

## Time, tumours and telomeres

## Meeting on Cancer and Aging

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The Centro Nacional de Investigaciones Oncológicas (CNIO) meeting on Cancer and Aging took place between 7 and 9 November 2005 in Madrid, Spain, and was organized by M. Blasco, K. Collins, J. Hoijmakers and M. Serrano.

to our Spanish hosts—a serving of intellectual tapas for the meeting participants. Central to many of the discussions were attempts to understand how and where the biology of cancer and ageing overlapped, and how each discipline could potentially inform the other. This report highlights just some of the interesting questions and new findings discussed at the meeting.

**Telomeres, telomerase, cancer and ageing**

The interface of cancer and ageing is traditionally located at the telomere. This complex of proteins and repetitive DNA sequences (TTAGGG in mammals) caps the end of eukaryotic linear chromosomes and prevents their degradation. In cancer cells, and in normal stem cells, the enzyme telomerase continually adds repeats to telomeres. Conversely, most somatic cells lack telomerase activity, which leads to a progressive shortening of the telomere with each cell division. When a small subset of telomeres becomes critically short, cells undergo permanent growth arrest. Erosion of telomere length is therefore viewed as an important checkpoint of somatic cellular lifespan. However, in keeping with what now seems to be a general theme in cancer and ageing, this potential anti-cancer mechanism might promote and possibly even cause some aspects of ageing (Fig 1).

The meeting began with a discussion by J. Shay (Dallas, TX, USA) about the role of telomeres and telomerase in cancer stem cells. A small subset of cancer cells are believed to be responsible for tumour maintenance and current cancer therapies might not be adequately targeting these cancer stem cells. Early results indicate that cancer stem cells express telomerase and have short but stable telomeres. Thus, telomerase inhibitors in current clinical trials can be predicted to target the bulk of cancer cells and also the more rare cancer stem cells. Several approaches to telomerase inhibition are now being evaluated and Shay and C. Harley (Menlo Park, CA, USA) provided an update on the preclinical and clinical progress of several of these trials. Harley emphasized the ongoing phase I/II clinical trials with GRN163L (a small oligonucleotide competitive inhibitor of telomerase) in patients with chronic lymphocytic leukaemia. The potential risk–benefit ratio of activating telomerase in older patients who do not have cancer was also examined.

M. Blasco (Madrid, Spain) followed this discussion with an overview of stem cells in mice. She indicated that it is now known that knocking out telomerase (Terc) in mice results in a reduced incidence

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**Introduction**

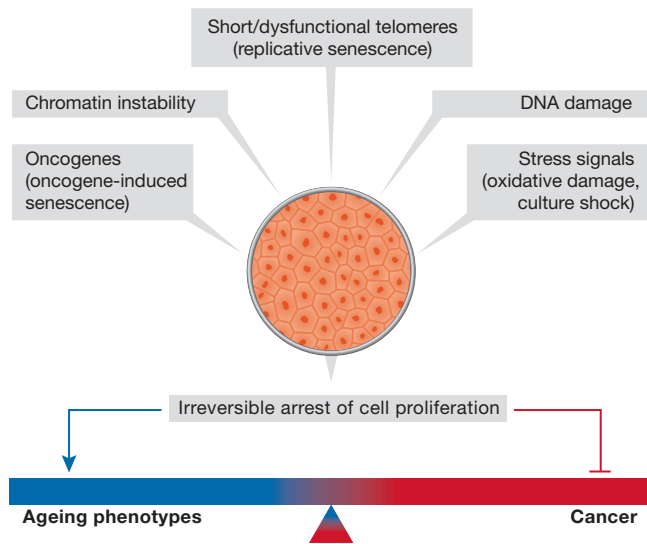
The crisp November air and beautiful Iberian city of Madrid served as the backdrop for the Cancer and Aging conference, held in the recently completed and ultra-modern CNIO (Centro Nacional de Investigaciones Oncológicas) facility. Thirty speakers and a similar number of invited guests heard presentations covering a range of topics including telomere biology, oxidative stress, genomic instability and the hormonal regulation of cancer and ageing. The presentations were short, lively and varied, providing—in deference

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**Fig 1** | Cellular senescence and the balance between cancer and ageing. In cultured cells and in tissues and organs, many stimuli seem to trigger induction of cellular senescence and also seem able to induce apoptosis. Both senescence and apoptosis lead to the reduction in the pool of rapidly proliferating cells. The short-term consequence of this removal might be to interrupt the malignant process whereas the long-term consequence might be to accelerate the ageing process.

