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Dietary restriction ameliorates haematopoietic ageing independent of telomerase, whilst lack of telomerase and short telomeres exacerbates the ageing phenotype

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ABSTRACT

Ageing is associated with an overall decline in the functional capacity of tissues and stem cells, including haematopoietic stem and progenitor cells (HSPCs), as well as telomere dysfunction. Dietary restriction (DR) is a recognised anti-ageing intervention that extends lifespan and improves health in several organisms. To investigate the role of telomeres and telomerase in haematopoietic ageing, we compared the HSPC profile and clonogenic capacity of bone marrow cells from wild type with telomerase-deficient mice and the effect of DR on these parameters.

Compared with young mice, aged wild type mice demonstrated a significant accumulation of HSPCs (1.3% vs 0.2%, $P = 0.002$) and elevated numbers of granulocyte/macrophage colony forming units (CFU-GM, 26.4 vs 17.3, $P = 0.0037$) consistent with myeloid "skewing" of haematopoiesis. DR was able to restrict the increase in HSPC number as well as the myeloid "skewing" in aged wild type mice. In order to analyse the influence of short telomeres on the ageing phenotype we examined mice lacking the RNA template for telomerase, $TERC^{-/-}$. Telomere shortening resulted in a similar bone marrow phenotype to that seen in aged mice, with significantly increased HSPC numbers and an increased formation of all myeloid colony types but at a younger age than wild type mice. However, an additional increase in erythroid colonies (BFU-E) was also evident. Mice lacking telomerase reverse transcriptase without shortened telomeres, $TERT^{-/-}$, also presented with augmented haematopoietic ageing which was ameliorated by DR, demonstrating that the effect of DR was not dependent on the presence of telomerase in HSPCs. We conclude that whilst shortened telomeres mimic some aspects of haematopoietic ageing, both shortened telomeres and the lack of telomerase produce specific phenotypes, some of which can be prevented by dietary restriction.

1. Introduction

Ageing is associated with an increased spectrum of haematological diseases including anaemia, myelodysplastic syndrome and myeloid leukaemia. An important underlying factor of ageing is immunosenescence, which is a progressive disruption of innate and adaptive immune function. This decline in adaptive immunity results in a persisting low-grade chronic inflammation during ageing which is referred as inflamm-ageing (Franceschi et al., 2000). On a cellular level, ageing leads to an increase in the number of haematopoietic stem and progenitor cells (HSPCs) and elevated myeloid differentiation (Beerman et al., 2010; Benz et al., 2012; Cho et al., 2008; Dykstra et al., 2011; Pang et al., 2011), impaired regenerative potential of HSPCs (Morrison et al., 1996; Rossi et al., 2005), and disrupted HSPC homing efficiency (Dykstra et al., 2011; Liang et al., 2005). Myeloid skewing during ageing is associated with an up-regulation of genes associated with myeloid specification in the HSPC compartment (Rossi et al., 2005). Furthermore, the increase in myeloid precursors with ageing is associated with a decrease in the oxidative burst and the phagocytic capacity of neutrophils and macrophages (Kuranda et al., 2011; Plowden et al., 2004). On a molecular level, dysfunction of telomeres and telomerase is a key aspect of ageing in haematopoietic cells. Ageing HSPCs show an accumulation of DNA damage and telomere dysfunction that leads to age-related dysfunction of stem cells (Flores et al., 2008; Rossi et al., 2007; Song et al., 2012; Yahata et al., 2011).

Telomeres are protective structures at the end of mammalian chromosomes. Telomeres consist of TTAGGG repeats bound by a multiprotein complex known as shelterin. Telomerase is a reverse transcriptase responsible for the addition of TTAGGG repeats de novo onto telomeres at chromosome ends. Telomerase is composed of a catalytic subunit TERT (telomerase reverse transcriptase) and an associated RNA component (TERC) which contains the template for telomere

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synthesis (Saretzki, 2009). Progressive telomere shortening leads to critically short telomeres that limit the replicative capacity of cells (de Lange, 2002). In mouse models lacking one of the telomerase components, telomeres shorten progressively during successive generations of telomerase-deficient TERC$^{-/-}$ and TERT$^{-/-}$ mice, however, no significant abnormal phenotypes have been observed in early generations to date (Blasco et al., 1997; Chiang et al., 2004; Lee et al., 1998). However, telomere-independent roles for the telomerase protein TERT have been described recently (Saretzki, 2009). Notably, most mouse strains have exceptionally long telomeres compared to those of humans. In contrast, heterozygous mutations in TERT or TERC in humans are linked to some diseases characterised by premature loss of tissue renewal and death, such as idiopathic pulmonary fibrosis and dyskeratosis congenita (Armanios et al., 2007; Vulliamy et al., 2004).

