Reviews

TELOMERASE: A NOVEL TARGET OF ANTITUMOR AGENTS

ZHANG Ru-gang 张如刚[1], YUAN Jin-hui 袁金辉[1], WANG Xing-wang 王兴旺[1], XU Bin 胥彬[2], XIE Hong 谢弘[1]

[1]Shanghai Institute of Cell Biology, [2]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China

ABSTRACT

Telomerase activity was found to be high in various human cancers, but absent in most normal tissues. Its expression pattern made it a novel target for antitumor agents. Several strategies against telomerase were presented in this review. Targeting the telomerase RNA component by oligonucleotide/ribozyme was considered to be one of the most hopeful approaches. Some progresses were made in this area, such as the use of PANs and 2-5A antisense compounds. The relationships among telomerase activity and cell differentiation, signal transduction, oncogene, tumor suppressor gene as well as cell cycle modulation also provided a series of valuable ideas in designing anti-telomerase drugs for cancer therapy. In conclusion, although there is still a long way in understanding the mechanism and regulation of telomerase, the advance of studies on telomerase has allowed the development of numerous strategies for the treatment of cancer.

Key words: Telomerase, Antitumor agent, Antisense therapy, Cell differentiation, Signal transduction, Oncogene, Tumor suppressor gene, Cell cycle modulation

Anticancer drug screenings include cytotoxicity-based and mechanism-oriented strategies. Several reviews have covered the topic.[1-3] Although cytotoxicity-based systemic chemotherapy has had some success in selectively killing rapidly growing tumor cells, it has the major disadvantage of killing rapidly proliferating nonmalignant cells, resulting in serious side effects. The research of mechanism-oriented antitumor agents not necessary producing significantly better effectiveness than those that are already in clinical use is, therefore, of particular interest. The phenotypes of malignant cells have been found to differ in a number of respects from those of the nonmalignant cells and each of these differences may offer itself as a potential target of antitumor agents. Some cancer-specific targets defined at the molecular levels have been discovered in the recent years, including alpha-fetoprotein,[4] oncogenes,[5] the apoptotic process[6] and so on. The identification of these molecular targets has provided the possibilities of the development of novel antitumor agents by mechanism-oriented screenings.

The presence of telomerase in various human cancers and its absence in many normal cells[7] not only give us an insight into how changes of the biochemistry of cells can result in malignancies, but also indicate a novel target for antitumor agents. Thus, the discovery of telomerase will lead to the development of new antitumor agents. Several telomerase-targeting strategies have been used to investigate suitable cancer therapy. Some examples of the exciting possibilities of telomerase inhibition as an approach of the development of new effective antitumor agents are presented here, which become the main aspects of this review.

Telomere and Telomerase

Telomeres are the end caps of eukaryotic chromosomes which are made up of an array of tandem repeats of the hexanucleotide TTAGGG (5'→3' direction). Putative functions of telomeres include protecting the ends of the chromosomes against exonucleases and ligases, preventing the activition of DNA damage check points, countering the loss of terminal DNA segments that occurs when linear DNA is replicated, and so on.

Received September 30, 1999, accepted December 22, 1999
Correspondence to: WANG Xing-wang, Shanghai Institute of Cell Biology, Chinese Academy of Sciences, No. 320, Yueyang Road, Shanghai 200031, China.

However, the number of repeated subunits in telomeres differs between different cells in the same organism. Moreover, the number may also fluctuate in a given cell over time. Several specialized proteins are involved in the regulation of telomere metabolism, including the yeast double strand telomere-binding protein Rap 1 and single strand telomere-binding protein Rlf 6p, Cdc 13 and EST1, the human duplex telomere-binding protein TRF, telomerase, telomerase-associated protein TP 1 and so on. It is well known that lagging-strand DNA synthesis can not replicate the very ends of linear chromosomes, and this so-called end replication problem results in progressive shortening of telomeric repeats. When the telomere length is reduced to a critical point, a signal is given to stop further cell division, and cell death will take place. Thus, it has been proposed that telomere length may serve as a "mitotic clock". The structures and functions of telomeres will not be extensively discussed here, since they have been reviewed elsewhere.[8]

