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Methods of Telomerase Inhibition

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Summary

Telomerase is central to cellular immortality and is a key component of most cancer cells although this enzyme is rarely expressed to significant levels in normal cells. Therefore, the inhibition of telomerase has garnered considerable attention as a possible anticancer approach. Many of the methods applied to telomerase inhibition focus on either of the two major components of the ribonucleoprotein holoenzyme, that is, the telomerase reverse transcriptase (TERT) catalytic subunit or the telomerase RNA (TR) component. Other protocols have been developed to target the proteins, such as tankyrase, that are associated with telomerase at the ends of chromosomes. This chapter summarizes some of these recent advances in telomerase inhibition.

Keywords

Telomerase; inhibition; technique; method; telomerase reverse transcriptase (TERT); telomerase RNA (TR)

1. Introduction

Telomeres are sequences of DNA extending for many kilobases at the ends of chromosomes that in humans consist of hexameric 5′-TTAGGG-3′ tandem repeats. Telomerase is a ribonucleoprotein that maintains the lengths of chromosomal ends by synthesizing telomeric sequences. There are two major components of the telomerase holoenzyme: the telomerase reverse transcriptase (TERT) protein subunit that catalyzes the enzymatic reaction of DNA synthesis and the telomerase RNA (TR) component that serves as a template for the addition of deoxyribonucleotides to the ends of chromosomes. Although other proteins are associated with the holoenzyme, these two components are essential and sufficient for telomerase activity and telomere lengthening (1-3).

2. Importance of Telomerase in Cancer and Aging

Telomerase is expressed in germline and embryonic stem cells as well as most somatic stem cells but is barely detectable in the great majority of adult somatic cells (4). In actively dividing somatic cells, the telomeres shorten with each cell replication because of the paucity of telomerase activity eventually leading to replicative senescence after about 50 divisions (5). This phenomenon has led to the idea that reduced telomerase expression in somatic cells may set in motion a molecular clock that controls the aging process (5). In contrast, telomerase is up-regulated in the vast majority of cancer cells that are dependent upon this enzyme for maintaining their telomere lengths thereby conferring unlimited proliferative capacity or cellular immortality (4).

Because telomerase is active in most cancer cells and is almost undetectable in most normal somatic cells, analysis of telomerase activity has potential as a diagnostic marker of cancer. The increase in telomerase in cancer cells generally occurs very early during tumorigenesis and sensitive techniques such as the telomeric repeat amplification protocol (TRAP) assay can detect trace levels of this enzyme, a method that has obvious potential for cancer diagnosis (6).

3. Potential of Telomerase Inhibition in Cancer Therapy

The cancer therapeutic potential of telomerase inhibition is probably the area of telomerase research that has received the most attention. Telomerase activity can be inhibited in cancer cells and leads to a marked reduction in cellular viability and/or induces apoptosis of these cells (7,8). Anticancer approaches directed at telomerase inhibition are varied, and methods ranging from RNA interference (RNAi) of the TERT catalytic subunit to inhibition of the proteins associated with telomerase at the telomeres have proven to have efficacy against cancer. As the telomeres of the rare normal cells that express telomerase are longer than that in most cancer cells and the level of telomerase activity is generally lower in normal telomerase-positive cells as compared with cancer cells, the risks associated with possible telomere shortening in normal cells because of off-target telomere shortening are thought to be relatively minimal. Therefore, the efficacy of telomerase inhibition in inducing loss of viability or apoptosis of cancer cells combined with the relative low risks to normal cells of inhibition of telomerase have moved telomerase research to the forefront among anticancer approaches.

