Experimental Gerontology 71 (2015) 109–117

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Review

Comparative cellular biogerontology: Where do we stand?

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ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 24 August 2015
Accepted 29 August 2015
Available online 3 September 2015

Keywords:
Reptiles
Longevity
Fibroblast
Stress
Senescence

ABSTRACT

Due to the extreme variation in life spans among species, using a comparative approach to address fundamental questions about the aging process has much to offer. For example, maximum life span can vary by as much as several orders of magnitude among taxa. In recent years, using primary cell lines cultured from species with disparate life spans and aging rates has gained considerable momentum as a means to dissect the mechanisms underlying the variation in aging rates among animals. In this review, we reiterate the strengths of comparative cellular biogerontology, as well as provide a survey of the current state of the field. By and large this work sprang from early studies using cell lines derived from long-lived mutant mice. Specifically, they suggested that an enhanced resistance to cellular stress was strongly associated with increased longevity of select laboratory models. Since then, we and others have shown that the degree of stress resistance and species longevity is also correlated among cell lines derived from free-living populations of both mammals and birds, and more recent studies have begun to reveal the biochemical and physiological underpinnings to these differences. The continued study of cultured cell lines from vertebrates with disparate life spans is likely to provide considerable insight toward unifying mechanisms of longevity assurance.

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Aging and its associated decline in survival and reproductive fitness is an inherent feature of biological systems (Lemaitre et al., 2015). Even bacteria, once thought to possess "clonal immortality," exhibit signs of replicative aging due to the formation of a distinct "soma" and "germ line" after binary fission (Gomez, 2010). Moreover, the mechanisms that drive the aging process, that is the decline of physiological function with the passage of time (Grotewiel et al., 2005), are largely conserved from one species to another. Or at least it appears that way. The problem with making this generalization is that the vast majority of biogerontological research has been conducted in one of four species: baker's yeast (*Saccharomyces cerevisiae*), the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and house mice *Mus musculus domesticus*. Because these model organisms have been propagated and maintained under the highly artificial conditions of a laboratory setting it is impossible to know whether the regulation of the aging process within these species is a truly a reflection of the aging process under natural conditions or is simply an artifact of laboratory adaptation.

1. Model organisms for biogerontological research

Laboratory strains of rat, most notably Fischer 344 (F344) and Sprague–Dawley (SD), were the most commonly used rodent aging

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http://dx.doi.org/10.1016/j.exger.2015.08.018
0531-5565/

models up until a few decades ago. Now it is the laboratory mouse, *Mus musculus domesticus* that is undoubtedly the workhorse of vertebrate biogerontological research that is recognized by academics and the general public alike. Unfortunately, a prototypical laboratory mouse does not exist. There are literally hundreds of strains, each with a unique genotype and its associated variation in phenotypic traits, that are used for all aspects of biomedical research (Doetschman, 2009). Within biogerontological circles, the C57BL/6 and CB6F1 (i.e. a BALB/c × C57BL/6 F1 hybrid) strains are the mostly widely used. For example, a Pubmed search using the terms "aging," "C57BL/6," and "CB6F1" returned over 750 citations within the last 10 years alone.

The advantages of laboratory mouse strains are several-fold. Most notably: (1) due to standardized husbandry conditions they are relatively easy to maintain with minimal cost; (2) due to their short generation times and large litter sizes it is simple to generate large numbers of individuals for study quickly; and (3) due to their small size hundreds of mice can be kept in a room no larger than a typical bedroom. Most importantly, however, the complete genome of multiple strains has been sequenced and laboratory mice are readily amendable to genetic manipulation (Doetschman, 2009). Indeed, genetic manipulation, such as the generation of single, double, and triple gene knockouts, is often used to explore the effects of aging on a broad suite of traits, such as cognition or the DNA damage response (Yu et al., 2015; Lemos et al., 2010). Finally, since laboratory strains have a mean longevity of approximately two years, it is feasible to complete a life span study within the typical life cycle of a grant (Vanhooren and Libert, 2013).

It is also no secret that a given animal model is often chosen for its specific attributes. For example, there are more than 20 strains of mice available from the Jackson Laboratory that harbor mutations associated with both congenital and age-related deafness for those interested in hearing research, as well as dozens more that are susceptible to the development of muscular dystrophies, ocular disease or skin aging (http://jaxmice.jax.org/research/index.html). In addition, there is an ever increasing number of single-gene mutant strains that have been shown to be significantly long-lived relative to their wildtype counterparts (Bartke et al., 2013). Taken together, this means that laboratory mice have likely become the dominant vertebrate model for aging research more out of convenience rather than any preconceived appreciation for their utility; although the use of laboratory strains has undoubtedly moved the field of biogerontology forward by enormous leaps within the last two decades.