A variety of solutions for replication of DNA termini have been proposed. Telomerase, a telomere-specific terminal transferase, provides an excellent candidate for a telomere-specific polymerase. Telomerase can use the 3'-telomeric end as a primer and employ a RNA template for the synthesis of G-rich telomeric repeat. Thus, telomeres can be lengthened by the attachment of newly synthesized telomeric subunits. Telomerase is a ribonucleoprotein DNA polymerase. Like all polymerases, it consists mainly of protein. But it uniquely includes a single molecule of RNA that contains the critical nucleotide template for building telomeric subunits. Telomerase places the tip of one strand of DNA on the RNA, positioning itself so that the template lies adjacent to that tip. Then the enzyme adds one DNA nucleotide at a time until a full telomeric subunit is formed. When the subunit is completed, telomerase can attach another by sliding to the new end of the chromosome and repeating the synthetic process. Although the cloning of gene encoding the protein component of telomerase has not yet been reported for any vertebrate, three major components of human telomerase have been identified, i.e. human telomerase RNA (hTR), telomerase-associated protein (TP1/TLPI), and human telomerase catalytic subunit (hTRT/hEST2). The hTR gene has been cloned from several species including humans. TP1/TLP1, which is presumably the human homologue of the *Tetrahymena* telomerase p80 gene, has also been expressed. A recently described gene, hTRT/hEST2, is a human homologue of the yeast *Saccharomyces cerevisiae* gene EST2 that is the catalytic subunit of the yeast telomerase gene. The hTRT/hEST2 gene has been postulated to be the catalytic subunit of the human telomerase gene. A series of findings provide strong evidence that expression of hTRT/hEST2 is a rate-limiting determinant of the enzymatic activity of human telomerase. More data can be found in the references.[9-11]

Cancer Connection

Although the importance of telomeres and telomerase is indisputable, most normal somatic human cells lack telomerase. It is suggested that human cells might "count" division by tracking the number of telomeric repeats they lose, and they might stop dividing when telomeres decline to a critical length. However, if the deranged cells begin to make telomerase, they may replicate indefinitely. This property is known as cellular immortality. Cancers arise when a cell escapes from normal controls on division. As the cell and its offspring multiply uncontrollably, they can invade nearby tissues and travel to parts of the body where they do not belong, establishing new malignancies (metastases) at distant sites. In theory, a lack of telomerase would retard the growth of tumors by causing continually dividing cells to lose their telomere and to succumb before they do much damage. If cancer cells make telomerase, they would retain their telomeres and would potentially survive indefinitely. Thus, telomerase is thought to be required for the maintenance of many human tumors.

The cancer field started to take an interest in telomere and telomerase approximately 8 years ago. There has been the exponential increase in papers of the relationship between telomerase and cancer in the past 5 years. A series of investigations show that telomerase is active not only in cancer cell lines maintained in the laboratory but in tumor samples from the human body. Telomerase is found to be active in 85% of 400 tumor tissue samples.[12] The activation of telomerase in malignant cancers seems to be an important step in tumorigenesis.[13] In many instances, telomerase activity may indicate high proliferation rates of tumor cells.[14,15] In addition, many investigators have found that telomerase might also be related to tumor cell metastasis, differentiation, cell cycle events, apoptosis and so on. In a variety of cancers, evidence suggests that telomerase activity might be a useful diagnostic marker for their degree in malignancy. And, there is some indication that telomerase activity might be a useful prognostic marker for tumors. Further prospective and retrospective clinical studies must be carried out to assess the validity of telomerase as a diagnostic or prognostic marker in many cancer types. On the other hand, the fact that telomerase is absent and not required in most human somatic tissues but is necessary for tumor growth should make this enzyme an attractive target for the development of therapeutics for the treatment of cancer. As described in the introduction, one of the greatest challenges in cancer therapy is to achieve a high therapeutic effect by maximizing the desired reactions and minimizing the undesired side effects. The telomerase-targeting approach would appear to be less

toxic than conventional chemotherapy which affects all dividing cells and has undesirable side effects.

