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Calorie Restriction in Gerontological Experiments on Cell Cultures

The lifespan of model organism, including chronologically aging yeast, can be extended by calorie restriction (CR). There is little information on the effect of CR on cultured animal and human cells.

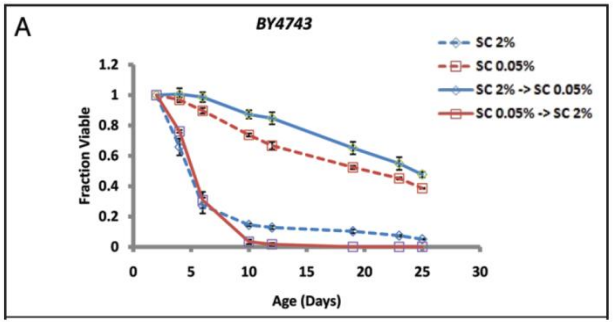


Fig. 1. CR extends yeast chronological lifespan (Burtner et al. *Cell Cycle*, 2009)

SC 2% (SC 0.05%) – synthetic complete medium with 2% of glucose (0.05%)

We studied how starvation, some mimetics of CR, and inhibition of autophagy affects the lifespan of Chinese hamster cells (CHC) in the "stationary phase aging" model, as well as the expression of *LDHa* and genes associated with autophagy.

The model of "stationary phase aging" is based on the idea that restriction of cell proliferation leads to the accumulation of macromolecular damage in cultured cells, similar to that in postmitotic cells of an aging multicellular organism (the same as chronological aging).

We have previously shown that a mammalian cell culture in the "stationary phase aging" model dies out in accordance with the Gompertz law (i.e., they age in true sense).

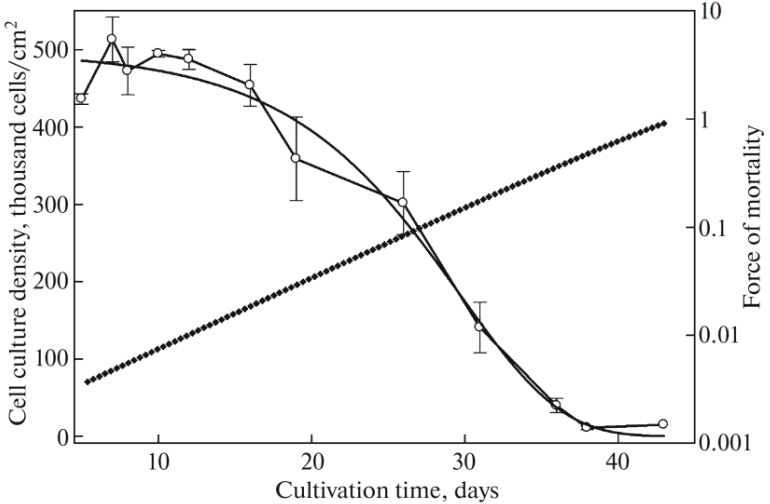
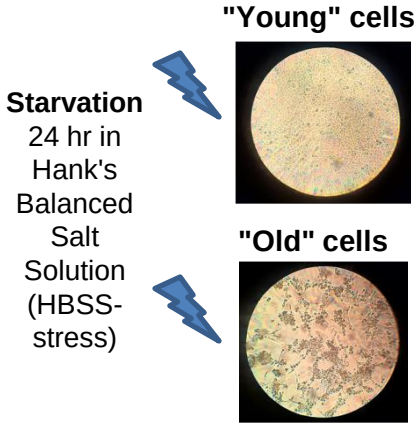
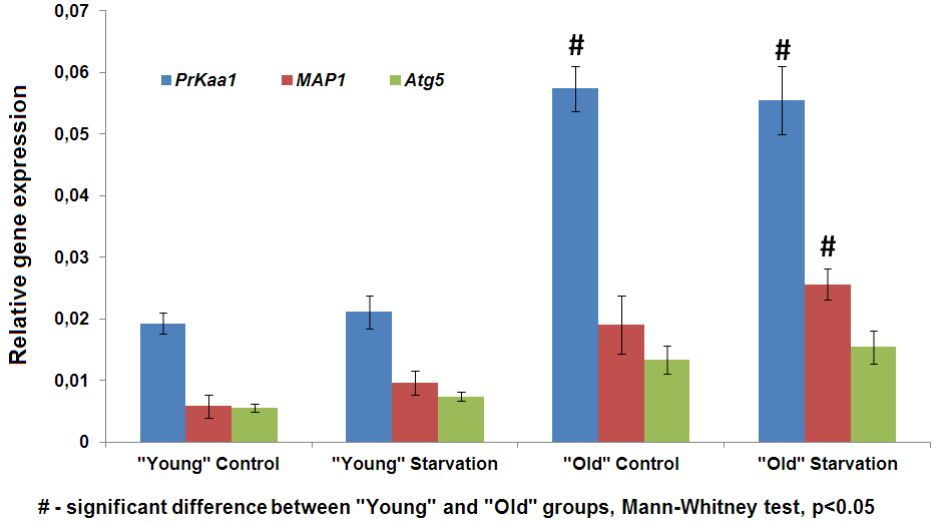


Fig. 2. Survival curve of a Chinese hamster stationary phase cell culture (Khokhlov et al. *Moscow Univ. Biol. Sci. Bull*. 2014; Khokhlov et al. *Moscow Univ. Biol. Sci. Bull*. 2019).

