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Original article

Genetic instability and aging under the scrutiny of comparative biology: a meta-analysis of spontaneous micronuclei frequency

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ABSTRACT

In gerontology, comparative biology of longevity offers a powerful observation point thus far underexploited. We use this approach to evaluate the role of genetic stability in longevity determination, extrapolating existing data from the literature. Screening eight pre-existing studies, we collected data from 47 mammalian species and analyzed the relationship of spontaneous micronucleated erythrocyte frequency to species maximum longevity and species adult body mass. Since in 26 of these species the spleen removes micronucleated erythrocytes from the peripheral circulation, we conducted further comparative analysis on the remaining 21 species. We demonstrate that spontaneous micronucleated erythrocyte frequency correlates primarily with body mass and not with maximum longevity. We suggest that other data on genetic stability could be collected from published works in different species and analyzed in a similar way to test further the role of genetic stability in aging.

1. Introduction

1.1. The DNA damage theory of aging

The idea that accumulation of DNA damage may be responsible for the aging process dates back to the late 1950s (Failla, 1958; Szilard, 1959). The key aspects of this theory are twofold: the idea that damage accumulates with time and the fact that damage to the DNA is of fundamental importance. The first concept is easily explainable if we look at many of the lifeless objects of our daily life that, after a period of service, accumulate some sort of damage (cars, buildings, etc.). These objects are designed to last for a specified time and they maintain themselves well for that period. However, damage eventually appears and accumulates due to intrinsic failure of their parts, harmful extrinsic factors, and lack of proper maintenance and repair. The result is a period of good function followed by a gradual decline. Biological systems are similar: there is a period of low mortality up to a point, followed by a gradual decline in fitness and increased mortality. Linking this concept to longevity, each species possesses an intrinsic design that ensures functionality for the life span of that species. Repair of DNA damage is critical to that design.

The second concept, that damage to the DNA is of fundamental importance, is also readily understandable thanks to a recent scientific breakthrough. Gibson et al., having created the first synthetic life form through the synthesis of a 1.08-megabase-pair mycoplasma genome, have de facto demonstrated that DNA has a master role in the hierarchy of organic molecules (Gibson et al., 2010). DNA damage will, in fact, carry consequences at any level of biological organization or function (RNA, protein, metabolism, etc.).

Szilard, in his theory paper "*On the nature of the aging process*", discussed "hits" to chromosomes that render genes inactive (Szilard, 1959). However, genetic instability may also be obtained in the absence of actual damage to the DNA molecule. Aneugens, for example, are substances that cause genetic instability by simply interfering with chromosome segregation. Genetic, genomic, or chromosome instability are thus broader terminologies that are being used progressively more in gerontology (Bürkle, 2002).

Abbreviations: MN, micronuclei; MNE, micronucleated erythrocytes; MNEF, micronucleated erythrocytes frequency; SSB, single-strand break; DSB, double-strand break; TE, total erythrocytes; BM, body mass; ML, maximum longevity; NHEJ, non-homologous end joining; HR, homologous recombination; TEs, transposable elements.

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Many observations and theoretical considerations support a role for genetic instability in aging. In rats, for example, inducing DNA damage with a single dose of ionizing radiation at a young age (6 weeks) shortens average life span by 10% for a dose of 1 Gy and 25% for a 3-Gy dose (Soffritti et al., 2015). The very existence of more than 150 genes involved in DNA damage repair attests to the importance of DNA integrity in sustaining life (Wood et al., 2005). Following this reasoning, the perturbation of one such repair gene should, to some degree, negatively affect life span. In support of this, the Ku heterodimer, comprising the Ku70 and Ku80 polypeptides, is critical for double-stand break (DSB) repair. Double mutants, as well as mutants of either Ku component, display an approximate 66% reduction in life span, with signs of early aging (Li et al., 2007). Despite a dramatic effect of the mutant Ku on life span, not all data are supportive of the theory. Kaya et al., for example, have monitored a mutant of *Saccharomyces cerevisiae* that accumulates high levels of mutations. Whereas old wild-type cells with few mutations were dying, young mutant cells with many more mutations continued budding (Kaya et al., 2015). In conclusion, although the DNA damage accumulation theory is among the "oldest" of the aging theories, discussion of its true relevance is still very active among gerontologists.