Targeting Telomerase RNA Component

Telomerase contains its own RNA template as an integral part of the enzyme. In *Tetrahymena* cells synthesizing mutant telomerase RNA, altered telomere sequences and lengths result in nuclear and cell division defects, and eventual cell death.[16] Disruption of the telomerase RNA gene in yeast results in shortened telomeres, a gradual increase in generation time, a decrease in viability and cell death. These results suggest that targeting the telomerase RNA component may become an effective cancer treatment approach.

There are two crucial parameters in drug design: the first is the identification of an appropriate target in the disease process, and the second is finding an appropriate molecule that has specific recognition and affinity for the target, thereby interfering the disease process. However, the majority of the drugs discovered to date recognize the targets by mechanisms that are not well defined. The knowledge gained by this drug discovery process can not be generalized for rational drug design. The oligonucleotide/ribozyme strategies provide novel approaches to drug design with two advantages over conventional drugs, including that the target gene has a defined sequence, and the oligonucleotide interacts with the target gene by Watson-Crick base pairing, providing specificity and affinity. Thus, although action mechanism of oligonucleotide/ribozyme is still under investigation, they have been considered to be one of the most hopeful approaches to targeting the telomerase RNA component.

Early report by Feng et al.[9] indicated that HeLa cells transfected with an antisense human telomerase RNA lost telomeric DNA and began to die after 23 to 26 doublings. In order to avoid destruction of the antisense sequences by nuclease and to increase their delivery into the cell, peptide nucleic acids (PNAs) were designed with a charge-neutral, pseudo-peptide backbone consisting of N-(2-aminoethyl) glycine units instead of a negatively charged deoxyribose-phosphate backbone.[17] The PNAs that have a sequence complementary to the RNA component of human telomerase are very specific and efficient inhibitors of telomerase *in vitro* with IC_{50} values in the picomolar to nanomolar range. The inhibition depends on targeting exact functional boundaries of the human telomerase RNA template and is 10- to 50-fold more efficient than the inhibition by analogous phosphorothioate oligomers. In contrast to high selectivity of inhibition by PNAs, phosphorothioate oligomers inhibit telomerase in a non-sequence-selective fashion.[18] Further experiments with cell cultures and tumor models will continue this path of investigation. In addition, a 2–5A antisense compound targeting telomerase under development by Atlantic Pharmaceuticals has also demonstrated the ability to inhibit the growth and survival of human malignant glioma cells *in vitro* and *in vivo*. The results showed that the vast majority of treated cells could be killed within 14 days. When the antisense molecules were applied to human tumors implanted in nude mice, tumor mass was significantly reduced. Ribozymes (catalytic RNAs, RNA enzymes) are RNAs that possess site-specific RNA cleavage activities. Especially, ham-merhead ribozymes have higher specificity and can cleave telomerase RNA in trans in a truly catalytic manner. Kanazawa et al.[19] prepared a hammerhead ribozyme directed against the RNA component of human telomerase. The ribozyme exhibited a specific cleavage activity against a synthesized portion of the telomerase RNA component used as the substrate. Furthermore, when added to cell extracts from HepG2 or HuH-7, human hepatocellu!ar carcinoma derived cell lines, the ribozyme inhibited the telomerase activity in both. The inhibition observed was dose-dependent and reached up to approximately 90% at 10 μM concentration. It is concluded that oligonucleotide/ribozyme strategy provides a rational basis for the development of specific antitumor agents aimed at the telomerase RNA component.

