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Research paper

Telomere regulation during ageing and tumorigenesis of the grey mouse lemur

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ABSTRACT

Telomere erosion leading to replicative senescence ageing has been well documented in human and anthropoid primates, and provides a clue against tumorigenesis. In contrast, other mammals, such as laboratory mice, with short lifespan and low body weight mass have different telomere biology without replicative telomere ageing. We analyzed telomere biology in the grey mouse lemur, a small prosimian model with a relative long lifespan currently used in ageing research. We report an average telomere length by telomere restriction fragment (TRF) among the longest reported so far for a primate species (25–30 kb), but without detectable overall telomere shortening with ageing on blood samples. However, we demonstrate using universal STELA (Single Telomere Length Amplification) the existence of short telomeres, the increase of which, while correlating with ageing might be related to another mechanism than replicative senescence. We also found a low stringency of telomerase restriction in tissues and an ease to immortalize fibroblasts *in vitro* upon spontaneous telomerase activation. Finally, we describe the first grey mouse lemur cancer cell line showing a dramatic telomere shortening and high telomerase activity associated with polyploidy. Our overall results suggest that telomere biology in grey mouse lemur is an exception among primates, with at best a physiologically limited replicative telomere ageing and closest to that observed in small rodents.

1. Introduction

Telomeres are nucleoprotein structures that protect the end of chromosomes from inappropriate DNA repair events or degradation [1]. In vertebrates, telomeres consist of TTAGGG repeats bound by the proteins from the shelterin complex, which regulate telomere length and replication, and control its protection under a capped state [2,3]. Two mechanisms have been reported for the maintenance of telomere length. The first involves telomerase activity to add telomere repeats at the 3′ telomeric G-overhang [1]. The second corresponds to homologous recombination between telomeres, but was only evidenced in immortalized or tumour cells in humans [4]. A recent work reported on the physiological existence of telomere recombination in mice somatic tissues, although it is not clear whether it really contributes to telomere length maintenance [5].

Telomere erosion leading to replicative senescence and the role of telomerase to counteract this erosion has been extensively studied in cell cultures and in human premature ageing diseases affecting telomerase components [6–8]. These studies led to the

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general concept that progressive telomere shortening from cell division (also known as replicative ageing) contributes to age-dependent processes through cell senescence. Conversely, telomere shortening is prevented in the germ line, and partially counteracted in stem cells and tissues with high renewal capacities, where telomerase activity is present [9–11]. Overall, loss of chromosome end protection through telomere erosion is one of the hallmarks of ageing and senescence, which contribute to provide a barrier against genome instability and cancer in human [12]. Afterwards, the mechanism of replicative ageing has been demonstrated for many species in mammals [13]. However, it is not a universal programme, since different telomere biology has been observed in some species such as laboratory mouse. Indeed, while telomerase activity in somatic tissue is mostly the signature of cancer in human, mice laboratory strains presented a detectable telomerase activity in many somatic tissues. Moreover, laboratory strains have very long telomere (~up to 50) compared to humans (~10 kb) and it is today admitted that they do not use replicative ageing as a mean of cell division counting [14,15]. Of note, a growth arrest occurs in mice cultures that do not correspond to replicative senescence, but rather to stress-induced senescence [7,16]. On the other hand, studies on mouse strains or species with shorter telomeres, such as *Mus spretus* or wild-derived castaneous mouse strains (CAST/EiJ), suggested that replicative senescence may take place at least in some tissues [17,18]. Interestingly, in other rodents, telomere length or telomerase activity did not correlate with lifespan. In contrast, telomerase activity in somatic tissues was found inversely correlated with the body mass, suggesting a coevolution to repress telomerase and to limit cancer risk when the body mass increases [19].

A larger comparative study of telomere biology on more than 60 mammalian species showed interesting relationships between telomere length, telomerase expression, body mass and lifespan [20]. This study point out that telomere length inversely correlated with lifespan and that telomerase activity inversely correlated with the body mass, with a clear tendency for species larger than 1 kg to have short telomeres (<20 kb) and repress telomerase. This study also suggested that the acquisition of homeothermy requires the onset of a mechanism to repress oxidative damages leading to the acquisition of replicative ageing for larger mammals, such as anthropoid primates, in order to increase longevity [21,22]. Interestingly, a resistance to oxidative damage was also found associated with decreased overall telomere length and increased body mass or lifespan among different mammal species [20].