1.2. Genomic stability and body mass

The somatic mutation theory of aging predicts that long-lived species will have superior genomic stability. In fact, if alterations at the level of DNA are supposed to be responsible for the aging process, these alterations must accumulate at a lower rate in longer-lived species. With similar reasoning, we can hypothesize that genomic stability will also be positively related to adult body mass. In fact, two species with similar longevity but different adult body mass will differ in their total number of cells in adulthood. Consequently, the number of total cellular divisions that the genome must sustain, from the creation of the zygote onward, can be very different. Additionally, body mass is positively correlated with longevity among mammals, birds, amphibians, and reptiles (Magalhães et al., 2007). In the multitude of cellular mechanisms involved in preserving genomic stability, some will be more essential in order to guarantee longevity, while others will be more essential in order to guarantee the development of greater body mass. For example, we have found that the capacity to bind DNA ends (a capacity of the Ku heterodimer) correlates with longevity but not with body mass in 12 mammalian species (Lorenzini et al., 2009). In contrast, we have found that the efficiency of the spindle assembly checkpoint correlates strictly with body mass and more loosely with longevity in six species of mammals (Lorenzini et al., 2011a). The efficiency of excision repair after UV damage and the activity of Parp, an enzyme involved in single-strand break (SSB) repair, are other examples where a correlation is found both with longevity and body mass (Grube and Bürkle, 1992; Promislow, 1994; Cortopassi and Wang, 1996; Stuart et al., 2013).

1.3. Advantages of the comparative approach

Life span studies may be extremely long lasting, being clearly linked to the life span of the tested species. Rats and mice, which require at least three to four years of study, are the most studied mammals in the laboratory. Nonetheless, these two rodents are not the best representation of the aging process in mammals since their longevities lay at the lowest extreme of the vast range of life spans exhibited by this class. Maximum longevity in mammals spans 100-fold, from 2.1 years for the forest shrew to 211 years for the bowhead whale (data from the AnAge database; Tacutu et al., 2013). Comparing characteristics of species known to differ significantly in longevity represents a unique and powerful approach to aging studies (Austad, 2009). Comparative cellular biology also gives the potential to manipulate the environment and challenge cells with different stressors to monitor their differential reactivity (reviewed in Stuart et al., 2013). In a recent and comprehensive review, Moskalev et al. analyzed evidence for and against the DNA damage theory cataloguing studies based on Koch-like criteria (Moskalev et al., 2013). The authors concluded, in part, that comparative biology data are thus far scarce in the literature (both for and against the theory). In the present article, we will demonstrate to the gerontological community that comparative biology data are readily available and may need only to be extracted from the literature. To this end, we conducted a meta-analysis on spontaneous *in vivo* micronuclei (MN) frequency in mammalian erythrocytes of species with different longevity and body mass. Our aim was to search for a possible relationship between these two parameters (longevity and body mass) and the propensity to this type of genomic instability.

1.4. Analysis of micronucleated erythrocyte frequency across mammals

MN are small cellular bodies containing genomic material enclosed in nuclear membranes. These small bodies are produced during mitosis when a whole chromosome or chromosome fragment is left behind by the mitotic spindle and fails to be incorporated into either one of the daughter cell nuclei. The spontaneous or induced formation of MN is used as a cytogenetic test using different cellular substrates: epithelial cells from mouth swab, lymphocytes from blood, and erythrocytes from blood, bone marrow, or spleen (Fenech et al., 2011; Abramsson-Zetterberg et al., 1999). Erythrocytes are particularly useful since they naturally expel the nucleus during maturation, but do not expel the MN (Schmid, 1975). With staining such as Giemsa or acridine orange, micronucleated erythrocytes (MNE) are scored to assess the possibility of genotoxicity *in vivo* (Krishna and Hayashi, 2000). Observing MN in bone marrow erythrocytes may testify to recent genotoxic damage, while the presence of MN in circulating erythrocytes is an indication of chronic exposure (Schlegel and MacGregor, 1982). If the aging process is seen as an accumulation of damage, then it may be likened to a chronic exposure. Consequently, we have screened the literature for reports on the spontaneous frequency of MN in circulating erythrocytes in different species of mammals.

2. Methods

Through a systematic PubMed search, we have found data on the spontaneous MNE frequency (MNEF) for 65 mammals. The age of the donor organism used in the MNEF determination was one problem that needed to be solved before collecting data. MNEF is known to be higher in erythrocytes of developing organisms and to decrease to a certain level in adult life (Zúñiga-González et al., 2001; Zúñiga-González et al., 2005). Additionally, studies performed in mice (Dass et al., 1997; Bhilwade et al., 2014) and humans (Peace and Succop, 1999) show that during aging there is again an increase in MNEF. According to Sato et al., this increase is not observed, or observed only very late in life, in some strains of mice (Sato et al., 1995). In light of these considerations, we collected data only for adult animals for which the approximate age was specified (47 species). Table 1 reports this filtered collected data and relative references. Additionally body mass (BM) and maximum longevity (ML) values are shown as taken from the AnAge Database (Tacutu et al., 2013).

The comparisons between these data could be weakened by factors related to peculiar differences in the circulatory systems of the species and to different modalities of erythropoiesis. For