Targeting Telomerase Activity Regulation

Although the regulation of telomerase activity in cancer is poorly understood, some exciting results have been available, which provides a series of valuable ideas of the development of anti-telomerase drugs for cancer therapy.

Cell Differentiation

Although a great variety of mechanisms may be responsible for the malignant transformation of cells, the common result is a loss of control of differentiation. Recently, telomerase activity has been found to be dramatically inhibited during tumor cell differentiation. Thus, down-regulation of telomerase activity by inducing cell differentiation is an interesting possibility and several differentiation-inducing agents have been tested with some success. HL-60 human promyelocytic leukemia cell line can be chemically induced to differentiate into a variety of cell types that functionally and morphologically resemble normal monocytes/macrophages and granulocytes.[20] To determine the effect of differentiation on telomerase activity, HL-60 cells were induced to differentiate with 12-o-tetradecanoylphorbol-13-acetate (TPA), and telomerase activity was assessed. It was found that telomerase activity declined rapidly during the course of differentiation to monocytes/macrophages. But the enzyme activity in extract from uninduced HL-60 cells

was not inhibited by adding TPA directly to the extract itself, indicating that the loss of activity was differentiation specific. When HL-60 cells were induced to differentiate along the granulocyte lineage with retinoic acid (RA) or DMSO. telomerase activity was also seen to decline rapidly over the course of treatment but the activity in the uninduced HL-60 cell extract was not inhibited by adding RA or DMSO at concentrations that induced cell differentiation. This loss of telomerase activity induced by three different inducers of differentiation was independent of differentiation-induced apoptosis, and occurred in the presence of unaltered expression of the RNA component of telomerase. In addition, reduction in telomerase activity was also observed during the differentiation of murine F9 teratocarcinoma and C2C12 myoblast cells by the above mentioned differentiation-inducing agents.[22] Recently, it was also noticed that TPA and RA, two different inducers of differentiation, caused the down-regulation of hEST2/hTRT expression.[22,23] Whether the down-regulation of hEST2/hTRT expression is a general consequence of differentiation induction and parallels the down-regulation of telomerase activity remains to be clarified.

Signal Transduction

Targeting some molecules of cellular signal transduction pathway may result in the inhibition of telomerase activity. Some studies show that telomerase activity is regulated by protein phosphorylation and dephosphorylation in human tumor cells since vertebrate telomerase is a ribonucleoprotein complex with at least two protein components containing multiple protein phosphorylation sites. Incubation of cell nuclear telomerase extracts with protein phosphatase 2A (PP2A) abolished the telomerase activity, and in contrast, cytoplasmic telomerase activity was unaffected. Inhibition of telomerase activity by PP2A was both concentration-and time-dependent, and was prevented by the protein phosphatase inhibitor okadaic acid and a specific antibody against PP2A catalylic subunit. In addition, nuclear telomerase activity inhibited by PP2A was reactivated by endogenous protein kinases in the presence of ATP. Furthermore, telomerase activity was stimulated by Okadaic acid, consistent with a role for PP2A in the regulation of telomerase activity in intact cells. These results indicate that PP2A is a telomerase inhibitor in some tumor cells and a potential target in controlling telomerase activity in cancer therapy.[24] Tyrosine phosphorylation of PP2A catalytic subunit by receptor-linked and non-receptor typrosine kinase inhibits the activity of PP2A,[24] suggesting the relationship between PP2A and protein kinases. Recently, two protein kinase C inhibitors (PKC) (bisindolylmaleimide I and H-7) were found to produce a potent inhibition of telomerase activity in treated nasopharyngeal carcinoma cells NPC-076. Staurosporine produced a moderate inhibition, and sphingosine had a small inhibitory effect. The inhibition of telomerase activity by the above mentioned PKC inhibitors appears to be specific since the treated cells were mostly viable and still retained significant levels of protein synthesis capability. No obvious inhibition of the enzyme activity was observed with cells treated with protein tyrosine kinase (PTK) inhibitors quercetin and herbimycin A or protein kinase A (PKA) inhibitor H-89. In addition, the enhanced telomerase activity in phyto-hemagglutinin-stimulated peripheral blood lymphocytes was also blocked by PKC inhibitors H-7, bisindolyl-maleimide and staurosporin.[25]