of spontaneous cancers and a corresponding increase in certain age-related symptoms such as accelerated greying of hair, alopecia and reduced wound healing. Blasco was then able to show that the mobility of epidermal stem cells in the skin from telomerase knockout mice was inhibited (Flores *et al*, 2005). By contrast, telomerase overexpression in mouse skin seemed to promote stem-cell mobilization and hair growth. Although these results do not directly address the role of inhibiting telomerase in cancer cells, one prediction is that when telomeres are short, there could be impaired mobilization of normal stem cells; this could improve the therapeutic window for treating cancer patients with telomerase inhibitors. Blasco also presented data on transgenic mice engineered to overexpress the telomeric repeat-binding factor 2 (TRF2). These mice exhibit a predisposition for cancer and an accelerated ageing phenotype.

P. Rabinovitch (Seattle, WA, USA) reported on the increased cancer risk associated with many diseases of chronic injury and inflammation. Examples are Barrett's oesophagus and ulcerative colitis. In these instances, inflammation is thought to increase oxidative damage to DNA and Rabinovitch showed that telomere length in cells obtained from Barrett's patients was shorter than age-matched controls. The telomere theme continued with W. Wright's (Dallas, TX, USA) analysis of what is found at the end of telomeres and, in particular, the differences between the G-strand and C-strand terminal nucleotides. Comparing telomerase-positive versus normal cells revealed variations in the terminal G-strand nucleotides, but curiously, not on the C-strand. He also reported that expressing a small hairpin RNA (shRNA) against protection of telomeres 1 (POT1, a single-strand telomere-binding protein) randomizes the last nucleotide. Finally, he noted that the MRN complex—consisting of the DNA-repair factors MRE11, Rad50, and NBS—can recruit

telomerase to the telomeres and that the size of the G-rich overhang after MRN knockdown decreases in tumour but not in normal cells.

Three talks discussed dyskeratosis congenita (DKC), a rare disease associated with shortened telomeres. The autosomal dominant form of this disease is caused by mutations in the gene that encodes TERC, the RNA component of telomerase. Patients with DKC accumulate much less telomerase RNA but remain healthy until young adulthood. Mortality primarily arises from progressive bone marrow failure, although there are accompanying defects in skin, hair, nails, epithelia and lung tissue. From these symptoms, it seems probable that the cells affected in DKC are progenitor cells in tissues with a high cell turnover. K. Collins (Berkeley, CA, USA) asked several questions about DKC including whether a telomerase RNA defect can account for DKC disease pathology. Collins showed that DKC cells grown in culture have defects in telomere-length maintenance. This defect could be remedied by expression of recombinant telomerase enzyme subunits, suggesting a possible intervention for future treatment of DKC patients.

M. Bessler (St Louis, MO, USA) discussed acquired (through, for example, drugs, viruses, radiation) versus inherited (DKC, Fanconi's anaemia) bone marrow failure syndromes. There are several genetic varieties of DKC, and she found that the X-linked recessive form occurring mostly in young males might have telomerase and ribosomal RNA defects; the autosomal dominant form affects mostly older adults with anticipation occurring in this form—that is, earlier age of onset in subsequent generations. She also addressed the possible relationship of dyskerin, the protein product of the other disease gene in DKC, with telomere metabolism. Dyskerin is a protein involved in the H/ACA small nucleolar ribonucleoprotein particles (snoRNPs) that have key roles in the synthesis of eukaryotic ribosomes. Dyskerin seems to be responsible for pseudo-uridylation of specific residues of nascent rRNA molecules. It is also part of the telomerase complex, but its role there is less understood. R. Perona (Madrid, Spain) showed that a dyskerin gene fragment rescues telomerase activity in X-linked recessive DKC cells and, interestingly, increased survival in response to the chemotherapeutic agent, cisplatin.

### Reactive oxygen species: a link between cancer and ageing?

Another component common to both cancer and ageing is the role that reactive oxygen species (ROS) seem to have in both processes. It has been nearly 50 years since Denham Harman initially proposed the 'free radical theory of ageing'. One of the central tests of this theory is that organisms would live longer if their oxidative burden were reduced. Rabinovitch described experiments using transgenic mice that overexpress the hydrogen peroxide scavenging enzyme catalase. Rabinovitch and co-worker directed this peroxide-scavenging enzyme to the nucleus, peroxisomes (its normal location) or mitochondria. The latter localization proved the most potent and led to an approximately 18% increase in lifespan (Schriner *et al*, 2005). The enzyme is abundantly expressed in the heart where physiological assessment indicated that age-related stiffening of the heart was delayed in the catalase-overexpressing mice. In contrast to these positive experiments, working with genetically engineered mice that are deficient in crucial antioxidants such as manganese superoxide dismutase or glutathione peroxidase, H. van Remmen (San Antonio, TX, USA) found many markers of increased oxidative stress but no shortening of lifespan. W. Orr (Dallas, TX, USA) showed that increasing the levels of glutathione (GSH) in neuronal tissues seems to be a powerful strategy

for life extension in *Drosophila*. Glutathione is a tri-peptide located intracellularly in millimolar concentrations, and one of its main functions is to reduce hydrogen peroxide levels. However, Orr was unable to show any benefit of catalase overexpression in this organism. Therefore, manipulating antioxidant levels seems to modulate lifespan in some—but clearly not all—situations.