Nevertheless, it can be argued that this approach is somewhat shortsighted. Just because a model organism is convenient it does not mean it's perfect. Even within mice, there is still much to be learned about what regulates the aging process, especially given the marked disparity among strains in their propensity to develop specific age-related pathologies (Miller and Nadon, 2000; Harper, 2008). In addition, there is a marked disparity in the lifespan of wild-derived mouse stocks relative to laboratory mice (Harper et al., 2006a, 2006c). If we go beyond mice and consider mammals as a whole, untangling the aging process becomes even more complex since specific taxa are clear outliers in terms of lifespan potential. For example, bats and primates, especially humans, live far longer than they "should" given their body size (Fischer and Austad, 2011; Austad and Fischer, 1991; Bronikowski et al., 2011).

Another consideration is the influence of laboratory husbandry on a species' genetics and physiology. Regardless of the model organism, all laboratory stocks or strains are adapted to the highly artificial conditions imposed by life in agar plates, plastic vials or shoebox cages under strict nutritional parameters and relatively invariant environmental conditions. Couple this with hundreds-to-thousands of generations of inbreeding for sexually reproducing species, or the continued maintenance of clonal lines for asexually reproducing species, and the conditions are set for very unusual genetic end products. This is a significant deviation from a species' norm and it should come as no surprise that the individual end products are very unlikely to occur in nature. Indeed, some well-known single gene mutations that lead to a dramatic increase in longevity fare poorly when maintained under "real world" conditions in both invertebrates (Walker et al., 2000) and vertebrates (Fabris et al., 1972; Giorgio et al., 2012). In fact, there is evidence to suggest that free-living populations of typical laboratory-adapted species age differently than their laboratory-adapted counterparts due to the need to contend with the stress of variable environments and social interactions with conspecifics under natural conditions (Authier et al., 2012; Bro-Jorgensen, 2012; Diamantidis et al., 2011; Duyck et al., 2010; Hamalainen et al., 2014; Hansen et al., 2012; Harper et al., 2006a; McFarland and Majolo, 2013; Reid et al., 2010; Reynolds and Phillips, 2013; Saino et al., 2012; Selman et al., 2013; Stumpferl et al., 2012; Sutphin and Kaeberlein, 2008; Turbill and Ruf, 2010; Yuan et al., 2013).

2. Natural populations of animals for aging research

The variation in maximum lifespans among animals is enormous and Mother Nature has provided a practically limitless array of test species that should garner significant biogerontological interest. For example, maximum life spans may be a 100-fold or more within the same taxonomic class (e.g. mammals, insects) and even within a single mammalian order, namely rodents, differences of at least 30-fold in maximum life span exist among individual species (Hulbert et al., 2007). This provides a rich palette from which to work and taking a comparative approach may hold the key to unraveling the complexity of the aging process while identifying the "public" mechanisms of aging long sought after by the biogerontological community (Martin et al., 1996).

In a perfect world, biogerontologists would be able to take a fully comparative approach; i.e. monitoring a diverse array of species with known differences in maximum life spans to catalog age-related changes in gene expression, protein activity and physiological function over chronological time. These data could then be used to search for commonalities among long-lived species, and by extension, the "winning" combination of genetic and biochemical markers that ensure long life. As beneficial as this scenario would be, it is not a practical reality for two reasons: (1) the infrastructure needed is daunting to say the least, even for an entire institution, much less any one research laboratory. (2) The very reason some species would be desirable, namely the fact that they are exceptionally long-lived, makes them impossible to study simply because no one individual is likely to be around long enough to see the study to its end. On the other hand, due to the relative ease of generating primary cell lines from a diverse array of vertebrate species (Miller et al., 2011) it has been possible to delve into the mechanisms underlying successful aging both within, and among, various vertebrate taxa using cell culture models.

3. Primary cell culture, stress resistance and rodent models of aging

Life history theory posits that organisms are forced to make physiological trade-offs in order to maximize reproductive fitness (Lemaitre et al., 2015). Or more specifically, if a species devotes more resources toward cellular repair and defense, fewer are available for reproduction and *vice versa*. Altogether, it is thought that the product of these tradeoffs is the incurrence of organismal senescence (Williams, 1957). Importantly, there is a clear physiological foundation for these tradeoffs and primary cell lines, especially fibroblasts, have been an attractive model system to dissect the specific pathways involved (Jimenez et al., 2014a).

Primary cultures of fibroblasts are readily obtainable from any organ that has a large proportion of connective tissue, but skin is the most common source. This is because of the relative ease with which skin samples can be collected using punch biopsies, ear punches and/or toe/tail clips. Moreover, since these methods are minimally invasive and do not require that individuals be sacrificed for biopsy collection they are suitable for sampling threatened or endangered species. In