

MOUSE RNAi SCREEN FOR INCREASED RESISTANCE TO OXIDATIVE STRESS

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Invertebrate genetic screen for longevity revealed a link between life-span extension and increased resistance to oxidative stress. Although the link is conserved in several mouse longevity mutants, mammals seems to have another pathway that controls oxidative stress resistance and longevity. For example, null mutation in mouse *p66shc* is known to increase resistance to oxidative stress and extend average life-span by 30%, however, mutation in its counterpart has not been found in invertebrate longevity screens. As it is nearly impossible to directly screen for longevity in mammals, increased resistance to oxidative stress is used as a longevity indicator in our genetic screen. We developed a cell-based genetic screen for increased resistance to tert-Butylhydroperoxide (tBHP), an hydrophobic analogue of hydrogen peroxide (H₂O₂), based on the fact that fibroblast from *p66shc*^{-/-} mouse shows increased resistance to H₂O₂ under culture condition. To perform genome-wide loss-of-function screen in cultured cells, we have generated a RNAi library from 15,000 mouse cDNA library by an enzymatic production method. Screening of 1x10⁶ NIH3T3 cells infected with the RNAi library allowed us to isolate a siRNA that gives NIH3T3 cells a potent protection from H₂O₂ and tBHP. The siRNA also protected mouse primary cultured fibroblast from the two peroxides without affecting growth rate, cell morphology, UV sensitivity and replicative senescence. Specificity of the target-siRNA interaction and correlation between mRNA suppression and phenotype change are under investigation.