Dietary restriction (DR) is a well-known anti-ageing intervention that increases lifespan as well as health in many organisms and decreases the incidence of cancer during ageing (Bronson and Lipman, 1991; Swindell, 2012; Weindruch et al., 1988). DR also delays some age-related diseases such as diabetes, brain atrophy, and cardiovascular disease (Colman et al., 2014). However, some studies have reported varying influences of DR on lifespan in a panel of recombinant inbred mouse strains (Liao et al., 2010) and rhesus monkeys (Mattison et al., 2012). The exact mechanism of DR is not clear, however, evidence is mounting for a protective effect of DR on cellular damage and oxidative stress by reducing cellular metabolism and ROS production (Bordone and Guarente, 2005). Moreover, studies have confirmed several nutrient-signalling pathways mediating DR effects including AMP kinase (Greer et al., 2007; Schulz et al., 2007), sirtuins (Li et al., 2008; Rogina and Helfand, 2004), insulin/insulin-like growth factor (IGF-1) (Arum et al., 2009; Honjoh et al., 2009), and the target of rapamycin (mTOR) pathway (Hansen et al., 2007; Kaeberlein et al., 2005; Kapahi et al., 2004), reviewed in Kenyon (2010). Although the beneficial effect of DR on HSPC ageing has been analysed previously using bone marrow transplantation (Ertl et al., 2008), little is known about HSPC ageing in situ, in the absence of bone marrow transplantation, particularly with respect to telomeres and telomerase in TERT$^{-/-}$ and TERC$^{-/-}$ mice.

The main focus of this study was to investigate the role of telomerase and the impact of DR on HSPC ageing. In order to analyse the role of telomerase, we used first generation TERT and TERC knockout mice. Both telomerase knockout mice (TERT and TERC) lack telomerase activity and are characterised by progressively shortening telomeres over consecutive generations (Blasco et al., 1997; Chiang et al., 2004; Sahin et al., 2011). First generation of mice was examined as a model of ageing based on the lack of telomerase and beginning of telomere shortening.

The aims of our study were to investigate how the lack of telomerase or short telomeres in TERT$^{-/-}$ and TERC$^{-/-}$ mice affects HSPC ageing and whether the lack of telomerase alters the effect of DR on HSPCs.

2. Methods

2.1. Animals

TERT and TERC mouse lines were purchased from the Jackson Laboratory. The TERT$^{-/-}$ strain was: B6.129S-Tert, tm1Yjc/J (Chiang et al., 2004) and the TERC$^{-/-}$ strain was: B6.Cg-Terc, tm1Rdp/J (Blasco et al., 1997). Heterozygous mice were bred to obtain wild type, first generation (G1) knockout and heterozygous mice for the TERT and TERC genotypes. Only male first generation mice were used for the experiments. Animals were housed in the same room and provided with sawdust, paper bedding, and had ad libitum access to water. Mice were housed at 20 ± 2 °C under a 12 h light/12 h dark photoperiod. Ethical approval was granted by the LERC Newcastle University, UK. The work was licenced by the UK Home Office (PPL 60/3864) and complied with the guiding principles for the care and use of laboratory animals.

2.2. Dietary restriction

The diet used was standard rodent chow (CRM (P); Special Diets Services, Witham, UK) for the AL mice whilst the DR mice received a smaller pellet size (4 mm). During dietary restriction experiments, the control group had ad libitum access to food (AL) whereas the dietary restricted (DR) group was limited by 40% as described previously (Cameron et al., 2011). DR started at 6 months of age and lasted for 16 months, i.e., mice were 22 months when used. Only males were used for the DR experiments. All work complied with the guiding principles for the care and use of laboratory animals in the UK.

2.3. Bone marrow isolation

Tibiae and femurs from both legs of mice were collected and carefully cleaned from adherent soft tissue. The tips of each bone were cut open and bone marrow was collected by inserting the needle into one end of the bone. Each bone was flushed with 3 ml PBS, after that cells were washed twice with 15 ml PBS and counted using a haemocytometer.

2.4. Flow cytometry

Single cell suspensions were prepared from bone marrow cells. Cells were incubated with 2 μl antibodies per 10^6 cells. Biotinylated primary antibodies against CD3e, CD11b, B220, Gr-1, and TER-119 were used and then 0.5 mg/ml PE-labelled streptavidin as described in the lineage specification kit (559971, BD Pharmingen, USA). Cells were then stained with 0.2 mg/ml c-Kit (PerCP) (46-1171-82, eBioscience, USA) and 0.5 mg/ml Sca-1 (PE-Cy7) (553334, BD Bioscience, USA) and analysed using a FACS Canto with FACS Diva software (BD Biosciences).

2.5. Clonogenic assays

8×10^5 cells from each bone marrow cell suspension were added to 4 ml of MethoCult M3434 medium and incubated in triplicate 35 mm dishes at 37 °C, 5% CO_2, with ≥95% humidity for two weeks as described by the manufacturer (Stem Cell Technologies, Canada). Erythroid (BFU-E), granulocytic (CFU-G), macrophage (CFU-M), granulocyte/macrophage mixed (CFU-GM) and the most primitive granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM) colonies were then scored using an inverted microscope (see Fig. 1D).

2.6. Telomere length (flow-FISH)

Telomere length measurements were carried out as previously described (Baerlocher et al., 2006; Spyridopoulos et al., 2004, 2008). In brief, each sample, containing 100,000 bovine thymocytes as an internal standard and 200,000 murine bone marrow cells, was resuspended in a hybridization mixture containing a telomere-specific N-terminal FITC-conjugated $(C_3TA_2)_3$ PNA probe, washed and counterstained with propidium iodide before analysing by flow cytometry on a FACS Canto with FACS Diva software (BD Biosciences). Relative telomere length is determined by comparing mean fluorescence intensity of the sample with that of the internal control. Samples used were from 6 and 22 month old TERT$^{-/-}$ mice and 12 month old TERC$^{-/-}$ mice.

2.7. Statistics

Statistical analyses were performed using GraphPad Prism software version 6.0. To calculate the P-values, either Mann–Whitney test or Kruskal–Wallis test followed by Dunn's test was used for comparison of two or more groups respectively. Error bars represent standard error of the mean in all figures.