Multiplex stress resistance in cells from long-lived dwarf mice[1]

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SPECIFIC AIM

Mutations that extend nematode longevity by interference with IGF-I/insulin sensing pathways lead to resistance to multiple forms of stress. The aim of the study was to test the hypothesis that the Snell dwarf mutation, already known to extend life span and delay aspects of aging in mice, also renders cells resistant to a variety of forms of stress.

PRINCIPAL FINDING

Fibroblasts from dwarf mice show resistance to a variety of forms of lethal injury, including ultraviolet light, heat, paraquat, H_2O_2, and the toxic metal cadmium

In Snell dwarf mice, mutation of the Pit-1 (pituitary transcription factor-1) gene leads to declines in growth hormone and IGF-I, thyroid hormones, and prolactin. This altered hormonal profile leads to dwarfism, deceleration of several aspects of aging, and a 40% increase in life span. However, the mechanism by which the altered hormonal profile leads to life extension is unknown.

We investigated whether fibroblasts from young Snell dwarf mice show resistance to cytotoxic stresses. Fibroblasts were grown from tail skin of young dwarf (dw/dw) mice and from their phenotypically normal littermates (dw/+). Third or fourth passage cells were arrested at the G0/G1 phase by a 27 h period of serum starvation, then exposed to agents that induce cellular injury. **Figure 1** shows LD_{50} values for fibroblasts from dwarf and control mice in responses to each of five forms of stress. Dwarf cells were significantly more resistant to each stress than were control cells. The effects on LD_{50} ranged from a 45% change for UV light to a 180% change for cadmium, and all were significant by ANOVA at $P < 0.01$. Additional details of the statistical analysis are shown in **Table 1**. Dose response curves also established that cells from the dwarf mice were relatively resistant to each stress over a wide dose range (not shown). An independent set of experiments using dwarf mice on a different genetic background produced similar outcomes (data not shown). The difference between dwarf and normal mice was also seen when the test for viability depended on uptake of tritiated thymidine into DNA rather than using measures of mitochondrial function as employed in Fig. 1. We also tested stress resistance of proliferating fibroblasts cultured without a period of serum starvation before stress exposure. Under these conditions we found no consistent or significant differences between dwarf and control cultures (data not shown).

TABLE 1. *Stress resistance of fibroblasts from normal and Snell dwarf mice*[a]

Stress	Strain	LD_{50} Mean ± SD	No. of mice	*P* value ANOVA	Increase
UV light	Normal	110 ± 19 (J/m^2)	10	—	—
	Dwarf	160 ± 29 (J/m^2)	13	<.0001	45%
H_2O_2	Normal	92 ± 23 (μM)	6	—	—
	Dwarf	227 ± 20 (μM)	8	.002	147%
Paraquat	Normal	1.28 ± 1.13 (mM)	7	—	—
	Dwarf	2.04 ± 0.90 (mM)	10	.01	53%
Cadmium	Normal	4.5 ± 3.8 (μM)	12	—	—
	Dwarf	12.6 ± 10.8 (μM)	15	.007	180%
Heat	Normal	14.1 ± 9.4 (min)	8	—	—
	Dwarf	28.6 ± 8.6 (min)	11	.006	102%

[a] Average LD_{50} across the set of mice tested.

CONCLUSIONS AND SIGNIFICANCE

Here we show that skin-derived fibroblasts from young dwarf mice are resistant to multiple forms of cellular injuries caused by UV light, heat, paraquat, H_2O_2, and cadmium. Thus, the altered hormonal profile in the dwarfs causes long-lasting physiological changes in cells, leading to multiplex stress resistance. The results support the theory that modulation of cellular stress resistance regulates life span in mammals. **Figure 2** summarizes our model for regulation of longevity and stress resistance.

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.02-1092fje; doi: fj.02-1092fje

