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The role of telomeres and telomerase in cancer

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Telomerase is a double-edged sword in biology, a kind of Jekyll and Hyde, in the words of the *American Association for Cancer Research* conference (7–11 December 2002, San Francisco, CA, USA) co-organizer Elizabeth H. Blackburn of the University of California, San Francisco (UCSF; http://www.ucsf.edu). Although it is the shortening of telomeres that limits the replicative lifespan of mammalian cells, perhaps setting an upper limit to human life, the enzymatic activity that maintains and lengthens those telomeres is itself a hallmark of cancer.

Capping the ends: function of telomeres and telomerase

Telomeres, the organelles at the ends of each chromosome, solve the 'end replication problem' – the erosion of the ends of replicating linear DNA molecules, a conundrum that results from the extension of DNA by polymerases only from a free 3′ end of an existing nucleotide chain and only in a 5′→3′ direction. In the absence or failure of telomere, chromosome ends 'stick' to each other, wreaking havoc on the fused chromosomes, which break during cell division.

The telomere consists of multiple copies of a repeating unit of DNA (in humans, the bases TTAGGG), which spans lengths of 6–12 Kb [1]. Preservation of the long telomeres requires the vigilance of an enzyme complex called telomerase, which consists of the catalytically active polypeptide telomerase reverse transcriptase (TERT), an attendant ribonucleic acid (telomerase RNA, TR; or telomerase RNA component, TERC) and at least two additional proteins that are essential for full function [2]. The yeast, mouse and human (hTERT, hTR) versions of these molecules are the subject of much study.

In time, however, a few critically short telomeres trigger a p53-mediated damage response in the cell. Cells then enter replicative senescence (M1 phase), in which they remain metabolically active but do not divide further. If checkpoints are impaired – for example, by the loss of p53 function – cells continue to divide until they reach 'crisis' or M2, in which telomeres are so short that rampant chromosomal end-joining and other events create a sufficiently unstable genome that cells become apoptotic. If, however, cells manage to stabilize their telomeres through the reactivation of telomerase or other mechanisms, cells become immortal and potentially cancerous.

The entry point for replicative senescence seems to involve a crucial stage at which perhaps 10% of the telomeres, according to Woodring Wright, of the University of Texas Southwestern Medical Center at Dallas (UT Southwestern; http://www.swmed.edu), are so short that the cell recognizes them as if they were double-stranded DNA breaks. Ling Qi, of Carol Greider's laboratory at Johns Hopkins University (http://www.jhu.edu), suggests that telomere malfunction induces p53, as would be expected if the chromosome end resembled a double-strand break. According to Qi, the shortest telomere, rather than the average telomere length, triggers apoptosis.

Capping cancer

Most tumours exhibit high levels of telomerase activity and the roles of telomeres and telomerases in cancer have attracted a great deal of attention (see [3]).

Most cells, confronted by chromosome damage, are arrested in the cell cycle until the necessary repairs can be completed. The passing of such essential 'checkpoints' is one of the initial steps in cancer transformation. However, these cells still face one inevitable end-point. The relentless shortening of the telomeres is the ultimate arbiter of cellular senescence. Only if telomerase itself is reactivated can the cancer cells achieve immortality. The potential for telomerase inhibitors as chemotherapeutic agents has engendered much excitement.

Alan K. Meeker, of Johns Hopkins University, found that telomere shortening is frequently found in pre-malignant epithelial precursor lesions. Adjacent normal tissue had longer telomeres. Meeker thinks that the shorter telomeres result in a 'mutator phenotype' in which chromosomal instability results in the subsequent genomic instability that is characteristic of epithelial tumours.

Ronald A. DePinho, of Boston's Dana Farber Cancer Institute (http://www.dfci.harvard.edu), observes that chronic epithelial tumours exhibit telomere attrition, then crisis and chromosome

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breakage. DePinho and colleagues used an assay in which genetically modified cells are injected into the tail vein of mice lacking mTERC, and the appearance of malignancies in the lung is monitored. Only those cells in which mTERC was supplied were highly malignant. DePinho concludes that 'telomerase-mediated telomere maintenance potentiates malignancy'.

According to Thea D. Tlsty (UCSF), the main problem with cancer is that it is found too late. Therefore, the answer could lie in the discovery of early molecular markers for cancer. Tlsty, together with Martha Stampfer of the Lawrence Berkeley National Laboratory (http://www.lbl.gov) and their colleagues, have observed the biology of mammary epithelial cells in detail. Human mammary epithelial cells (HMECs), according to Tlsty, do not behave like canonical human mammary fibroblast cells (HMFs) and the differences could give us important clues about cancer biology, early detection, prevention and therapy. Unlike the HMFs derived from the same tissue, HMECs reach a transient growth plateau rather than senescence [4]. HMECs emerge spontaneously from this stage at frequencies that are orders of magnitude higher than that seen in HMFs. The cells resume division, displaying a spectrum of chromosomal lesions similar to the translocations and breaks that characterize early breast cancer lesions. Although the first arrest stage is characterized by high expression of p16, cells escaping from this barrier lack p16 expression. Tlsty's continuing study of HMECs is leading not only to diagnostic markers and therapeutic targets, but also to possible agents that could prevent the progression of HMECs beyond the first growth plateau.

