (P < 0.0001) (94). Later, Katic *et al.* observed that *FIR*^{-/-} mouse showed an increased metabolic rate and higher oxygen consumption, compared to WT littermates (95). They also measured WAT transcriptome of *FIR*^{-/-} male mice at young (6 months old), middle (18 months old), and old (30-36 months

old) ages. The transcriptome data showed age-associated up-regulation of genes related to mitochondria, including OXPHOS and β -oxidation; and age-associated down-regulation of genes related to defense, external stimuli response, and protein prenylation (95). These results indicate that activated mitochondrial function and its maintenance during the aging process may be key mechanisms for the longevity of FIR^{-/-} mice.

Brain-specific reduction of IIS

As mentioned previously, the Igf1r^{-/-} mice were lethal because of postnatal respiratory failure (59). Kappeler *et al.* reported that brain (specifically, hypothalamus)-specific Igf1r heterogenic KO (bIGF1R^{+/-}) mice lived 13% longer than WT mice (96). The bIGF1R^{+/-} mouse model showed ~10% growth retardation, and increases in blood lipid, cholesterol, and free fatty acid levels at young ages (10 months old) (96). Similar to the GH-deficient mice (Snell, Ames and Little), bIGF1R^{+/-} mice showed small pituitary glands, low GH levels, and increased body fat mass (96).

Brain-specific Irs2^{+/-} (blrs2^{+/-}) and blrs2^{-/-} mice showed lifespan extension by 18% and 14%, respectively (79). During the aging process, these mice showed hyperinsulinemia and insulin resistance, as well as high glucose oxidation, and stable superoxide dismutase 2 (SOD2) levels in the hypothalamus, with stable diurnal rhythm (79). These results indicate that the metabolic rate, but not the obese state or insulin sensitivity might be associated with longevity. Sadagurski *et al.* reported that genetic intervention to reduce IRS2 signaling decreased incidence of age-associated Huntington disease (HD) (97). These results suggest that because brain-specific IRS2 reduction decreased incidence of neurodegenerative diseases, it could lead to longevity.

Heart-specific Igf1 overexpression

In contrast to the aforementioned studies, Li and Ren argued that aging-associated decreases of circulating IGF1 stimulates cardiac aging. They reported that cardiac-specific Igf1 (clgf1) Tg male mice showed a 60-80% increase in plasma IGF1 levels and extended their lifespan by 23% (98). clgf1 Tg mice exhibited improved cardiomyocyte function including increased Ca²⁺ homeostasis, reduction of protein damage, and decreased apoptosis (98). Indeed, long-lived Ames dwarf mice showed impairment of cardiac excitation-contraction coupling, compared to WT mice (99). Overall, these results suggest that the autocrine/paracrine role of IGF1 is important to cardiac-specific anti-aging effects.

mTORC1- AND MYC-REDUCED MICE

Both IIS and mechanistic target of rapamycin (mTOR) pathways could activate ribosomal protein S6 kinase 1 (S6K1), a regulator of growth and metabolism through activation of protein synthesis (100). Although male S6K1^{-/-} mouse

showed a similar lifespan to WT, female S6K1^{-/-} mouse showed a 20% longer lifespan than WT. Other phenotypes of female S6K1^{-/-} mice included lean and small body size, and improved insulin sensitivity (101). Interestingly, their total circulating IGF1, pituitary GH, TSH, and PRL levels were not changed compared to WT (101). Lamming *et al.* reported that mTOR and mLST8 double heterozygous (mtor^{+/-}::mlst8^{+/-}) female mice showed normal body weight and maintained insulin sensitivity (102). And mtor^{+/-}::mlst8^{+/-} female mice showed decreased mTORC1 activity, but not mTORC2, with a 14% extended lifespan (102). Although both S6K1^{-/-} and mtor^{+/-}::mlst8^{+/-} mice showed female-specific longevity, they showed different phenotypes. These results indicate that the small body size and improved insulin sensitivity in S6K1^{-/-} mouse are independent changes, compared to mTORC1-mediated longevity.

Selman *et al.* measured transcriptome in liver, skeletal muscle and WAT of young (10 months old) S6K1^{-/-} female mice (101). They identified 1,843 DEGs (q-value < 0.01) in the liver, 2,309 DEGs in muscle, and 1,970 DEGs in WAT. Across these three tissues, DEGs related to adenosine monophosphate-activated protein kinase (AMPK)-mediated signaling were commonly up-regulated.

p66Shc, an isoform of SHC-transforming protein 1, binds to both IRS1 and S6 kinase and can bridge the two signalings (103). p66Shc^{-/-} and p66Shc^{+/-} mice showed 28% and 7% increased median lifespans, respectively (104). The p66Shc^{-/-} mice showed less ROS production, high oxidative stress resistance, and prevention of obesity (104).