Starvation experiment design



RT-PCR
Map1lc3 (LC3),
atg5, *Prkaa1*
(AMPKα1)



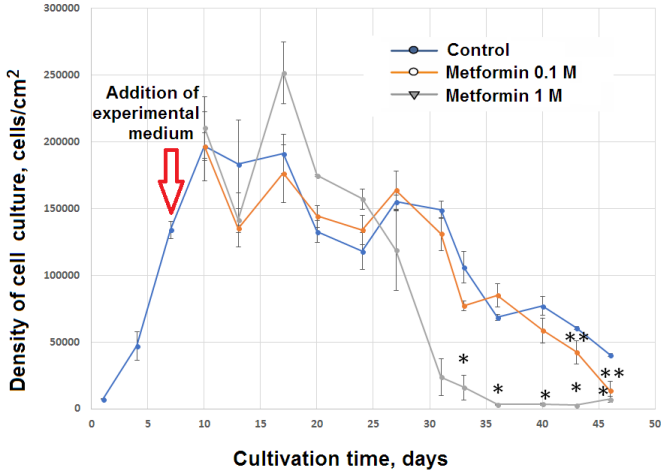


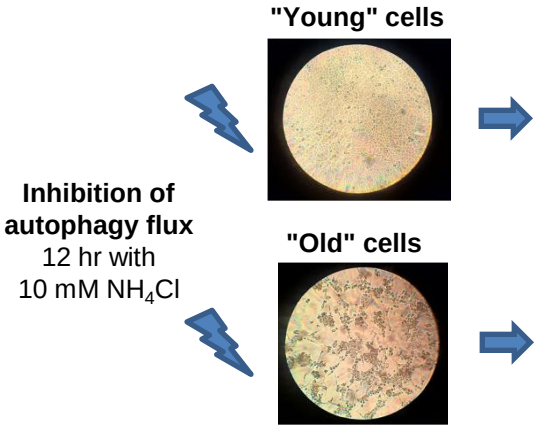
Fig. 3. Effect of metformin at concentrations of 0.1 M and 1 M on the kinetics of growth and subsequent death of the transformed CHC culture

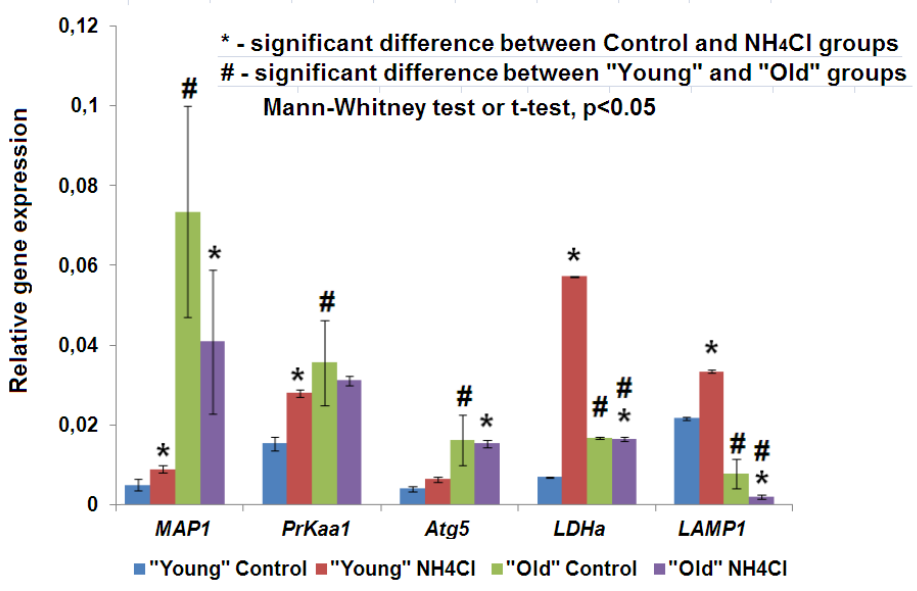
***** - significant difference between 0.1 M and Control groups, p<0.05;
****** - significant difference between 1 M and Control groups, p<0.05

Starvation for 24 hours
Starvation does not affect the expression of genes associated with AMPK and autophagy both in "Young" and "Old" cell culture. The expression level of *Prkaa1* in "Old" cells is higher than in "Young" ones (in Control as well as in Starvation group).

Mimetics of CR
2,4-Dinitrophenol in "mildly" uncoupling concentration did not affect CHC lifespan. Metformin at a concentration of 1M reduced CHC lifespan.

Design of experiment with NH_4Cl





Expression profile

In "Old" CHC, gene expression levels of all investigated genes increase (except *LAMP1*).

Inhibition of autophagy flux

Long culturing cells with NH_4Cl did not shorten lifespan of CHC. The addition of 10 mM NH_4Cl for 24 hr increases the level of expression of all genes (except *atg5*) in "Young" cells but reduces the level of expression of the same genes in "Old" cells (except *Prkaa1*).

It can be assumed that the response to stress in "Old" cells is disturbed.

Conclusion

- Mimetics of CR (metformin and 2,4-dinitrophenol) do not increase the lifespan of "stationary phase aged" CHC.
- In "Old" CHC, the level of expression of *LDHa* and genes associated with autophagy is higher than in "Young" cells.
- Inhibition of autophagy flux changes the gene expression profile (*LDHa*, autophagic and lysosomal genes) in both "Young" and "Old" CHC.

Acknowledgments

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