Oncogene and Tumor Suppressor Gene

The discovery of oncogenes and tumor suppressor genes lead to the realization that many types of cells have the potential to become malignant through activation of their endogenous genetic material and the inactivation of tumor suppressor genes can lead to having some types of cancer.[26] Moreover, it has been found that there is a link between oncogenes, tumor suppressor genes and telomerase activity. A report showed that antisense pentadreadeoxynucleotides targeted against c-*myc* mRNA inhibited telomerase activity in human leukemic cell lines, whereas cells treated with c-*myc* sense oligomers exhibited essentially no change in telomerase activity.[27] The functional wild-type p53 gene has been shown to transactivate several cellular genes that contain p53-binding sites including $p21^{WAF1}$ and many of the biological activities attributed to p53 are due to the activities of such downstream effector genes. It was found that $p21^{WAF1}$ down regulated human telomerase RNA component mRNA expression in human immortalized kerati-nocytes.[28] Moreover, normal human mammary epithelial cells immortalized by a p53 mutant have been reported to exhibit activation of telomerase, and the human papillomavirus type 16E6 protein that targets p53 for degradation activates telomerase in early-passage human keratinocytes and mammary epithelial cells.[29] These results suggest that wild-type p53 gene contributes the regulation of telomerase activity. However, telomerase activity was found to be unaffected during p53-induced apoptosis of the immortalized endothelial cell line ECV-304.[29]

Cell Cycle Modulation

Recent studies indicate that an association with an active cell cycle has been implicated for telomerase activity. In human breast cancer cells, the enzyme activity is regulated in a cell cycle-dependent manner, with increased telomerase activity in the S phase and

decreased telomerase activity in the G2/M phase.[24] In the tissue of human breast carcinoma, the relationship between telomerase activity levels and defects in the cell cycle machinery was analyzed with a focus on the retinoblastoma protein (pRB) pathway(s). Overexpression of Cyclin D1 and/or Cyclin E in combination with a normal pRB, was a typical feature of tumor with high telomerase activity levels. Downregulation of $p16^{INK4}$ was not related to telomerase activity, but tumors with low $p16^{INK4}$ in combination with Cyclin D or Cyclin E overexpression demonstrated high telomerase activity, further suggesting that telomerase activity levels may depend on certain specific cell cycle regulatory proteins.[30] In addition, although blocking of cells at G1/S phase by methotrexate and 5-fluorouracil did not produce marked influence on telomerase activity in the treated human nasopharyngeal cancer cells NPC-076,[25] adriamycin and nocodazole, two blockers of G2/M phase, were found to inhibit the activity of telomerase completely.[31] It is concluded that G2/M phase may become a target of screening telomerase inhibitors.

