Meeting Report: The Role of Telomeres and Telomerase in Cancer

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Abstract
The role of telomeres and telomerase in cancer is an area of much recent interest. This conference sponsored by the American Association of Cancer Research provided a timely opportunity to bring together basic and clinical scientists interested in the field of telomeres and telomerase cancer biology. The meeting included over 250 attendees with 150 oral and poster presentations focused on understanding telomere and telomerase biology for the development of cancer therapeutics. The meeting chairpersons were Dr. Jerry W. Shay, University of Texas Southwestern Medical Center, Dallas, TX; Dr. Elizabeth H. Blackburn, University of California San Francisco, CA; and Dr. Maria A. Blasco, Spanish National Cancer Center, Madrid, Spain. The meeting provided an update on the field, pointing to areas in which our knowledge is deficient, and explored how the most promising areas may be advanced into translational research. This conference brought together cell and molecular biologists with clinicians interested in fundamental cancer mechanisms as they relate to telomeres and telomerase. The symposium consisted of formal presentations by prominent scientists working in these areas and by participants selected from submitted abstracts. In addition, there were two poster sessions. Whereas there were many basic research advances presented, the focus of this overview will be the areas of clinical advances. (Cancer Res 2005; 65(9): 3513-7)

Background
The understanding of the role of telomere biology, the end replication problem leading to genomic instability and the reactivation of telomerase is absolutely critical to our understanding of cancer, and more so, to our ability to develop novel therapies and cancer prevention approaches. It is well established that the up-regulation or re-expression of telomerase is important for continuous tumor cell growth. In contrast to normal cells, tumor cells show no net loss of average telomere length with cell division, suggesting that telomere stability may be required for cells to escape from replicative senescence and proliferate indefinitely. Most, but not necessarily all, malignant tumors may need to become immortal to sustain their growth, thus, telomerase may be a rate-limiting step required for the continuing proliferation of advanced cancers. The telomere-telomerase hypothesis of aging and cancer is based on the findings that most human tumors have telomerase activity, whereas normal human somatic cells do not. Telomere length is maintained by a balance between processes that lengthen telomeres (telomerase) and processes that shorten telomeres (lack of complete lagging DNA strand synthesis and end-processing events). Telomerase is a cellular ribonucleoprotein enzyme that stabilizes telomere length by adding TTAGGG repeats to the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomeres that occurs in its absence. The enzyme is expressed in embryonic cells and in adult male germ line cells, but is undetectable in normal somatic cells except for proliferative cells of renewal tissues (e.g., hematopoietic stem cells, activated lymphocytes, basal cells of the epidermis, and intestinal crypt cells). In all normal somatic cells, even those with detectable telomerase activity, progressive telomere shortening is observed, eventually leading to greatly shortened telomeres and to a limited replicative capacity. It has been proposed that telomere shortening may be a molecular clock that counts the number of times a cell has divided and determines when cellular senescence occurs, and in the absence of other genetic or epigenetic changes, may serve as a potent tumor suppressor mechanism.

In summary, human telomerase is tightly repressed in most normal somatic cells, transiently inducible in certain stem or progenitor cells, and constitutively activated in germ line and tumor cells. There are now thousands of publications supporting the association between tumorigenesis and activation of telomerase. Targeting telomerase for potential therapeutics is starting to emerge. The following is an overview of recent preclinical and clinical findings in the telomerase field that was presented at this second American Association for Cancer Research–sponsored meeting on “The Role of Telomeres and Telomerase in Cancer”.

Progress on GRN163L
In the opening session of the meeting, Dr. Jerry Shay (UT Southwestern Medical Center, Dallas, TX) provided an overview of the importance of telomerase as a universal and highly specific target for the development of novel cancer therapies, and reviewed the breadth of approaches to kill telomerase-positive tumor cells while sparing normal tissue. The drug development status of GRN163L (a 13-mer N3-P5’ oligonucleotide thio-phosphoramidate fully complementary to the template region of hTR and a potent telomerase antagonist) was presented by Dr. Calvin B. Harley, Geron’s chief scientific officer (Menlo Park, CA). Dr. Harley indicated that “scaled-up production of GRN163L using Good Laboratory Practices standards has been achieved at the multi-hundred gram scale, enabling the ongoing preclinical studies. In addition, similar quantities of GRN163L have been made at higher purity under Good Medical Practices and the first lot of phase I clinical trial drug product has been successfully manufactured.” Dr. Harley reviewed the safety, toxicology, pharmacokinetic, biodistribution, and pharmacodynamic studies that have been conducted in multiple animal species. These studies, together with the growing body of human xenograft efficacy data in
rodents, suggest that intermittent i.v. dosing of GRN163L should achieve therapeutic tissue levels of the drug in cancer patients, while maintaining an acceptable safety profile.

According to Dr. Harley "the novel backbone chemistry of GRN163L provides greatly enhanced stability and extremely specific and high affinity binding to telomerase, while the lipid modification on GRN163L significantly improves its potency and biodistribution. These properties increase the chances of effectually inhibiting telomerase in cancer cells throughout the body with relatively low doses of the drug." In summary, GRN163L is on the path to be the first telomerase inhibitor for the treatment of cancer, and current data suggests that it has the potential to be a universal anticancer agent with an acceptable safety profile.