Tomilov *et al.* measured transcriptome in liver, spleen, lung, WATs (retroperitoneal and epididymal), and peritoneal macrophages from young p66Shc^{-/-} mice (3 months old males, and 12 months old males and females) (105). They found that commonly down-regulated DEGs (P < 0.05) were enriched in PI3K signaling, antigen processing, chronic myeloid leukemia, adipokine signaling, anti-apoptosis, and heme transcripts by gene ontology analysis (105). During phagocytosis, phagocytes, including neutrophils, eosinophils and macrophages exhibit an intense consumption of oxygen for NADPH oxidase (PHOX), in a process called a respiratory burst (106). In a comparison of peritoneal macrophages, p66Shc^{-/-} mice showed 31% less PHOX activity and 40% less production of PHOX-dependent superoxide than WT mice (105).

Myc transcription factor, known as a protooncogene regulates various downstream functions, including energy metabolism, ribosome biogenesis, cell cycle, apoptosis, differentiation, and stem cell maintenance (107). Recently, Hofmann *et al.* reported that heterozygous Myc^{+/-} mice showed a 15% longer lifespan than WT mice, while Myc null mice resulted in an embryonic lethal phenotype (108). Myc^{+/-} mice showed 15-20% less body mass, 56-64% lower serum IGF1 levels, higher metabolic rates, and higher AMP levels in liver, whereas there was no difference in adipose tissue mass, reproductive ability, or

incidence of deaths due to cancer, compared to WT mice (108).

Hofmann *et al.* (2015) measured transcriptome in young (5 months of age) and old (24 months of age) *Myc*^{+/-} male mice and identified 307, 160 and 412 DEGs (FDR < 0.05 and FC > 1.5) in the liver, skeletal muscle, and gonadal WAT, respectively, compared to those of WT mice (108). The altered DEGs showed tissue-specific functions without a common DEG across the three tissues. In liver, down-regulated DEGs at old age were involved in cholesterol biosynthesis, and *Myc*^{+/-} mice were protected from age-associated enlargement of hepatic lipid droplets (108). Comparing hepatic DEGs from *Myc*^{+/-} mice to long-lived Snell, Little, and *GHR*^{-/-} mice, the DEGs for xenobiotic metabolism enzymes, including CYP genes, *Fmo3*, *Fmo4*, *Hao2*, *Mt1*, *Mt2*, and *P450* (cytochrome oxidoreductase (Por)) were commonly up-regulated, but the fold changes were orders of magnitudes smaller in *Myc*^{+/-} mice (108). In addition, *Myc*^{+/-} mice showed decreases in S6 kinase and AKT activities in both liver and muscle, an increase of AMPK in liver, and decreased serum IGF1 levels, whereas none of the genes related to these pathway components were identified as DEGs (108). Based on these observations, the authors hypothesized that the reduced available energy in the liver of *Myc*^{+/-} mice activates AMPK, and then reduces mTOR and S6 kinase activities (108), promoting longevity.

CONCLUDING REMARKS

GH signal-deficient mice, including Snell, Ames, Little, *GHR*^{-/-}, and *Fgf21* Tg dwarf mice, showed increased lifespans and smaller body masses than WT mice. Therefore, body size was strongly dependent on GH action. This consistent trend suggests an inverse correlation between size and lifespan (109). However, small size can not be used as a general indicator of longevity, because *Kl Tg*, *Irs2*^{+/-}, *p66*^{Shc-/-}, and *mtor*^{+/-}::*mlst8*^{+/-} mice had normal body masses like WT mice, but showed longer lifespans than WT. In addition, *GHA Tg* mice had normal lifespans like WT mice, and *Kl*^{-/-} and *Irs2*^{-/-} mice showing dramatically shorter lifespans also had a dwarfism phenotype.

Snell, Ames, Little, and *GHR*^{-/-} mice exhibited obesity, caused by loss of lipolytic and anti-lipogenic activities of GH. Especially, long-lived *bIGF1R*^{+/-}, *blrs2*^{+/-}, and *blrs2*^{-/-} mice had high body fat. These results indicate brain-mediated endocrinological control of body fat and the IGF1 feedback loop. However, excess weight and obesity are known risk factors of aging and cause critical diseases, including diabetes and heart failure (110). Indeed, *LI-Igf1*^{-/-}, *FIR*^{-/-}, *Irs1*^{-/-}, *S6K1*^{-/-}, and *p66*^{Shc-/-} mice showed less body fat than WT animals. In contrast, *bGH Tg* mice producing excess GH had lean bodies, but shortened lifespans. Surgical removal of visceral fat in *GHR*^{-/-} mice decreased the fat-induced beneficial effects (92). Therefore, under the different endocrine circumstances, regardless of the body fat mass, adipose tissue itself contributes effects beneficial to longevity.