4. Contents of this Book

4.1. Methods of Inhibiting the TERT Catalytic Subunit of Telomerase

The TERT catalytic subunit has been a major target for the development of anticancer methods because of the high concentration of TERT in almost all cancer cells, the dependency of most cancer cells on TERT activity, and the scarcity of TERT in most normal cells. The approach of transcript knockdown has utilized antisense oligodeoxyribonucleotides as a mainstay, whereas newer advancements have relied on small-interfering RNA (siRNA) molecules. Both these techniques involve synthetic nucleic acids that can bind to specific mRNA targets and both have been effective in anticancer approaches to knockdown TERT expression as described in Chapter 2. The use of double-stranded RNA (dsRNA) has also been quite effective in ablating or greatly reducing transcripts from genes such as *TERT* (*see* Chapter 3). These dsRNA sequences can be used to generate an RNAi response in cells of embryonic origin such as human embryonic kidney (HEK) cells, a popular cell type used in cancer research. This technique is especially effective for short-term analyses of *TERT* knockdown because the dsRNA is degraded in the cells in the long term. RNAi of *TERT* has also been successful with the use of plasmid constructs that exogenously express short hairpin RNA sequences complementary to the *TERT* transcript. This technique (*see* Chapter 4) allows analysis of downstream effects of *TERT*, serves as an alternative approach to gene therapy using viral vectors, and allows long-term and permanent gene knockdown. Also effective for long-term knockdown of *TERT* is the use of retroviral vectors that express short hairpin RNA specific to a segment of the *TERT* transcript. This RNAi-based technique (*see* Chapter 5) involves incorporation of the anti-telomerase sequence into the host genome and can provide effective knockdown of *TERT*.

Small molecules such as 3′-azido-2′, 3′-dideoxythymine (AZT), which is a nucleoside analogue, can be effective in targeting the active site of TERT, but this approach lacks the desired selectivity of many other approaches. Small non-nucleosidic synthetic compounds can be quite effective in inhibiting the catalytic activity of the TERT protein component as described in Chapter 6. One compound that has shown promise in this regard is BIBR1532 that inhibits the in vitro processivity of telomerase. The inhibition of TERT activity with BIBR1532 occurs in a dose-dependent manner, and higher concentrations of this telomerase inhibitor can be cytotoxic to cancer cells of the hematopoietic system such as HL-60 cells with little effect on normal cells.

Anticancer immunotherapeutic approaches have also focused on TERT (*see* Chapter 7). These methods involve the use of peptides derived from TERT. The peptides are presented by major

histocompatibility complex (MHC) class I molecules to T lymphocytes. The result is that CD8 + cytotoxic T lymphocytes specific for the TERT-derived antigenic peptides lyse cancer cells that express TERT. These immunotherapeutic approaches directed against TERT epitopes can be carried out in the absence of toxicity and are showing great promise as anticancer agents.

It can be a challenge to identify small molecule compounds that affect the expression of TERT, and the use of cell-based reporter systems for the analysis of TERT expression has been developed to enhance these endeavors as described in Chapter 8. For example, the *TERT* promoter can be linked to two different reporter genes encoding green fluorescent protein (GFP) and secreted alkaline phosphatase (SEAP). The transfection of these reporter constructs results in stable clones that allow analysis of *TERT* expression. Ultimately, some level of inhibition of *TERT* is the goal of many anticancer approaches, and Chapters 2–8 provide many of the most promising and effective methods for actively knocking down the *TERT* transcript, ablating its catalytic activity, directing the immune system to lyse telomerase-positive cancer cells, or using expression constructs to identify small molecule components that affect the expression of telomerase.

4.2. TR Inhibition as an Anticancer Approach

The RNA component of telomerase has also been a popular and effective target for inhibiting telomerase activity in cancer cells. As in the case for *TERT* transcript knockdown, antisense oligonucleotides against the human TR template can be employed to reduce or eliminate telomerase activity as described in Chapter 9. In this approach, a 2′,5′-oligoadenylate (2–5A) antisense system can be used as a mediator of interferon actions through RNase L activation. The result of this approach is that single-stranded templates, such as the TR component, are specifically cleaved. The anticancer utility of this approach has been proven not only in vitro but also in vivo.