Primates are subdivided into 2 great clades, the Haplorrhini suborder that contains the Simiiformes infraorder (*i.e.* anthropoid primate) and the Strepsirrhini suborder that contains the Lemuriformes infraorder (such as grey mouse lemur) which would have diverged 87 Million Years Ago (MYA) [23]. Telomere biology has been studied in several anthropoid primates and a robust control of replicative ageing was observed [22,24,25]. In contrast, prosimian lemurs seem to display an intermediate situation regarding their telomere biology. Fibroblasts from ring-tailed lemur (*Lemur catta*) displayed heterogeneous telomeres length (15–50 kb) that shortened during cell division until cultured cells stopped dividing after 80 population doublings (PD). After a plateau of the cell population doubling, some cells overgrew and immortalized with a slower growth rate. Surprisingly, this escape was not accompanied by a reactivation of telomerase but a high chromosomal instability was observed. A recombination-based alternative pathway (ALT) was evocated [22]. Therefore, telomere biology in ring-tailed lemur represents an interesting situation that deserved to be investigated in others Lemuriformes.

The grey mouse lemur (*Microcebus murinus*) has diverged from ring-tailed lemur 39 MYA [23]. Interestingly, grey mouse lemur is the only lemur with important breeding colony that could be used in ageing research with several advantages as compared to other classical biological models, such as rodents, including its closer phylogeny to human than rodents [26]. First, they share physiological and anatomical features, such as a complex nervous system, and ageing processes in common with humans. Second, the size of the breeding colony mimics the heterogeneity found in the human population, but their observation can worthy be performed under controlled experimental conditions. Furthermore, grey mouse lemur is exceptionally long-lived when compared to other small mammals (8–12 years *versus* 3 years for laboratory mouse) but lower than other non-human primates, thus allowing easier and faster observations. Therefore, the position of mouse grey lemur at an interesting evolutionary transition between small and large body mammals and its interest for ageing studies prompted us to study their telomere biology.

We report here the analysis of the telomere biology during ageing of the grey mouse lemur and the characterization of the cell growth and telomere behaviour of fibroblasts and mammary tumour cell cultures. Our results, using different telomere techniques including universal STELA (Single Telomere Length Amplification) suggest that grey mouse lemur presents a telomere metabolism without obvious relationship to replicative ageing and with features closer to laboratory mice than to other primates. Transversal ageing studies in blood samples using universal STELA also revealed the presence of a fraction of very short telomeres increasing with ageing, that classical TRF did not easily detect, which might arise from abrupt telomere shortening. These findings suggest that grey mouse lemur represent an interesting situation among primates for its telomere biology regarding its relative long longevity.

2. Materials and methods

2.1. Ethic statement

All experiments were performed in the laboratory breeding colony of Brunoy (UMR 7179 CNRS/MNHN, France; agreement n° E91-114-1 from the Direction Départementale de la Protection des Populations de l'Essonne). Under veterinary supervision (DVM F. Aujard n°17–145), blood samples were collected from quick sampling of the saphenous vein in heparinized glass capillaries and euthanasia was performed using Pentobarbital (1 ml/100 g body mass). These procedures received the legal authorization from the Cuvier Committee in Animal Experiment (registered to the National Ethic Committee n° 68-018) and the agreement from the Internal Review Board of the UMR 7179. All efforts were made to minimize nociception during the experiments.

2.2. Animals and housing conditions

All the studied grey mouse lemurs (*M. murinus*) were born in the laboratory breeding-colony of the National Museum of Natural History in Brunoy, France from a stock originally caught in southern Madagascar 45 years ago. In captivity, seasonal variations of physiological and behavioural functions were entrained by alternating between 6 months of a long-day (LD) photoperiod (14:10 light:-dark) and 6 months of a short-day photoperiod (SD) (10:14 light:-dark), under artificial light (white light, 250 lux, wavelength peak at 488 nm). Animals were studied during the LD period. To minimize social influences, animals were housed individually in cages (50 × 40 × 30 cm) with branches and a nest box, visually separated from each other.

General conditions of captivity were maintained constant: ambient temperature (24–26 °C), relative humidity (55%), food in

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