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Calorie restriction in gerontological experiments on cell cultures

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***Abstract* — Lifespan can be increased by the calorie restriction. However, it is not entirely clear whether this effect is manifested at the cellular level. Recently, it has been shown that calorie restriction extends the lifespan of yeast. We conduct similar experiments on mammalian cell cultures.**

Keywords* — *calorie restriction, cell aging, glucose metabolism, survival curves, RT-PCR

Motivation and aim

Motivation

The lifespan of yeast undergoing chronological aging can be extended by calorie restriction (CR) [1, 2]. CR in this case means reducing the amount of glucose or replacing glucose with non-fermentable carbon sources. In addition, it was also possible to increase the lifespan of yeast with the help of some drugs that mimic this effect (for example, rapamycin) [3].

Aim

We studied how CR affects the lifespan of mammalian cell culture in the "stationary phase aging" (similar to chronological aging) model, as well as the expression of genes associated with autophagy and glucose metabolism.

Methods

Transformed Chinese hamster cells were grown at 37°C in Dulbecco's modified Eagle's medium (or Minimum Essential Medium, or Medium 199) supplemented with 10% bovine serum. The influence of various ways of dilution of medium and addition of CR mimetics on viability of the culture, its growth and subsequent dying out in the stationary phase as well as gene-expression profile was analyzed. The resulting cell survival curves were approximated using the Gompertz equation. Differences were considered statistically significant at $p<0.05$. Mathematical calculations and statistical data processing were performed using SigmaPlot 12.0 software (Systat Software Inc., United States).

Results

We found no dependence of lifespan of "stationary phase aging" mammalian cell culture on glucose content; in some cases, the cells lived longer on media with high glucose content. Drugs that could presumably cause CR either did not affect (2,4-dinitrophenol) or reduced (metformin) the lifespan of the culture. Dilution of the culture medium with the isotonic Quinton Marine Plasma increased lifespan, however, this effect can be associated not so much with CR, but with a more successful combination of components in the new medium. Despite the lack of the same encouraging results as in experiments with yeast, it cannot be denied that the effect of CR is realized at the cellular level. Inhibition of autophagy, one of the main mechanisms through which CR is realized, reduced the lifespan of a cell culture. Moreover, the expression of some genes responsible for the synthesis of glucose metabolism enzymes and autophagy-related proteins (LDHa, AMPK, LC3, LAMP1) differs in "young" and "old" cells.

Conclusion

The results of experiments on yeast, at first glance, confirm that CR increases cell lifespan. However, the interpretation of such experiments seems to us not always correct [4, 5]. Yeast are grown in a nutrient-rich medium, and then transferred to a medium with a lack of nutrient components or with CR mimetics. In such experiments, it is necessary to determine the metabolic activity of "aging" yeast; it is likely that they become metabolically inactive. This way of increasing lifespan is not suitable for actively functioning postmitotic cells such as neurons and myocytes. Based on our experiments, we can conclude that the "old" cells are metabolically active (according to the results of changing the expression level of some genes and the kinetics of cell culture dying out), but we do not always get the same results as in experiments with yeast.

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