High insulin sensitivity could be associated with improved health. Long-lived GH signal-deficient mice, as well as *FIR*^{-/-} and *S6K1*^{-/-} mice, showed high insulin sensitivity. However, some long-lived mice with a mutation in IIS components, including *LI-Igf1*^{-/-}, *Igf1r*^{+/-}, *bIGF1R*^{+/-}, and *Irs1*^{-/-}, as well as the *Kl Tg* mice displayed insulin resistance. In the case of systemic *Irs2* deficiency, *Irs2*^{-/-} mice had diabetes, whereas brain-specific mutant *blrs2*^{-/-} mice showed both insulin resistance and longevity. Interestingly, *R6/2::Irs2*^{+/-}::*Irs2* β Tg mice, genetically modified to be *Irs2*^{+/-} heterozygous except in β cells, showed a slow Huntington disease (HD) progression (97). In addition, insulin resistant (e.g., *IR*^{+/-}) male mice showed a 20% extended maximum lifespan, but high insulin sensitivity mice, including *PTP-1B*^{-/-} and *PGC-1 α* Tg displayed shortened or similar lifespans compared to WT mice, respectively (62). It is clear that manipulation of insulin sensing signaling produces beneficial effects on longevity, however, either impaired or hyper-sensitive insulin signaling can lead to shortened lifespans.

Snell, Ames, Little, *GHR*^{-/-}, and *bIGF1R*^{+/-} mice showed both high-stress resistance and less tumor incidence. *Pappa*^{-/-} mice also showed less tumor incidence, and *Kl Tg*, *Igf1r*^{+/-} and *p66*^{Shc-/-} mice showed high-stress resistance. Hepatic DEGs of Snell, Ames, Little, and *GHR*^{-/-} mice showed up-regulation of DEGs for xenobiotic metabolism (e.g., *Fmo3*, CYPs, GSTs, and Sulfotransferases) and oxidative stress response (e.g., *Hao2* and *Hao3*), representing improved oxidative stress response and toxin defense. In addition, similar hepatic DEG patterns were observed in DNA repair-deficient progeroid mice, and these changes might be a compensatory response (46). FoxO genes were up-regulated by inhibition of IIS signaling, and FoxOs regulated gene expression for stress response and DNA damage repair (111) (88). Genetically mTORC1-reduced mice, including *mtor*^{+/-}::*mlst8*^{+/-} and *S6K1*^{-/-} showed extended lifespans with up-regulation of FoxOs (101). And *p66*^{Shc-/-} mice showed less ROS production and high oxidative stress resistance (104). In addition, hepatic DEGs from Ames, Little, *GHR*^{-/-}, and *Irs1*^{-/-} mice commonly showed up-regulation of genes for mitochondrial respiration, including the TCA cycle, OXPHOS, and β -oxidation. Collectively, the gene expression analyses of IIS and mTORC1 reduction commonly represent improved stress response that is a signature of longevity. High resistance to oxidative stress and DNA damage in these mice might be contributing factors to low tumor incidence and increased longevity.

Snell, Ames, Little, *GHR*^{-/-}, and *Fgf21* Tg dwarf mice showed female-biased lifespan extension, and commonly showed STAT5-related gender-different hepatic gene expression. Moreover, female *Igf1r*^{+/-}, *LI-Igf1*^{-/-}, *Irs1*^{-/-}, *mtor*^{+/-}::*mlst8*^{+/-}, *S6K1*^{-/-}, and *Myc*^{+/-} mice also lived longer than their male counterparts. These results suggest that the GH-IIS axis is regulated in a sex-specific manner. Gender is a critical factor in lifespan explained by genes that work differently in males and females in a concept known as sexual antagonistic

pleiotropy (SAP) (112). STAT5-mediated liver gene expression analysis showed altered genes in xenobiotic metabolism (e.g., CYPs, GSTs, and sulfotransferases), oxidative stress response, and serine protease inhibitors. They overlap with the aforementioned longevity-associated liver gene expression data. In particular, *Hsd3b5* encoding a ketosteroid reductase for steroid hormone biosynthesis, including testosterone was commonly down-regulated in Snell, Ames, Little, *GHR*^{-/-}, and *Fgf21* Tg mice. The expression of *Hsd3b5* was associated with activation of GHR-STAT5 signaling, suggesting testosterone inactivation might contribute to the female-biased longevity.

On the other hand, *Myc*^{+/-} mice showed up-regulated DEGs for xenobiotic metabolism and inhibition of mTOR and S6 kinases, however their tumor incidence was similar to WT mice (108). In addition, *Kl* Tg, *bIGF1R*^{+/-}, and *Irs1*^{-/-} mice showed similar lifespan extension in both males and females. Therefore, more work is needed to elucidate factors contributing to the lifespan of these mice.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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