Update on Telomerase Immunotherapy/Vaccine Phase I/II Trials

Because survival of telomerase expressing (hTERT+) tumor cells requires functionally active telomerase, hTERT is believed to be a prototypic immune target for which mutation or loss as a means of escape may be incompatible with sustained tumor growth. Dr. Robert Vonderheide (University of Pennsylvania School of Medicine, Philadelphia) showed that CTLs recognize peptides derived from hTERT which are expressed on the surface of tumor cells but not normal cells. These CTLs kill hTERT+ tumor cells in vitro. In a feasibility trial, vaccination of advanced prostate and breast cancer patients with hTERT-derived peptide safely induced functional antitumor CTL following vaccination. In recent work, Dr. Vonderheide reported the immunologic and biological effects of vaccinating metastatic breast cancer patients with a non–cell-based vaccine using an HLA-A2-restricted hTERT peptide. Under the auspices of an investigator-sponsored IND, 11 HLA-A2+ women with metastatic breast cancer have been vaccinated s.c. No serious adverse events were observed, including any bone marrow toxicity. Grade 1 and 2 injection site reactions were observed in most patients. Interestingly, some patients reported a syndrome of pain or itchiness at the site of metastatic tumor after three or four vaccines, and in one patient, there seemed to be a clinical response. In summary, vaccination of metastatic breast cancer patients against hTERT induces hTERT-specific T cells that can be identified in peripheral blood and tumors without major toxicity. Tumor necrosis is observed after vaccination and dose escalation results in an enhanced immunologic response.

In another telomerase vaccine trial, Dr. Thomas B. Okarma, Geron’s president and CEO of Geron, presented an in-depth update of the completed phase I/II trial of Geron’s telomerase vaccine in 20 prostate cancer patients conducted at Duke University Medical Center by Dr. Johannes Vieweg and colleagues. Dr. Okarma indicated that “the first challenge to the development of a clinically useful cancer vaccine is breaking self-tolerance. Telomerase can be expressed transiently by several normal cell types thereby posing not only the challenge of self-tolerance with respect to initiating an immune response, but also the challenge of maintaining that response long enough to be clinically effective, yet avoiding autoimmunity.” To meet these challenges, Geron and collaborators chose to use an RNA-transfected autologous dendritic cell technology developed by Dr. Eli Gilboa at Duke and exclusively licensed to Geron for telomerase. Dr. Okarma emphasized “the improved cellular immune response seen in the study subjects who received a modified version of the hTERT antigen that enabled the generation of antitelomerase CD+ helper T cells, as well as telomerase-specific CD8+ killer T cells. High frequencies of telomerase-specific CD8 T cells were induced in all but one subject, yet no adverse reactions were observed in any of the patients. Evidence suggestive of a clinical impact included reduction or clearance of circulating prostate cancer cells in 9 of 10 subjects and a highly statistically significant prolongation of PSA doubling time associated with the presence of antitelomerase T cells.” Ongoing new clinical studies testing other modifications of the vaccine protocol were described along with progress in automating the vaccine production process.

In his introductory remarks, Dr. Shay (UT Southwestern Medical Center) also mentioned the ongoing studies being conducted at the Norwegian Radium Hospital and the Ulleval University Hospital in Norway led by Dr. Gustav Gadernack. Using two different hTERT peptide vaccines and interdermal injections along with granulocyte macrophage colony-stimulating factor, Dr. Gadernack and collaborators have enrolled ~98 advanced pancreatic, lung and melanoma patients during the past 3 years. The protocol developed is to give weekly vaccines for the first 4 to 6 weeks, and then followed by monthly booster vaccines. The results to date have not revealed any serious adverse effects. There is no evidence of bone marrow stem cells effects, and no evidence of autoimmune disease in long-term survivors who received monthly booster vaccines (some in excess of 2 years). These studies were not blinded, placebo-controlled trials, but in advanced pancreatic cancer patients, there seemed to be a dose or immune response–related survival benefit compared with historical controls. Current plans in Norway are to conduct a randomized phase II trial that will involve approximately 200 to 300 patients with advanced pancreatic cancer.

The development of these promising approaches for a telomerase-based universal cancer vaccine is highly encouraging and hopefully will be even more effective when used to treat patients with less advanced disease.

