Pharmacology & Therapeutics 163 (2016) 24–47

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B Teicher

Functional and mechanistic analysis of telomerase: An antitumor drug target

Yinnan Chen [b], Yanmin Zhang [a,*]

[a] School of Pharmacy, Health Science Center, Xi'an Jiaotong University, Xi'an, Shaanxi Province 710061, PR China
[b] School of Molecular Sciences, Arizona State University, Tempe, Arizona 85287, USA

ARTICLE INFO

Available online 23 April 2016

Keywords:
Function
Mechanism
Telomerase
Drug target
Tumor inhibitors

ABSTRACT

The current research on anticancer drugs focuses on exploiting particular traits or hallmarks unique to cancer cells. Telomerase, a special reverse transcriptase, has been recognized as a common factor in most tumor cells, and in turn a distinctive characteristic with respect to non-malignant cells. This feature has made telomerase a preferred target for anticancer drug development and cancer therapy. This review aims to analyze the pharmacological function and mechanism and role of telomerase in oncogenesis; to provide fundamental knowledge for research on the structure, function, and working mechanism of telomerase; to expound the role that telomerase plays in the initiation and development of tumor and its relationship with tumor cell growth, proliferation, apoptosis, and related pathway molecules; and to display potential targets of antitumor drug for inhibiting the expression, reconstitution, and trafficking of the enzyme. We therefore summarize recent advances in potential telomerase inhibitors for antitumor including natural products, synthetic small molecules, peptides and proteins, which indicate that optimizing the delivery method and drug combination could be of help in a combinatorial drug treatment for tumor. More extensive understanding of the structure, biogenesis, and mechanism of telomerase will provide invaluable information for increasing the efficiency of rational antitumor drug design.

Contents

Abbreviations: TERT, Telomerase reverse transcriptase; hTERT, Human telomerase reverse transcriptase; RT, Reverse transcriptase; TR, Telomerase RNA; hTR, Human telomerase RNA; RNP, Ribonucleoprotein; TEN, Essential N-terminal domain; TRBD, Telomerase RNA binding domain; CTE, C-terminal extension domain; CR4/5, Conserved region 4 and 5; TBE, Template boundary element; ROS, Reactive oxygen specie; G4, G-quadruplexes; ALT, Alternative lengthening of telomeres; EGF, Epidermal growth factor; bFGF, Basic fibroblast growth factor; EMT, Epithelial–mesenchymal transition; TERRA, Telomeric repeat-containing RNA; PKC, Protein kinase C; HDAC, Histone deacetylases; MM, Multiple myeloma; Hsp90, Heat shock protein 90; ODN, Antisense oligodeoxynucleotides; TAA, Human tumour-associated-antigens; CTL, CytotoxCLLic T lymphocytes; VCA, Viscum album L. coloratum agglutinin; CLL, Lymphocytic leukemia; NSCLC, Non–small-cell lung cancer; mTOR, Mammalian target of rapamycin; TRF1, Telomeric repeat-binding factor 1; POT1, Protection of telomeres; TNKS1, TRF1-interacting ankyrin-related ADP-ribose polymerase 1; DOX, Doxorubicin; RHPS4, 3,11-difluoro-6,8,13-trimethyl-8H-quino[4,3,2-kl]acri-dinium methosulfate; DNA-PK, DNA-dependent protein kinase; IR, Immune response; Sp1, Specificity protein 1; ER, Estrogen receptor; GKN1, Gastrokine 1.

* Corresponding author at: School of Pharmacy, Health Science Center, Xi'an Jiaotong University, No. 76, Yanta West Street, #54, Xi'an, Shaanxi Province 710061, PR China.
E-mail address: zhang2008@mail.xjtu.edu.cn (Y. Zhang).

http://dx.doi.org/10.1016/j.pharmthera.2016.03.017
0163-7258/

1. Introduction

Telomerase is a special reverse transcriptase enzyme that adds DNA repeats to the ends of chromosome to offset the loss (Greider & Blackburn, 1987). For the past few decades, since the discovery of telomerase, progress has been made in the identification of core components from evolutionary groups of species across eukaryotes, the catalytic telomerase reverse transcriptase (TERT) and telomerase RNA (TR). TERT contains the catalytic site for DNA synthesis and TR provides the template (Greider & Blackburn, 1989; Shippen-Lentz & Blackburn, 1990). A variety of accessory proteins, while dispensable for enzyme activity *in vitro*, play important roles in regulation, biogenesis, and localization (Egan & Collins, 2010; Podlevsky, Bley, Omana, Qi, & Chen, 2008).

