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"Short Take": Resistance of Skin Fibroblasts to Peroxide and UV Damage Predicts Hearing Loss in Aging Mice

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Summary

Those mice whose skin-derived primary fibroblast cell lines resist lethal injury induced by hydrogen peroxide or UV light show lower age-related decline in hearing. Skin cell lines may provide an easily accessible surrogate index of intrinsic stress resistance that varies among individuals and influences the pace of neurosensory decline in aging mice.

Keywords

biomarkers; mouse; aging rate; oxidation; DNA damage

Primary fibroblasts derived from skin of long-lived mouse mutants, such as Ames and Snell dwarf, and GHR knock-out mice, resist the lethal effects of UV light, hydrogen peroxide, and cadmium (Salmon et al., 2005; Murakami et al., 2003). Age-associated loss of sensory cells in the inner ear is accompanied by, and potentially caused by, increased oxidant stress in mice (Jiang et al., 2007). We therefore hypothesized that differences among mice in overall cellular stress resistance might influence both cultured fibroblasts and the rate of age-related decline of auditory function of the ear. To test this idea, we bred a group of genetically heterogeneous mice ("UM-HET4 stock") whose inbred grandparents were from inbred stocks (MOLF/Ei, 129/S1/SvImJ, C3H/HeJ and FVB/NJ) lacking the *Cdh23*^{753A} allele at the *Ahl1* locus, which would have led to hearing loss in the first third of life. Primary tail skin fibroblast lines were established for 250 female mice at 16 months of age and tested for resistance to death induced by UV, cadmium, and peroxide using published methods (Salmon et al., 2005; Murakami et al., 2003). Each mouse was evaluated for hearing at 8 and then again at 22 months of age, using the auditory brainstem response (ABR), as previously described (Sha et al., 2008). The increase in ABR threshold from 8 to 22 months was calculated at each of four frequencies (4, 12, 24, and 48 KHz), and the mean rank for each mouse, averaged across the test frequencies, was taken as the best single estimate of age-associated hearing loss. The mean rank scores for hearing were then compared to fibroblast stress resistance values, expressed as the logarithm of the LD50, i.e. the dose of peroxide or UV needed to kill 50% of the fibroblasts. The average rank score,

rather than average raw score, was used because each of the four test frequencies has its own variance and skew.

The mice with the best preservation of hearing at 22 months were those whose fibroblasts at age 16 months were most resistant to death induced by hydrogen peroxide ($p = 0.0006$; for pairwise correlation of log LD50 vs. ranked hearing change score) or by UV light ($p = 0.003$). These associations are relatively weak ($R = 0.22$ for peroxide and $R = 0.19$ for UV). This is not unexpected, given the measurement uncertainty in the estimates of ABR and estimates of LD50, as well as the strong likelihood that age-related loss of hearing is modulated by many factors unrelated to systematic, shared resistance to lethal cellular stresses. Hearing rank scores for mice in the upper and lower halves of each fibroblast LD50 distribution are shown in Figure 1. We found no indication of an association of hearing loss with cellular resistance to cadmium (not shown).

Resistance of long-lived *C. elegans* mutants to multiple forms of stress suggests that nematode longevity may be at least in part controlled by the set-point of cellular defenses against injury (Johnson et al., 1996; Lithgow et al., 1995; Lithgow and Miller, 2008). Stress resistance of fibroblasts from long-lived mammals, rodents, and birds (Kapahi et al., 1999; Harper et al., 2007; Miller et al., 2010) is consistent with the idea that similar links between longevity and cell biology may apply to homeothermic vertebrates. Our data support this concept by showing that the pace of age-related decline in hearing is correlated with inter-individual differences in skin fibroblast resistance to two stresses, one (peroxide) that induces oxidative damage and one (UV) that induces DNA damage through anti-oxidant independent mechanisms (Salmon et al., 2005). Skin cells do not regulate the pace of hearing loss, but may provide an easily accessible surrogate index of intrinsic stress resistance that varies among genetically heterogeneous mice and also influences preservation of hearing in aging mice. It would be interesting to learn if skin fibroblast stress tests predict the pace of age-related functional decline in other neurosensory organs, or predict lifespan in mice or humans. Similarly, it would be worthwhile to determine whether those mice, or humans, which exhibit relatively slow development of presbycusis in middle age are also protected against lethal and non-lethal illnesses of aging.

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Figure 1.

Mice whose tail-derived fibroblasts are resistant to death induced by hydrogen peroxide (left panel) or UV light (right panel) also resist age-related hearing loss. ABR thresholds were measured at four frequencies, 4 KHz, 12 KHz, 24 KHz, and 48 KHz, at each age. Then the ABR value at 8 months was subtracted from the value at 22 months to give an estimate of the extent of age change in hearing for each frequency. To provide an overall estimate of age effects, combined across all four test frequencies, we converted each change score into a rank, with high ranks corresponding to the largest age effect, and then calculated the mean rank score (averaged across the four frequencies) for each mouse. The histograms compare those mice in the upper vs lower halves for fibroblast stress resistance, showing mean rank for ABR change scores (between 8 and 22 months). A high rank score corresponds to greatest loss of hearing.