TELOMERES AND TELOMERASE GROUP

Maria A. Blasco
Group Leader

Staff Scientists
Isabel López de Silanes, Rosa M. Marión, Paula Martínez, Marinela Méndez (until April)

Post-Doctoral Fellows
Giuseppe Bosso (since June), Sergio Piñeiro (since June), Sarita Saraswati (since October), Kurt Whittemore

Graduate Students
Leire Bejarano, Iole Ferrara, José Carlos González (since November), Jessica Louzame (since September), Juan José Montero, Miguel Ángel Muñoz, Raúl Sánchez

Technicians
Aksinya Derevyanko (since February), Rosa M. Serrano

Visiting Students
Pawel Kordowitzki (May-September)

(Polish Academy of Sciences), Jessica Louzame (until August) (Univ. of Amsterdam, The Netherlands), Lydia T. Poluha (until August) (Univ. of Salford, UK), Tommaso Vicanolo (since October) (Sapienza Univ. of Rome, Italy)

OVERVIEW

We study the mechanisms by which tumour cells are immortal and normal cells are mortal. Immortality is one of the most universal characteristics of cancer cells. The enzyme telomerase is present in more than 95% of all types of human cancers and is absent in normal cells in the body. Telomeres are nucleoprotein complexes located at the ends of chromosomes and are essential for chromosome protection and genomic stability. Progressive shortening of telomeres associated with organism ageing leads to ageing. When telomeres are altered, adult stem cells have a maimed regenerative capacity.

“We have demonstrated that telomerase activation in mouse models of pulmonary fibrosis can stop the progression of this fatal disease in mice.”

Our research focuses on:

- → Generating mouse models to validate telomeres and telomerase as therapeutic targets for cancer and age-related diseases.
- → The interplay between telomeres and DNA repair pathways.
- → The role and regulation of non-coding telomeric RNAs or TERRA.
- → Testing telomerase gene therapy in ‘telomere syndromes’ and age-related diseases.
- → The role of telomerase and telomeres in adult stem cell biology and in nuclear reprogramming of differentiated cells to iPS cells.

RESEARCH HIGHLIGHTS

Telomerase gene therapy to cure pulmonary fibrosis in mice

Pulmonary fibrosis is a fatal lung disease that currently lacks effective treatment and is characterised by fibrotic foci and inflammatory infiltrates. Short telomeres can impair tissue regeneration and are found both in hereditary and sporadic cases. We have shown the therapeutic effects of AAV9-telomerase gene therapy in a mouse model of pulmonary fibrosis from a combination of bleomycin-induced lung damage short telomeres. AAV9 targets preferentially regenerative alveolar type II cells (ATII). Treated mice show improved lung function and lower inflammation and fibrosis at 1-3 weeks after treatment, and improvement or disappearance of the fibrosis at 8 weeks post treatment. Treatment results in longer telomeres and increased proliferation of ATII cells, as well as lower DNA damage, apoptosis, and senescence. We have provided a proof-of-principle that telomerase activation may represent an effective treatment for pulmonary fibrosis provoked by or associated to short telomeres.

Telomerase gene therapy does not increase risk of cancer in cancer-prone models

Short and dysfunctional telomeres result in various age-related conditions, including a group of diseases collectively known as "telomere syndromes" that are provoked by extremely short telomeres arising from germline mutations in telomere genes. This opens the possibility of using telomerase activation as a potential therapeutic strategy to rescue short telomeres, maintaining tissue homeostasis and ameliorating these diseases. In 2012, we designed a highly innovative strategy: a gene therapy that reactivates the telomerase gene using adeno-associated viruses (AAV9). We have since shown its therapeutic efficacy in mouse models of cardiac infarct, aplasic anaemia, and pulmonary fibrosis. Although we did not observe, in any of the former models, an increased cancer incidence as a consequence of telomerase overexpression in any of those models, the potential medical use of telomerase still clashes with fears surrounding a possible increased cancer risk. We now tested the safety of AVV9-telomerase gene therapy in the context of a cancer-prone mouse model owing to expression of oncogenic *K-ras*. We found that telomerase overexpression resulted in longer telomeres in the targeted tissue (lungs; FIGURE 1) but does not accelerate carcinoma onset or progression. Telomerase activation by using AAV9-mediated telomerase gene therapy has no detectable cancer-prone effects in the context of oncogene-induced mouse tumours.