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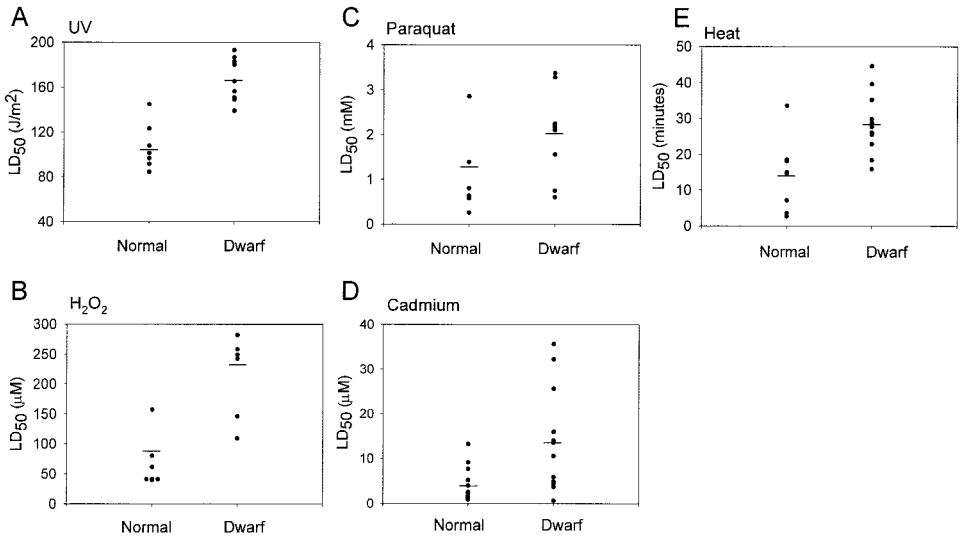

Figure 1. Skin-derived fibroblasts from dwarf mice are resistant to multiple forms of stress. Each symbol represents an individual mouse of the indicated genotype; the horizontal dash indicates the mean value in each case. The results for each stress are compiled from 3–5 separate experiments, each containing a balanced number of samples from dwarf and control mice. Percent increases in LD_{50} and P values were as follows: UV light (45%, $P<0.0001$), hydrogen peroxide (147%, $P=0.002$), paraquat (53%, $P=0.01$), cadmium (180%, $P=0.007$), and heat (102%, $P=0.006$).

Our findings have several interesting implications. First, they support the idea that the extended longevity and delayed aging of Snell dwarf mice may reflect increased resistance of cells to stress. It is noteworthy that a mutation at the p66-shc locus that renders mouse fibroblasts resistant to UV-induced apoptosis also extends longevity in homozygous mice. It will be worthwhile to delineate the steps that connect cellular stress resistance to its pathological result, disease resistance, in these and other models of decelerated aging. Second, the data suggest that resistance to multiple varieties of stress may be coordinately regulated by hormone-sensitive pathways in mice. Defining the molecular basis for this coordinate control may lead to important insights into the relation of cellular differentiation to organismal aging and provide biochemical targets for potential interventions. Ames dwarf mice, similar in many respects to the Snell dwarfs, have higher levels of catalase in liver and kidney, and it will be of interest to determine what other forms of cellular defense may be bolstered in these and other cells in dwarf mice. Third, the data suggest that the stress resistance property of slow-aging mice extends to cells, like dermal fibroblasts, that may play little role in resistance in most forms of lethal disease but which are easily accessible and thus useful for tests of genetic and pharmacological antigenic interventions.

Last, the parallelism between the worm and mouse results suggests that the connections between cellular stress resistance and modulation of life history trajectories may have ancient evolutionary roots. FJ

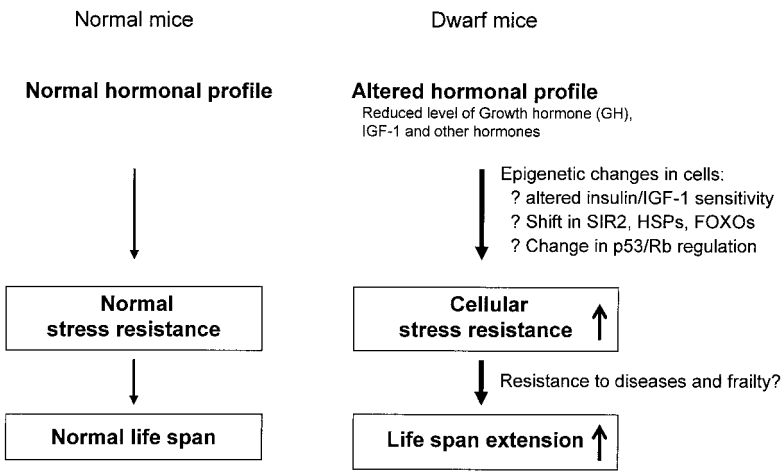

Figure 2. Schematic diagram of a model linking hormone levels to longevity via effects on cellular stress resistance. In dwarf mice, altered hormonal profile (low growth hormone, IGF-1, and/or other hormones) causes long-lasting physiological changes in cells that render them resistant to multiple forms of injury. This cellular stress resistance may lead to resistance to late-life diseases and frailty, and thereby increase longevity.