Direct Effect

A reverse transcriptase inhibitor, 3'-azido-3'-deoxythymidine (AZT), was shown to suppress the activity of *Tetrahymena* telomerase.[32] AZT triphosphate inhibited the telomerase activity in extracts of Swiss 3T3 mouse cells. Spontaneous transformation of mouse embryonic fibroblasts in the presence of the reverse transcriptase inhibitors (RTI) AZT and carbovir led to the formation of telomerase-free clones, indicating that RTI could block telomerase activity in mammalian cells.[33] Telomerase activity in the B cell line JY616 and T cell line Jurkat E6-1, two immortalized human cell lines, was also inhibited by ddGTP and AZT triphosphate.[34] Using cell-free biochemical telomerase assay, it was found that 7-deaza-2'-deoxyguanosine-5'-triphosphate (7-deaza-dGTP) and 7-deaza-2'-deoxyadenosine-5'-triphosphate (7-deaza-dATP) were potent telomerase inhibitors. IC_{50} values were 11 and 8 μM for 7-deaza-dGTP and 7-deaza-dATP, respectively. Additional studies showed that both 7-deaza-dGTP and 7-deaza-dATP were also incorporated into telometric DNA by telomerase, which may also be an effective approach in the design of new telomerase inhibitors.[35] Other studies with deoxy-nucleoside analogues indicated that AZT triphosphate exhibited much more inhibitory action on telomerase than 2', 3'-dideoxy 2', 3'-didehydrothymidine triphosphate, and the cytidine analogue ddCTP was not inhibitory. ddGTP was the most potent inhibitor among all dideoxynucleosides studied.[36] These nucleoside analogues may cause their telomere-shortening effects by binding to and competing for the nucleoside triphosphate-binding site of telomerase rather than by being incorporated and causing chain termination.[34] In addition, a new series of pyridine aldehyde, thioaldehyde, acetal or thioacetal derivatives with telomerase-inhibitory activity including the exemplified compound GRN-53804, have recently been prepared by the Geron pipeline. The human telomerase-inhibitory activity of a series of 2, 6-disubstituted amidoanthracene-9, 10-diones such as BSU- 1021, BSU-1032 and BSU-9048 and their resulting antitumor activities have also been demonstrated by some investigators at CRC.

Other Approaches

The introduction of a normal chromosome 3 to an immortal human renal cell carcinoma cell line can inhibit the activity of telomerase, suggesting that a gene(s) on human chromosome 3 repressing telomerase activity is present.[37] Some other putative genes involved in telomerase inhibition have also been mapped to different chromosomes.[38] In addition, investigators have noticed telomere-binding proteins (TBP) play an important role in regulating the activity of telomerase.[39] The approaches targeting the gene encoding the protein component of telomerase, and structures and functions of telomere will also be available in the near future.

Conclusions

Telomerase is a RNA-dependent DNA polymerase that can compensate for the telomeric losses of DNA that occur at each cell division. Telomerase activity in the tissues of a variety of carcinomas has been found, but the enzyme is inactive in most normal somatic cells. So the expression of telomerase and the ensuing stabilization of telomeres appear to be associated with the ability of tumor cells to achieve immortality. Opportunities are now opening up where this basic knowledge can he translated into a novel approach to the development of antitumor agents. At present, a number of agents have been proven effective against telomerase, although the structure and functions of telomerase have not been fully clarified to date. Agents able to affect telomerase might kill tumor cells by allowing telomeres to shrink and disappear without disrupting the function of many normal cells. It is predicted that rapid progress in the field of telomerase will allow us to utilize the numerous telomerase-targeting strategies for clinical cancer therapy.

However, many issues need to be overcome for clinical use of anti-telomerase approaches. In the oligo-nucleotide/ribozyme-based telomerase-targeting therapy, pharmacokinetics (i.e., absorption, distribution, metabolism and excretion), mutagenicity and toxicity studies should be carefully designed and accomplished before their clinical uses can be developed. The rational development of new drugs, based upon the knowledge of

the telomerase to be targeted, requires the availability of selective, mechanism-oriented screenings. Therefore, it is hoped that specific cellular models aimed at the enzyme could be developed in the future. Some normal somatic cells are telomerase-positive. Telomeric DNA in these cells would he lost during an anti-telomerase therapy. On the other hand, although telomerase activity in most tumor samples has been detected, the presence of tumor samples lacking detectable telomerase[40] suggests that telomerase-independent pathways are available to tumors for telomerase-length maintenance or telomerase-negative tumor cells contain a gene or genes functioning as a telomerase regressor.[41] If telomerase is to be a target for antitumor agents, it will he very important to know whether a particular tumor type requires the enzyme, and perhaps the development of combined therapies is necessary to disrupt telomere maintenance in these tumors.