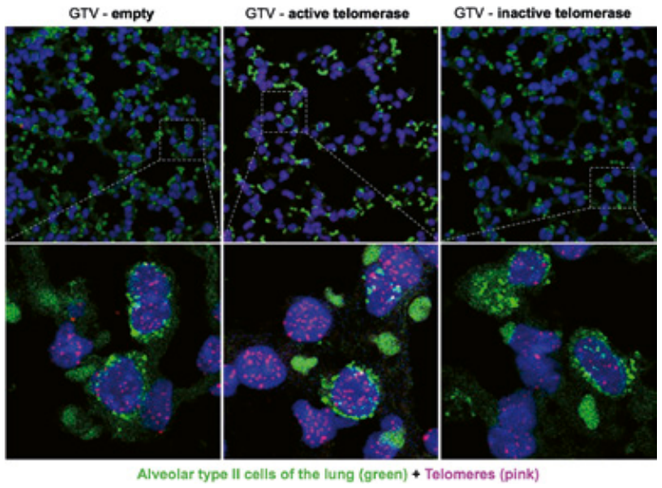

Figure 1 Representative images of lungs treated with gene therapy vectors (GTV). Nuclei are in blue, alveolar type II cells in green and telomeres in red. Lung cells treated with telomerase present the most intense telomeres, indicating that they are the longest of all three scenarios.

TERRAs are important epigenetic regulators

TERRAs are long non-coding RNAs that protect the telomeres. Our Group had already identified chromosome 20q as one of the main origins of human TERRAs and demonstrated that, by generating the first 20q-TERRA knockout models, they are essential for telomere length maintenance and protection. Using 20q-TERRA knockout cells we have now addressed the direct role of TERRAs in telomeric heterochromatin formation. We discovered that TERRAs interact with components of the polycomb complex (PCR2), an important epigenetic regulator of gene expression, thus facilitating the assembly of telomeric heterochromatin. We analysed telomere heterochromatin marks in cells deficient for 20q-TERRA and observed that their telomeres had decreased heterochromatic marks; we discovered that they had lost a histone mark not previously recognised at telomeres (H3K27 trimethylation) that is catalysed by PCR2, a master regulator of gene silencing, which we found locates to the telomere in a TERRA-dependent fashion. Establishment of trimethylation marks in other histones (H3K9 and H4K20) and HP1 binding at telomeres required PRC2-dependent H3K27me3 at telomeres. Our findings demonstrated an important role for TERRAs in telomeric heterochromatin assembly (FIGURE 2). ■

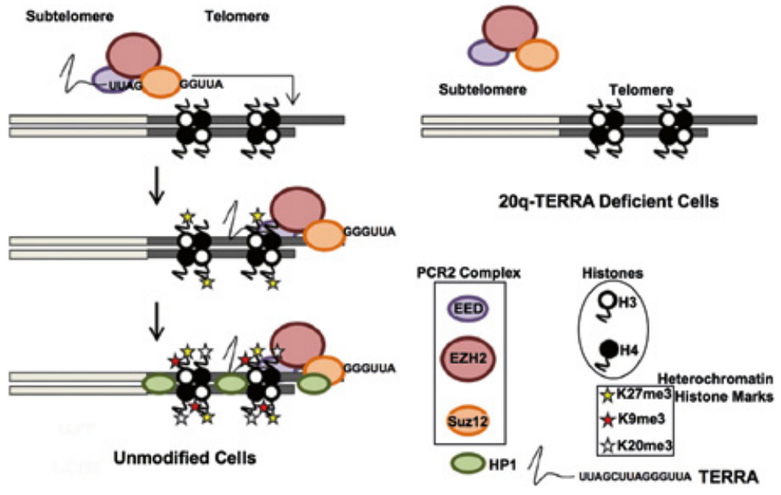

Figure 2 TERRAs regulate the status of telomeric chromatin. After binding to the PRC2 complex TERRAs bring PRC2 to the telomere. Binding of PRC2 makes possible the deposition of heterochromatin marks. These events cannot take place when TERRAs are absent.

