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Physiological deterioration associated with breeding in female mice: A model for the study of senescence and aging ☆

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Abstract

Longevity is a complex and dynamic process influenced by a diversity of factors. Amongst other, gestation and lactation contribute to organismal decline because they represent a great energetic investment in mammals. Here we compared the rate of senescence onset observed in primary fibroblast obtained from the lungs of retired female breeder mice (12 months old), with the senescence arrival observed in fibroblasts derived from age-matched nulliparous mice. Two-month-old animals were also used as controls of young, fully-developed adults. Cell proliferation, DNA synthesis, and expression of senescence-associated β-galactosidase activity were evaluated as senescent parameters. In order to test differences in energetic competence at a systemic level, mitochondrial respiration was also evaluated in mitochondria isolated from the livers of the same animals used for the primary cultures. Our data indicated that the cells derived from female mice subjected to the physiological stress of breeding onset into replicative senescence prior than the cells from female mice age-matched without that particular stress. Thus validating the use of retired breeders as a model to study aging and senescence at the cellular level.

Keywords: Aging; Gestation; Mitochondrial respiration; Nulliparous; Primary cultures

1. Introduction

Longevity is a complex dynamic process influenced by a diverse array of intrinsic and environmental factors in which the cells must coordinate various biochemical and molecular mechanisms and, at the same time, deal with damage and stress (Bree et al., 2002). For that reason aging studies must take into account a very large number of variables. As one mean of minimizing that variability, aging research is often performed with inbred strains of animals housed under carefully controlled conditions. However, longevity is not a trait that exists in isolation; it develops as part of a complex life history, involving a wide range of physiological mechanisms and, among other things, chronic disease processes (Phelan and Rose, 2005). There is abundant evidence proposing that wild animals are exposed to predation, early starvation and hazardous habitats that modify their lifespan when compared to laboratory animals that are aged with all their physiological needs satisfied (Culter, 1982; Austad and Kristan, 2003).

In particular, longevity is well-known to be affected by reproduction, an effect that is often mediated by the utilization of calories from reserves (Finch et al., 1990; Rose and Graves, 1989); but reproductive effort does not end with gestation. Energetically, lactation in mammals represents a far greater

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investment than gestation, yet, a significant physiological wear or decline that can commonly be observed in cattle and poultry, as well as in laboratory animals is the deterioration associated with breeding. It has been demonstrated that in 14 of 15 tested inbred mice strains (all descended from the Foundation Stocks of the Jackson Laboratory), virgin females survived significantly longer (20–25%) than did their bred sisters (Russel, 1964).

The same principle could be applied for human populations (Westendrop and Kirkwood, 1998) where aging rarely occurs under ideal conditions; in addition, a significant number of humans are subjected to a great diversity of physiological stressors. In particular, the link between breeding and aging has been postulated for rural populations where birth rate index is high and women healthcare during puerperium is poor, as occurs in Latin American and African countries (Tuirán et al., 2002; Zúñiga et al., 2002). However, great care has to be taken when extrapolating the results obtained in rodent models to primates, because mice and rats invest a great deal of energy during reproduction, compared to primates (Phelan and Rose, 2005).

Normal primary cells in culture show physiological and molecular changes at the end of their replicative life span defined as replicative senescence. Replicative senescence has been postulated to be a contributor to aging (Faragher and Kipling, 1998) and a tumor suppression mechanism (Campisi, 2005), in a pleiotropic antagonistic manner. Early in life, senescence may protect organisms from cancer; but, as senescent cells accumulate in tissues later in life, it may actually promote carcinogenesis and contribute to a variety of pathological changes seen in the aged (Campisi, 2000). Senescent cells were originally described in primary cultures (Hayflick and Moorhead, 1961), but their presence has also been documented *in vivo* (Dimri et al., 1995). Hence, determining cellular proliferation and replicative senescence have been used as a successful tool for estimating cellular aging and longevity.

We have previously reported that breeder female mice showed not only phenotypical and morphological characteristics of aging as well as cellular and molecular features of replicative senescence (Königsberg et al., 2004). Considering the cellular changes associated with senescence in vitro as a correlative to organismal aging, we were interested in comparing the senescence onset in primary fibroblast cultures obtained from the lungs of retired female breeder mice with the ones obtained from nulliparous female mice of the same age (12 months), to validate a cellular model to study cellular senescence associated with breeding. Two-month-old animals with vaginal openings were also used as controls of young, fully-developed adults.

The lung was chosen because of its high pO_2 (López-Torres et al., 1993) that might induce an oxidative stress response not found in other tissues. Cell proliferation, DNA synthesis, and the expression of senescence-associated β-galactosidase activity (SA-β-Gal) were evaluated as senescent parameters. To test differences in energetic competence, mitochondrial respiration was evaluated in mitochondrial preparations isolated from the livers of the same animals used for the primary cultures. Our data indicated that the cells derived from breeder female mice onset into replicative senescence earlier than the cells derived from age-matched nulliparous mice.

2. Materials and methods

2.1. Animals

Female CD-1 mice (*Mus musculus*) were obtained from the closed breeding colony at the Universidad Autonoma Metropolitana-Iztapalapa (UAM-I). For regular reproductive purposes at the breeding colony, 3 or 4, 2-month-old female CD-1 mice are placed with one male in what is called a "reproductive unit". All the females in these units become pregnant, give birth and start nursing their offspring approximately at the same time. Normally, there are no fights between the females and they even share the lactation. The gestation time for CD-1 mice is 19 days and the nursing time is 21 days. However, these kind of mice enter into estrus immediately after giving birth, therefore, they can be pregnant and nursing at the same time. Usually the mice live in the reproductive units for 6 to 7 months resulting in a total of 6–8 litters per female. Normally when the females finish their reproductive period (around 9 months old), they are sacrificed. In this work, 9-month-old breeder females were taken out of the reproductive unit and housed together until they were 12 months old.

To compare the wear out of the old retired breeder, 12-month-old female nulliparous mice were used as controls, along with young 2-month-old female mice, (CD-1 mice normally live 24 months in laboratory conditions) (Lang, 1995).

Mice were cared for according to the principles of the Mexican official ethics standard 062-ZOO-1999.

2.2. Cell culture

Primary cultures of normal lung fibroblasts were obtained from CD-1 female mice according to Doyle's protocol (Doyle et al., 1998) with minor modifications (Königsberg et al., 2004). Cells were maintained routinely in Dulbecco's modified essential medium (DMEM; GIBCO-BRL, Gaithersburg, MD, USA) supplemented with 10% inactivated fetal bovine serum (FBS; GIBCO-BRL), 1% nonessential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin (GIBCO-BRL). The medium was replaced every 2 to 3 days. Cells were trypsinized upon reaching confluence and re-plated to continue time in culture. Cells were grown at 37 °C in 60-mm-diameter culture multiwell plates (Corning, Acton, MA, USA) in an atmosphere of 95% air and 5% CO_2.

From here on, cells derived from 2-month-old mice are denominated young cells (YC), whereas cells from 12-month-old retired breeders and 12-month-old nulliparous mice are denominated old breeder (OBC) and old nulliparous (ONC) cells, respectively.