In summary, although we are still in an early stage of understanding of the mechanism and regulation of telomerase, the advance of studies on the relationship between telomerase and cancer has allowed the development of numerous strategies for the treatment of cancer.[42-46] It is likely that the next few years will still be an exciting time in telomerase research and may see a real breakthrough in the development of novel antitumor agents which target telomerase.

REFERENCES

[1] Wang XW, Xu, B. Screening models and methods of antitumor drugs. Chin J Pharmaceu 1 997; 28:39.

[2] Robert J. Anticancer drug screening with *in vitro* models. Drugs Fut 1997; 22:739.

[3] Jonhson RK. Screening methods in antineoplastic drug discovery. J Natl Cancer Inst 1990; 82:1082.

[4] Wang XW, Xu B. Stimulation of tumor cell growth by alpha-fetoprotein. Int J Cancer 1998; 75:596.

[5] Wang XW, Xu B. Informational drugs and gene therapy of human diseases. Chin Pharmacol Bull 1996; 12:289.

[6] Wang XW, Xu B. Several new targets of antitumor agents. Acta Pharmacol Sin 1997; 18:289.

[7] Blackburn EH. Telomerase. Ann Rev Biochem 1992; 61:113.

[8] Zakian VA. Structures and function of telomeres. Ann Rev Genet 1989; 23:579.

[9] Feng J, Funk WD, Wang SS, et al. The RNA component of human telomerase. Science 1995; 269:1236.

[10] Harrington L, Mcphail T, Mar V, et al. A mammalian telomerase-associated protein. Science 1997; 275: 973.

[11] Counter CM, Meyerson M, Eaton EN, et al. The catalytic subunit of yeast telomerase. Proc Natl Acad Sci USA 1997; 94:9202.

[12] Rhyu M. Telomeres, telomerase, immortality. J Natl Cancer Inst 1995; 87:885.

[13] Dahse R, Fiedler W, Ernst G. Telomeres and telomerase: biological and clinical importance. Clin Chem 1997; 43:708.

[14] Greider CW. Telomerase activity, cell proliferation and cancer. Proc Natl Acad Sci USA 1998; 95:90.

[15] Lee HW, Blasco MA, Gottlieb, GJ, et al. Essential role of mouse telomerase in highly proliferative organs. Nature 1998; 392:569.

[16] Yu GL, Bradley JD, Attardi LD, et al. *In vivo* alteration of telomerase sequences and senescence caused by mutated Tetrahymena telomere RNAs. Nature 1990; 344:126.

[17] Wittung H, Nielsen P, Buchardt, O, et al. DNA-like double helix formed by peptide nucleic acid. Nature 1994; 368:561.

[18] Norton J, Piatyszek M, Wright W, et al. Inhibition of human telomerase activity by peptide nucleic acids. Nature Biotechnol 1996; 14:615.

[19] Kanazawa Y, Ohkawa K, Ueda K, et al. Hammerhead ribozyme-mediated inhibition of telomerase activity in extracts of human hepatocellular carcinoma cells. Biochem Biophy Res Commun 1996; 225:570.

[20] Collins SJ. The HL-60 promyelocytic leukemia cell line: proliferation, differentiation and cellular oncogene expression. Blood 1987; 70:1233.

[21] Bestilny LJ, Brown CB, Miura Y, et al. Selective inhibition of telomerase activity during terminal differentiation of immortal cell lines. Cancer Res 1996; 56:3796.

[22] Meyerson M, Counter CM, Eaton EN, et al. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell 1997; 90:785.

[23] Ramakrishuan S, Eppenberger U, Mueller H, et al. Expression profile of the putative catalytic subunit of the telomerase gene. Cancer Res 1998; 58:622.

[24] Li H, Zhao LL, ,Funder JW, et al. Protein phosphatase 2A inhibits nuclear telomerase activity in human breast cancer cells. J Biol Chem 1997; 272:16729.