Three reports focused on the regulation of intracellular ROS formation. T. Finkel (Bethesda, MD, USA) reviewed evidence that oxidants might act as signalling molecules, challenging the prejudice that ROS are only random and destructive. He also provided preliminary evidence that many longevity genes seem to function as regulators of mitochondrial oxygen consumption. One regulator that is central to both cancer and ageing is the tumour suppressor p53. Interestingly, P. Hwang (Bethesda, MD, USA), using both p53-deficient cell lines and *p53*<sup>-/-</sup> mice, showed a role for p53 in regulating aerobic capacity and exercise thresholds in mice. This effect seems in part to be mediated by the ability of p53 to regulate cytochrome assembly. P. Pelicci (Milan, Italy) presented a follow-up of his fascinating observation that deletion of the p66shc adapter protein results in extension of lifespan in mice. Previous studies in animals and cells had shown that deletion of p66shc reduces oxidative stress. Pelicci reported that a fraction of p66shc localizes to the mitochondria where it seems able to react with cytochrome C and generate hydrogen peroxide (Giorgio *et al*, 2005). Interestingly, this p66shc-dependent mitochondrial oxidant generation seems to be required for several diverse properties including apoptosis, adipocyte differentiation and neo-angiogenesis.

### Senescence as a barrier to cancer

Eight years ago, S. Lowe (Cold Spring Harbor, NY, USA), D. Beach (London, UK) and M. Serrano (Madrid, Spain) collaborated on a landmark study showing that Ras oncogene expression in primary diploid fibroblasts triggered a telomere-independent form of cellular senescence. Although these *in vitro* results were clear, the *in vivo* implications remained ambiguous. D. Peeper (Amsterdam, The Netherlands) and Serrano presented recent data suggesting that cellular senescence might be an important *in vivo* tumour suppressor mechanism (Fig 1). Peeper was studying human nevi, typical skin moles that have a low but statistically significant risk of developing into deadly melanomas. Interestingly, these pre-malignant lesions often harbour mutations in B-Raf, a downstream effector of Ras proteins. Careful examination of the melanocytes that make up the nevi revealed that these cells exhibited evidence of senescence (Michaloglou *et al*, 2005). Serrano came to similar conclusions using an inducible Ras mouse expression system (Collado *et al*, 2005). Pathological examination showed that these mice first develop non-malignant lung adenomas followed by lethal lung adenocarcinomas. Again, in the pre-malignant adenoma stage, there was evidence that a large fraction of the adenoma cells underwent oncogene-induced senescence. However, a question remains in both models whether cancer occurs directly or independently from the senescent cells that predominate in these pre-malignant lesions. Nonetheless, these results suggest that senescence-induced withdrawal from cell division, similar to apoptosis, might be an important mechanism to remove potentially pre-malignant cells. Interestingly, whereas oncogenic Ras induces senescence, M. Barbacid (Madrid, Spain) showed that the growth of mouse embryonic fibroblasts (MEFs) lacking H-, K- and N-Ras was also arrested. Morphologically, these 'Ras-less' cells looked senescent

but interestingly they exhibited none of the known characteristics found in other senescent cells. The molecular details of this unique form of growth arrest remain to be discovered.

Although senescent cells do not divide, they clearly are not inert. J. Campisi (Berkeley, CA, USA) provided evidence that senescent cells secrete a plethora of factors including proteases and cytokines that might contribute to the ageing phenotype. She argued that this secretory phenotype might be important in disrupting tissue homeostasis. Campisi showed that, in contrast to normal proliferative fibroblasts, senescent fibroblasts seem to stimulate pre-malignant epithelial cells to form tumours. It is still uncertain what triggers the secretory phenotype of senescent cells, but her evidence suggests that the tumour suppressor p53 might have a main role. This was strengthened by V. Rotter (Rehovot, Israel), who attempted to understand the role of p53 using a bioinformatics approach. Her data suggest that p53 regulates hundreds of genes, with one class including both cytokines and metalloproteinases.

