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A new chronological survival assay in mammalian cell culture

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The question of whether aging can be modeled in cell culture has been highly debated among specialists in the field ever since the seminal publication that primary fibroblasts undergo a finite number of population doublings before entering a non-dividing state.[1] Although unable to divide, senescent cells can be maintained in a metabolically active state indefinitely if handled appropriately. Initial thoughts were that an accumulation of senescent cells in aging individuals, a now generally accepted phenomenon, would deplete the ability of tissues to repopulate; however, more recent findings indicate that senescent cells have altered secretion profiles that may promote inflammation (and aging itself) in an autocrine fashion.[2] Further suggesting a role for senescent cells in aging, a recent report finds that eliminating senescent cells in mice enhances survival of a mouse progeria model.[3]

But are studies of replicative senescence the only way to model aging in cell culture? Motivated by studies of chronological life span (CLS) in yeast showing that survival in the nutrient-depleted nonproliferaive state was limited by acidification of the culture medium resulting from accumulation of acetic acid,[4,5] Leontieva and Blagosklonny[6] report in a recent issue of *Aging* that, at least for cancer cells, acid-induced chronological senescence may also exist for mammalian cells in culture.

Chronological aging in yeast is typically studied by culturing cells into stationary phase, maintaining the non-dividing cells in the expired culture medium and periodically measuring cell viability.[7] When standard 2% glucose synthetic defined medium is used, the pH of the culture drops from around 4 to below 3 within 2–4 d. Buffering the medium to 6.0 extends CLS as robustly as calorie restriction,[4] which is accomplished by reducing initial glucose concentration of the medium.[8] Interestingly, calorie restriction itself causes the culture pH to become more alkaline, suggesting that the CLS extension from calorie restriction occurs from reduced acetic acid accumulation and toxicity.[9] Although it had been previously reported that alkalinization of the culture medium by addition of NaOH could prolong chronological lifespan,[10,11] this observation was not widely known outside of the yeast chronological aging community.

The mammalian phenotype is brought about by leaving confluent cancer cells in spent media, which, as any scientist with experience in cell culture knows, turn from red to yellow, and can lead to a rapid loss of cell viability as assessed by plating the cells to new media and counting clones. Leontieva and Blagosklonny show that the color change reflects a reduction in pH brought about by an accumulation of lactic acid. As with yeast, buffering pH extends survival and addition of acid (lactic instead of acetic) shortens the survival period of cells in unspent media.

The parallels between yeast CLS and mammalian chronological senescence are intriguing. Both acids are produced primarily as a by-product of fermentative metabolism, and both are known to induce an apoptosis and/or necrosis.

Notably, Leontieva and Blagosklonny[6] showed that inhibition of mTORC1 by treatment with rapamycin is sufficient to protect cancer cells against lactic acid-induced senescence, a finding strikingly similar to the effect of rapamycin on chronological life span in yeast.[12,13] In both cases, the positive effect of rapamycin may be mediated by reduced acid accumulation. It will be of interest to learn whether similar metabolic changes mediated by rapamycin underlie its effects in both systems and, perhaps, in whole animals.

A key question is whether the effects of acid on cultured cells is relevant for understanding aging in people. Are there environments in the organism that become more acidic with age, perhaps through cellular release of fermentation-derived acids? It has also been suggested that the burst of reactive oxygen species caused by acetic acid in yeast may mimic aspects of normal aging.[14] Interestingly, classic scavengers of free radicals, like NAC, also extend survival in the mammalian chronological senescence assay.[6] While the relationship to aging organisms remains unclear, findings in the Leontieva and Blagosklonny study indicate that at least at the cellular level, excess fermentative production of organic acid may be a conserved contributor to the aging of post-replicative cells.

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