[25] Ku WC, Cheng AJ, Wang TC. Inhibition of telomerase activity by PKC inhibitors in human nasopharyngeal cancer cells in culture. Biochem Biophy Res Commun 1997; 241:730.

[26] Whartenby KA, Abboud CN, Marrogi AJ, et al. The biology of cancer gene therapy. Lab Invest 1996; 72:131.

[27] Fujirmoto K, Takahashi M. Telomerase activity in human leukemic cell lines is inhibited by antisense pentadecadeoxynucleotides targeted against c-*myc* mRNA. Biochem Biophy Res Commun 1997; 241:775.

[28] Kallassy M, Martel N, Damonv O, et al. Growth arrest of immortalized human keratinocytes and suppression of telomerase activity by $p21^{WAF1}$ gene expression. Mol

Carcinog 1998; 21:26.

[29] Maxwell SA, Capp D, Acosta SA. Telomerase activity in immortalized endothelial cells undergoing p53-mediated apoptosis. Biochem Biophy Res Commun 1997; 241:642.

[30] Landberg G, Nielsen NH, Nilsson P, et al. Telomerase activity is associated with cell cycle deregulation in human breast cancer. Cancer Res 1997; 57:549.

[31] Zhu X, Kumar R, Mandal M, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc Natl Acad Sci USA 1996; 93:6091.

[32] Strahl C, Blackburn EH. The effects of nucleoside analogs on telomerase and telomeres in Tetrahymena. Nucleic Acids Res 1994; 22:893.

[33] Yegorov YE, Chernov DN, Akimov SS. Reverse transcriptase inhibitors suppress telomerase function and induce senescence-like processes in cultured mouse fibroblasts. FEBS Letters 1996; 389:115.

[34] Strahl C, Blackburn EH. Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. Mol Cell Biol 1996; 16:53.

[35] Fletcher TM, Salazar M, Chen SF. Human telomerase inhibition by 7-deaza-2'-deoxypurine nucleoside triphosphates. Biochem 1996; 35:15611.

[36] Pai RB, Pai SB, Kukhanova M, et al. Telomerase from human leukemia cells: properties and its interaction with deoxynucleoside analogues. Cancer Res 1998; 58: 1909.

[37] Ohmura H, Tahara H, Suzuki M, et al. Restoration of the cellular senescence program and repression of telomerase by human chromosomes. Jpn J Cancer Res 1995; 86:899.

[38] Shimura M, Barrett JC. Multiple pathways to cellular senescence: role of telomerase repressors. Eur J Cancer 1997; 33:703.

[39] Nugent CZ, Hughes TR, Liu NF, et al. Cdc13p: a single-strand telomeric DNA-binding protein with a dural role in yeast telomere mainitenance. Science 1996; 274:249.

[40] Kim NW, Piatyszek MA, Prowse R, et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266:2011.

[41] Katoh M, Kameyama M, Kugoh H, et al. A repressor function for telomerase activity in telomerase-negative immortal cells. Mol Carcinog 1998; 21:17.

[42] Zhang RG, Wang XW, Yuan JH, et al. Human hepatoma cell telomerase activity inhibition and cell cycle modulation by its RNA component antisense oligodeoxyribonucleotides. Acta Pharmacol Sin 2000; (in press).

[43] Zhang RG, Yuan JH, Xie H. Telomerase and tumor. Chin J Cell Biol 2000; 22:25.

[44] Zhang RG, Wang XW, Yuan JH, et al. Using a non-radioisotopic, quantitative TRAP-based method detecting telomerase activities in human hepatoma cells. Cell Res 2000; (in press).

[45] Zhang RG, Wang XW, Xie H. Progress on mechanism of telomerase activation. Prog Biochem Biophy (China) 2000; (in press).

[46] Yuan JH, Xie H. Progress in research on telomerase. Chin Bull Life Sci 1999; 11:111.