PUBLICATIONS

- Montero JJ, Lopez de Silanes I, Megias D, Fraga MF, Castells-Garcia A, Blasco MA (2018). TERRA recruitment of polycomb to telomeres is essential for histone trYmethylation marks at telomeric heterochromatin. *Nat Commun* 9, 1548.
- Martínez P, Blasco MA (2018). Heartbreaking telomeres. *Circ Res* 123, 787-802.
- Povedano JM, Martínez P, Serrano A, Tejera A, Gómez-López G, Bobadilla M, Flores JM, Bosch F, Blasco MA (2018). Therapeutic effects of telomerase in mice with pulmonary fibrosis induced by damage to the lungs and short telomeres. *ELife* 7, pii: e31299.
- Muñoz-Lorente MA, Martínez P, Tejera A, Whittemore K, Moisés-Silva AC, Bosch F, Blasco MA (2018). AAV9-mediated telomerase activation does not accelerate tumorigenesis in the context of oncogenic K-Ras-induced lung cancer. *PLoS Genet* 14, e1007562.
- Freitas-Simoes TM, Cofán M, Blasco MA, Soberón N, Foronda M, Corella D, Asensio EM, Serra-Mir M, Roth I, Calvo C, Valls-Pedret C, Casaroli-Marano RP, Doménech M, Rajaram S, Sabaté J, Ros E, Sala-Vila A (2018). The red blood cell proportion of arachidonic acid relates to shorter leukocyte telomeres in Mediterranean elders: A secondary analysis of a randomized controlled trial. *Clin Nutr*, pii: S0261-5614(18)30074-8.
- Ferrara-Romeo I, Martínez P, Blasco MA (2018). Mice lacking RAP1 show early onset and higher rates of DEN-induced hepatocellular carcinomas in female mice. *PLoS One* 3, e0204909.
- Freitas-Simoes TM, Cofán M, Blasco MA, Soberón N, Foronda M, Serra-Mir M, Roth I, Valls-Pedret C, Doménech M, Ponferrada-Ariza E, Calvo C, Rajaram S, Sabaté J, Ros E, Sala-Vila A (2018). Walnut consumption for two years and leukocyte telomere attrition in Mediterranean elders: results of a randomized controlled tial. *Nutrients* 10, pii: E1907.

PATENTS

- Pastor JA, Blasco MA, Martinez S, Blanco-Aparicio C, García AB, Gómez-Casero E, Bejarano, L, Méndez-Pertuz M, Martínez P, García-Beccaria M (2018). Novel TRF1 modulators and analogues thereof. *EP18382659.3*.
- Blasco MA, Pastor JA, Bejarano L, Méndez-Pertuz M, Martínez P, Blanco-Aparicio C, Gómez-Casero E, García-Beccaria M (2018). Modulation of TRF1 for brain cancer treatment. *EP18382658.5*.

AWARDS AND RECOGNITION

- *Doctorate Honoris Causa*, *Universidad de Murcia,* Murcia, Spain.
- Member of the Board of Trustees, *Principe Felipe* Research Centre, Valencia, Spain.
- Member of the Board of Trustees, *Víctor Grífols i Lucas* Foundation, Barcelona, Spain.
- Member of the Advisory Board for Research Centres of the Regional Government of Galicia, Spain.
- Member of the Scientific Committee of "Telos" magazine (published by *Fundación Telefónica*), Madrid, Spain.
- Editorial Board Member, *Mechanisms of Ageing and Development*.