Oncolytic Virus Update

In addition to direct enzyme inhibition and telomerase immunotherapy for cancer treatment, approaches to kill telomerase-positive cancer cells included methods to block telomerase production, disrupt telomere function, and use telomerase promoter-driven genes or viruses to trigger suicide or cell death. Conditionally replicating oncolytic viruses offer a promising modality for cancer treatment. Among this novel group of therapeutics are oncolytic adenoviruses engineered with tumor-specific transcriptional response elements controlling essential genes. The key to the development of such viruses is the determination of a transcriptional control strategy and the selection of a tumor- or tissue-specific transcriptional response element. Dr. D.C. Yu, Director of Oncolytic Virus and Principal Scientist at Cell Genesys (San Francisco, CA), presented data demonstrating the high degree of specificity and effectiveness of one of their preclinical cancer therapeutic candidates, the oncolytic virus, CG5757. This virus was generated by replacing the E1a and E1b endogenous promoters with promoters derived from the human E2F-1 and telomerase reverse transcriptase (hTERT) genes, respectively. The E2F-1 promoter is activated in retinoblastoma-defective tumor types, a pathway mutated in ~85% of all cancers. Likewise, telomerase is aberrantly expressed...
in ~90% of tumors. CG5757 shows strong tumor selectivity and antitumor efficacy. In vitro, expression of E1a and E1b genes was restricted to retinoblastoma-defective and hTERT-positive cancer cells. In normal cells, no E1 expression could be detected from infection with CG5757. The transcriptional control of E1 gene expression also correlated with selective viral replication in target cells. CG5757 replicates similarly to wild-type virus in tumor cells, but its replication is, on average, 1,000 times less efficient in normal cells. In a viral cytotoxicity assay, CG5757 in nude mice with s.c. A549, Hep3B, LoVo, and 253J B-V xenografts. In the 253J B-V model, 4 weeks after treatment, the average tumor volume in animals treated with four consecutive daily intratumoral injections of CG5757 (4 × 10^9 particles/mm^3 of tumor) decreased to 72% of baseline, whereas the control group had an increase to 94% of baseline. Furthermore, 50% of treated animals had complete regression of the 253J B-V tumor xenografts. These data showed the potential therapeutic efficacy of such dual promoter–controlled oncolytic adenoviruses in cancers that are retinoblastoma-defective and hTERT (telomerase)-positive.

Small Interfering RNA Targeting Telomerase RNA

Recent work from the laboratory of Dr. Elizabeth Blackburn (University of California, San Francisco, CA) has found that inhibition of the up-regulated telomerase activity in cancer cell growth using telomerase RNA knockdown approaches slows cell growth. Work by Dr. Shang Li in the Blackburn laboratory reported that, unexpectedly, a hairpin small interfering RNA specifically targeting human telomerase RNA (not hTERT but hTERC) rapidly inhibited growth of human cancer cells independently of p53 or telomere length, and without bulk telomere shortening. They reported that such telomerase RNA knock-downs in cancer cells did not cause telomere uncapping, but rather, induced changes in the global gene expression profile indicative of a novel response pathway, which includes suppression of specific genes implicated in angiogenesis and metastasis, that is distinct from the expression profile changes induced by telomere-uncapping mutant-template telomerase RNAs.

Small Molecules Targeting Telomeres and Telomerase

In work presented by Dr. Lloyd Kelland (St. Georges Hospital Medical School, London) and Dr. Stephen Neidle (University of London), a small molecule, BRACO-19 was discussed. Kelland indicated that whereas most approaches for antitelomerase therapy have targeted only telomerase activity, they have chosen to disrupt the telomere-telomerase complex. The maintenance of telomere length is an essential property of malignant tumors and has gained recognition as being critically involved as one of the hallmarks of cancer, that of limitless replicative potential. The Kelland/Neidle approach involved targeting the 3′ single-stranded overhang telomeric DNA substrate by inducing it to fold into a four-stranded guanine quadruplex structure (G4). Dr. Kelland showed that this folding is incompatible with telomerase activity and induces telomere disruption/uncapping, rapidly leading to apoptosis and/or senescence. A series of 3,6,9 trisubstituted acridines, exemplified by BRACO-19, were reported to exhibit potent (nanomolar) inhibitory activity against telomerase in vitro as well as strong, selective binding to G-quadruplex DNA. BRACO-19 exhibited selective arrest of cell growth at subcytotoxic concentrations, together with induction of senescence and apoptosis in several different human cancer cell lines. Analogous cellular effects were also observed in cells that maintain telomeres by the alternate pathway. In vivo, BRACO-19 showed significant antitumor activity against human tumor xenografts, both as monotherapy (in prostate cancer xenografts) and in combination with paclitaxel. Pharmacokinetic and metabolism experiments in mice reveal that levels of BRACO-19 known to be sufficient to inhibit telomerase and initiate apoptosis/senescence in vitro are achievable. Lead optimization around the trisubstituted acridine series is ongoing in order to select an optimal candidate for phase I clinical evaluation.

Summary

Research into the regulation of telomerase is leading to the development of methods for the early and accurate diagnosis of cancer and of novel antitelomerase cancer therapeutics. Although not described in this overview, there were several poster presentations on methodologic areas actively being investigated including better telomerase quantitation methods, such as the development of standardization protocols. There were methods described measuring telomerase RNA levels, development of in situ telomerase activity assays, comparing various telomerase antibodies, and measuring and quantitating telomere lengths in human tissues. Even though there have been many recent significant developments in the telomere/telomerase fields of research, there are still some gaps in our understanding and more preclinical proof of efficacy and early stage clinical trials are warranted. The progress made in the last 2 years has been impressive and there was general consensus that the telomerase-targeted therapies are likely to provide a promising and novel approach to cancer therapeutics.

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Appendix

List of Invited/Key Speakers

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