Ending the caps: telomerases as drug targets

Telomerase activity is absent in most normal somatic cells but is a hallmark of many cancers, therefore, hTERT and hTERC (or hTR) present attractive targets for therapy.

Conference co-organizer Jerry W. Shay (UT Southwestern), envisions traditional cancer therapy, including surgery, chemotherapy and radiation treatment, not replaced with but supplemented by anti-telomerase therapies. Inhibition of angiogenesis and telomerase activity would be key factors in preventing or delaying tumour re-growth. Shay cites earlier work from his laboratory showing that there are at least two classes of neuroblastoma, and that stage IVS (stage 4 special) tumours, which have a high spontaneous remission rate, have little or no telomerase activity, whereas the aggressive tumours are characterized by high telomerase activity [5].

Anti-telomerase therapies can target hTERT or the essential RNA component (hTERC or hTR), either by binding directly to one of these components or to the telomere sequence on the chromosome. The approaches include direct interference by drug molecules, including inhibitory RNAs (RNAi), the production of vaccines conferring immunity to various components of the telomerase machinery, and the construction of oncolytic viruses targeting telomerase-positive cells.

Not surprisingly, Geron Corporation (http://www.geron.com) highlighted several approaches to cancer therapy, which were extraordinarily advanced and promising.

Geron's telomerase inhibitor, GRN163, has passed key efficacy and safety benchmarks in animal tests. Calvin Harley explained that GRN163 is a 13-mer thio-phosphoramidate oligonucleotide that is targeted to the template region of hTR, where it binds with high affinity and inhibits telomerase activity. Because the drug binds to a component of the telomerase holoenzyme, its action should be quite specific. Moreover, it is selective for abnormal cells because few normal cells are telomerase-positive.

Working with scientists from the Memorial Sloan Kettering Cancer Center (http://www.mskcc.org), Allison Chin and David Karpf (Geron) presented data on the inhibition of growth in myeloma and lymphoma cell lines, and *in vivo* in mice and rats with malignant glioma, prostate cancer, lymphoma, myeloma and cervical cancer. Controls in studies use saline or a nonsense oligonucleotide that does not bind the telomerase RNA component target. Geron plans an investigational new drug (IND) filing for the use of GRN163 in glioblastoma treatment.

As Harley explained, Geron is also pursuing a telomerase vaccine in which a plasmid or adenovirus vector harboring the hTERT gene is injected directly into mice. Melanoma growth was slowed significantly in treated, compared with control, mice. Jane Lebkowski (Geron) described work, in collaboration with scientists at McMaster University (http://www.mcmaster.ca), in which mouse dendritic cells modified with hTERT DNA induce a potent, telomerase-specific cytotoxic T-cell response by *in vitro* immunization. Vaccinated mice with bone-marrow derived dendritic cells modified to express hTERT were able to break tolerance and induce immunity to hTERT in mice. This resulted in a delay in tumour growth. A Phase I clinical trial of the Geron vaccine, currently under way at Duke University (http://www.duke.edu), shows promise, with no safety problems after six vaccinations and substantial induction of telomerase-specific cytotoxic T lymphocyte and T-helper cell responses. Vaccination in the human trials was *ex vivo*, and clinical response is still under evaluation.

Oncolytic therapies

Geron's oncolytic virus approach, discussed by John Irving, uses the adenovirus E1A gene driven by the

hTERT promoter. The promoter renders the virus relatively specific to tumour cells, and E1A expression results in tumour cell lysis, viral replication, subsequent infection and killing of adjacent tumour cells. The oncolytic virus killed 12 different tumour lines in culture; normal cell controls were unaffected.

W. Nicol Keith (University of Glasgow; http://www.glasgow.ac.uk) used another exciting approach to achieve tumour cell lysis. The laboratory employed an adenovirus vector using an hTR or hTRT promoter to drive a bacterial nitroreductase (NTR) gene, in combination with the drug CB1954. The safety of CB1954 in pharmacologically relevant doses has already been demonstrated in clinical trials, but CB1954 is metabolized to a cytotoxic alkylating agent by NTR. The virus, in combination with the drug, is therefore a cytotoxic agent with specificity for telomerase-positive cells.

Telomerase inhibitors

Several additional investigations into the use of telomerase inhibitors are under way. David R. Corey, of UT Southwestern, described his work on Isis Pharmaceuticals (http://www.isip.com) compound 24691. This 2′-O-methoxymethyl (MOE) oligonucleotide binds the telomerase RNA template, and anti-telomerase activity has been demonstrated.

Shang Li, from the Blackburn Laboratory at UCSF, used a mutated template in a lentivirus-based system, introducing MT-hTR (mutant telomerase RNA component) or control WT-hTR (wild-type RNA) into cancer cells. Used with hTERT, MT-hTR results in cell inhibition and apoptosis.

Concluding remarks

All of this, however, is not the end of the telomere biology-based therapy story. Recent work of Seger *et al.* [6] emphasizes that the transformation of human cells in the absence of a telomere maintenance mechanism does occur, and suggests that alterations in telomere biology are not necessary for the transformed phenotype. Nonetheless, anti-telomerase therapies represent an area of great promise in medicine, and are a subject of great excitement in science.

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