Lowe further explored the molecular mechanisms underlying cellular senescence. Working with IMR90 human fibroblasts, Lowe showed that RAS-induced cellular senescence is associated with DNA foci with features of heterochromatin, so-called senescence-associated heterochromatic foci (SAHF). These SAHFs are associated with the downregulation of E2F target genes and might be crucial for the maintenance of cell-cycle arrest during senescence. His group also showed that the protein composition of chromatin in senescent cells is markedly different from quiescent or growing cells and identified some new molecules that might contribute to these changes.

Beach continued the cellular senescence theme by presenting some fascinating data indicating that enhancing glycolytic activity could allow cells to escape from Ras-induced senescence. It has been known for many years that tumour cells rely heavily on aerobic glycolysis for energy needs, the so-called Warburg effect. Using a genetic screen, the Beach lab isolated phosphoglycerate mutase (PGM) as a candidate gene that allows MEFs to overcome Ras-induced senescence (Kondoh *et al*, 2005). Expression of PGM or another enzyme involved in cytosolic glycolysis (glucose-6-phosphate isomerase) increased glycolytic flux and potentially decreased mitochondrial metabolism and ROS levels in cells. This is similar to results from Finkel's laboratory showing that Ras induces senescence through ROS, and previous work by the Campisi laboratory showing that the early growth arrest of normal MEFs can be bypassed by passaging the cells in low oxygen conditions.

H. Scrabble (Charlottesville, VA, USA) described her experience with a transgenic mouse that overexpresses a naturally occurring short form of p53 that lacks exon 1 and 2 of the tumour suppressor. Expression of this p44 isoform results in an overall increase in p53 activity and a form of accelerated ageing. Analysis of the neural stem-cell population of these mice suggests a rapid ageing of this cellular compartment. Interestingly, p44 mice show gross increases in anxiety levels as measured by their willingness to explore a new environment. In contrast to these results, accelerated ageing was not observed when Serrano overexpressed normal p53. His data suggest that in mice with three copies of wild-type p53, there was clearly a cancer-resistant phenotype but there was no evidence that lifespan was altered. The ensuing discussion revolved around whether the differences in lifespan between these two transgenic models might relate to the degree that p53 is constitutively activated (in Scrabble's p44 mouse) versus normally regulated (as in Serrano's extra-p53 animals).

### DNA damage, repair and recombination

DNA damage and mutations have long been implicated in both cancer and ageing. J. Hoeijmakers (Rotterdam, The Netherlands) showed that inactivation of certain main DNA-repair processes are not always associated with increased cancer, but can also lead to reduced spontaneous tumour formation and the appearance of a variety of symptoms of premature ageing. Hoeijmakers discussed nucleotide excision repair processes and, more specifically, mouse mutants that mimic the human segmental progeroid disorders Cockayne syndrome and trichothiodystrophy (TTD). Mice with an engineered TTD mutation show various symptoms of premature ageing, including kyphosis, osteoporosis and increased lipofuscin accumulation. The animals also have a reduced spontaneous cancer rate, indicating that some aspects of ageing might protect against the development of cancer. J. Vijg (San Antonio, TX, USA) provided evidence for increased stochasticity of gene expression as a potential mechanism for ageing-related cellular degeneration and death. He measured transcript levels of a randomly chosen panel of genes in individual cardiomyocytes from fresh heart samples of young and old mice, using a new global mRNA amplification method in combination with a real-time polymerase chain reaction. The expression levels of most genes varied among cardiomyocytes from young hearts, but this heterogeneity was significantly elevated at old age. Such increased stochasticity of gene expression could also be induced in MEFs in culture treated with hydrogen peroxide. These results suggest that ROS derived from mitochondria might ultimately contribute to ageing by directly altering nuclear gene expression.

O. Fernandez-Capetillo (Madrid, Spain) discussed DNA double-strand breaks and the role of chromatin remodelling in the repair of such lesions. On the basis of his results with histone H3 methylation during the repair of double-strand breaks, he presented a model of the role of a DNA-repair-specific histone code. J. Bartke (Copenhagen, Denmark) presented work related to the role of the DNA-damage response in cancer and ageing. His results indicate that early in tumorigenesis—before genomic instability and malignant conversion—human cells activate an ataxia telangiectasia mutated (ATM) and Rad3-related (ATR)-regulated DNA damage response network that delays or prevents cancer. Mutations that compromise this checkpoint, including defects in the ATM–CHK2–P53 pathway, might allow cell proliferation, survival, increased genomic instability and tumour progression. Interestingly, Bartke also showed that in clinical specimens from different stages of human tumours, the early precursor lesions—but not normal tissues—commonly express markers of an activated DNA-damage response.