In addition to antisense oligonucleotides, hammerhead ribozymes and RNAi can be directed to the RNA component of telomerase as delineated in Chapter 10. Both these methods also lead to degradation of the RNA component of telomerase. The effect is immediate growth inhibition of cancer cells both in vitro and in vivo independent of telomere length of the target cancer cell. The advantage of this technique is that it greatly reduces the lag period that is often encountered in approaches that are dependent upon the shortening of telomeres to inhibit cancer cell growth. Thus, methods directed at the RNA component of telomerase using antisense oligonucleotides, hammerhead ribozymes, or RNAi also show great promise as anti-telomerase approaches to cancer therapy.

4.3. Targeting Proteins Associated with Telomerase Activity

Approaches to telomerase inhibition have been developed that do not directly inhibit the TERT or TR components of telomerase but rather inhibit target proteins that are associated with telomerase activity. For example, Chapter 11 describes the details of monitoring the telomeric function of tankyrase I, a telomeric poly(ADP-ribose) polymerase (PARP) that can affect telomerase inhibition in cancer cells. The use of Southern blot analysis to screen tankyrase I inhibitors as well as direct monitoring of tankyrase I PARP activity is described.

Signalling pathways such as those carried out by mitogen-activated protein (MAP) kinase can result in stimulation of the *TERT* gene. For example, Ets and AP-1 may play a role in MAP kinase signaling of the *TERT* gene and inhibition of this pathway could be a novel approach to reducing *TERT* expression and telomerase activity as described in Chapter 12. It is apparent that many additional techniques will be developed to impact the proteins or pathways associated with telomerase activity in cancer cells, and Chapters 11 and 12 provide some important approaches for this avenue of potential anticancer therapy.

4.4. Screening of Telomerase Inhibitors

Finding novel inhibitors of telomerase is an important aspect of increasing the tools that we have for anti-telomerase approaches to cancer therapeutics. Chapter 13 proposes a strategy for determining the therapeutic potential of telomerase inhibitors using a screening system in one cell type. For example, four completely different compounds, BRACO19, BIBR1532, 2′-*O*-methyl RNA, and peptide nucleic acids, were chosen for detailing the methods of screening telomerase inhibitors. Additionally, methods are outlined in this chapter for determining the effectiveness of telomerase inhibition through TRAP assays or assessment of telomere lengths using Southern blot telomere restriction fragment analysis.

4.5. Telomerase Inhibition Combined with Other Chemotherapeutic Agents

Chapter 14 reviews the utility of telomerase inhibition in combination with other chemotherapeutic reagents to enhance anticancer effects. For instance, there is an indication that imatinib, a selective inhibitor of the BCR-ABL tyrosine kinase, can enhance the telomerase inhibition of a dominant-negative form of human telomerase (DN-*hTERT*). In a completely different approach, telomestatin, a natural telomerase-inhibiting product isolated from *Streptomyces anulatus*, was combined with imatinib, daunorubicin, mitoxantrone, or vincristine and was shown to enhance the sensitivity of these chemotherapeutic agents. Therefore, approaches to telomerase inhibition may also be merged with completely different anticancer approaches such as chemotherapeutic agents to render more effective modes of cancer therapy.

5. Conclusion

Many of the newest as well as established methods for telomerase-based anticancer approaches are provided in this book. Protocols are presented that involve inhibition of the TERT catalytic subunit of telomerase as well as the TR component of this ribonucleoprotein enzyme. Additional approaches involve intervention directed at the proteins that are associated with telomerase or pathways that modulate the *TERT* gene. Methods for the screening of telomerase inhibitors as well as the potential of merging telomerase inhibition with more conventional chemotherapy are also delineated in this volume. This last concept, that is, combination therapy, may be the most promising approach, and it is likely that many new advances will develop that merge different types of anti-telomerase methods or combine telomerase inhibition with other proven modes of anticancer therapy. The continued development of novel tools will likely be at the forefront of cancer therapy, and this book is intended to provide a synopsis of many different anti-telomerase approaches that may revolutionize cancer therapeutics in the future.

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