Continuing the theme of DNA damage and recombination, D. Sinclair (Boston, MA, USA) discussed the biology of silencing information regulator 2 (Sir2). Sir2 is the founding member of a phylogenetically conserved family of nicotinamide-adenine dinucleotide-dependent histone deacetylases, called sirtuins. In yeast, Sir2 is required for transcriptional silencing—transcriptional inactivation by altering chromatin structure through the deacetylation of histones. The enzyme also suppresses intrachromosomal recombination of rDNA. Sinclair proposed that, similar to Sir 2, its human orthologue SIRT1 acts to maintain the genome, possibly through the heterochromatinization of DNA. Using chromatin immunoprecipitation, Sinclair showed that SIRT1 binds to DNA at sites of mobile genetic elements—for example, long interspersed nuclear element 1—and satellite III repeats, both known to be in a permanent heterochromatic state. Interestingly, genotoxic stress or heat

shock causes SIRT1 to be released from the DNA. Sinclair speculated that the return of SIRT1 to again bind to the repeat elements is imperfect, which explains why levels of repetitive transcripts increase with age. These experiments might also provide insight into the increased stochasticity of gene expression reported by Vijg.

C. Lopez-Otin (Oviedo, Spain) ‘switched gears’ by moving from genotoxic stress and DNA-damage responses to the nuclear envelope and its connection with accelerated ageing and tumour suppression. His lab has been studying a protease known as Zmpste24. This protein is involved in the processing of prelamin A, encoded by the *LMNA* gene. Mutations in this gene have been linked to several diseases, including most recently Hutchinson Gilford progeria syndrome (HGPS). Prelamin A normally undergoes multi-step processing to make lamin A, a structural protein of the nuclear envelope. This process involves both farnesylation of a carboxy-terminal cysteine and cleavage of the carboxyl terminus. The latter is absent in patients with HGPS and seems to require the Zmpste24 protease. The Lopez-Otin lab created mice that were deficient in Zmpste24, and these animals recapitulated many of the progeria phenotypes. Microarray results of this mouse model indicate that many of the induced genes are regulated by p53 (Valera *et al*, 2005). Indeed, crossing the *Zmpste24*<sup>-/-</sup> mice into a p53-null background partially rescued the animals, whereas crossing into a *LMNA*<sup>-/-</sup> background completely abolished the progeria phenotype. He also speculated that because lamin A requires farnesylation, inhibitors of this post-translational process—initially developed as anti-cancer agents—might turn out to be useful in the treatment of this progeria.

### Hormonal regulation of cancer and ageing

The sessions concluded with discussions on hormonal regulation in both cancer and ageing. A. Bartke (Springfield, IL, USA) described long-lived mouse strains (Ames and Snell) with mutations that result in the absence of a fully functional pituitary gland. These mice lack normal amounts of several pituitary factors including growth hormone (GH), which is a secreted hormone that regulates tissue insulin-like growth factor-1 (IGF1) production. The absence of GH and lower IGF1 levels make the Ames and Snell mice smaller in size and increase their lifespan. Interestingly, these mice also have a cancer-resistant phenotype. Similarly, J. Kopchick (Athens, OH, USA) engineered a GH-receptor knockout mouse (*GHR*<sup>-/-</sup>), which also exhibited increased lifespan in addition to decreased rate of cancer. Although these long-lived mice all seem similar, Bartke showed that caloric restriction further increased the lifespan of the Ames mice, whereas it had no effect on the lifespan of the *GHR*<sup>-/-</sup> mice. This divergence is unexplained at present. Another secreted factor that might be important is oestrogen. In Western countries, women live considerably longer than men. Even though life expectancy has nearly doubled over the course of the past hundred years, the lifespan differences between the sexes has been maintained. J. Vina (Valencia, Spain) showed that mitochondria from female rats produced less ROS than male mitochondria and that there were also differences in antioxidant levels. These changes in female rats disappeared after ovariectomy, providing one possible explanation for the difference in longevity between males and females. The session concluded with a futuristic presentation by A. de Grey (Cambridge, UK), who spoke about potential strategies to simultaneously extend lifespan and avoid cancer formation. He proposed to engineer stem cells

that lack telomerase activity. These cells theoretically would be resistant to cancer but also short-lived. Nonetheless, de Grey proposed that repeated administration of these cells could maintain tissue homeostasis. Clearly, many obstacles exist before such ideas can be implemented and at this point, such approaches resemble an 'impossible dream'. This was perhaps a fitting way to end the conference, given that we were in the land of Don Quixote.

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