Tumor cell growth is a complicated progression, which is regulated by multiple factors including proliferation, cell cycle, and apoptosis (Y. Zhang et al., 2014). An increase in telomerase activity is often directly correlated with uncontrolled growth of cells, a known hallmark of cancer (Ruden & Puri, 2013). According to the Hanahan–Weinberg model of the hallmarks of cancer, to successfully achieve oncogenesis, cancer cells have to sustain proliferative signaling, evade growth suppressors, avoid immune destruction, enable replicative immortality, promote inflammation, activate invasion and metastasis, induce angiogenesis, establish genome instability, resist cell death, and deregulate cellular energetics (Hanahan & Weinberg, 2011). The hallmark enabling replicative immortality demonstrates the ability to grow endlessly, synonymous with reactivation of TERT. Telomerase, and specifically its catalytic subunit TERT, is overactive in 85–90% of cancers and has become a widely acceptable tumor marker and a popular target for anticancer therapeutics (Ruden & Puri, 2013). The discovery of the relationship between telomerase, telomeres, aging, and tumors has broadened the avenue in tumor biology research (Calado & Young, 2009; Low & Tergaonkar, 2013). In humans, telomerase is inactive in most of the somatic cells, which stop division when their telomeres are critically short. This is known as replicative senescence. Most of the human cells cannot overcome the senescence and crisis, which restricts cell growth and protects against oncogenesis (Martinez & Blasco, 2011). Many human cells remain in the crisis period, as balance between cell growth and death, unless occasionally acquiring a mechanism such as up-regulation of telomerase. Cells that escape crisis can grow continuously and are usually characterized with telomere stability and telomerase activity (Feldser & Greider, 2007). This is believed to be a pivotal step in carcinogenesis (Mocellin, Pooley, & Nitti, 2013). Cancer cells that develop chromosomal aberrations show activation or reactivation of telomerase upon exposure to a DNA damage signal, thereby bypassing cell cycle checkpoints and leading to uncontrolled growth and proliferation (Rankin, Faller, & Spanjaard, 2008; Tian, Chen, & Liu, 2010). In addition to the canonical role of maintaining telomere length in malignant cells, telomerase has also been recognized to take part in tumor promoting pathways. Telomerase activity is prominent in highly proliferative cells such as stem cells, germ line cells, as well as 90% of human cancers (Kim et al., 1994), which makes it as a main target for cancer treatment. There are two general strategies of targeting telomerase in cancer treatment. One directly inhibits telomerase catalytic activity leading to telomere shortening. The other strategy is down-regulating the expression of the telomerase subunits or blocking the access of telomerase to its substrates.

The current trend in research on anticancer drugs is to exploit particular traits or hallmarks unique to cancer cells (Olaussen et al., 2006). The ideal cancer treatment would target specifically at cancer cells but have a minimal adverse effect on the normal cells. This review highlights recent advances in our understanding of mammalian telomere biology, analyzing the functional and mechanism of telomerase and how it relates to cancer. Meanwhile, we discuss the current approaches that exploit this knowledge to develop novel antitumor drugs targeting telomerase.

2. Biological aspects of telomerase

2.1. Structural and components of telomerase

Telomerase is unique reverse transcriptase (RT) by functioning as a ribonucleoprotein (RNP). The catalytic core of telomerase is minimally composed of TERT and TR (Collins, 2006; Weinrich et al., 1997). Between the two essential components of telomerase RNP complex, the catalytic TERT protein is highly conserved among all eukaryotes, while the TR is extremely divergent, suggesting that telomerase initially emerges as a protein enzyme and acquires an integral RNA component during the early evolution of eukaryotes (Lingner et al., 1997; Peng, Mian, & Lue, 2001; Podlevsky et al., 2008). TERT is the catalytic component of the enzyme that comprises four conserved structural domains: the telomerase essential N-terminal domain (TEN), the telomerase RNA binding domain (TRBD), the RT domain, and the C-terminal extension domain (CTE) (Fig. 1A) (Lingner et al., 1997). The catalytic domains of TERT and other reverse transcriptases have the common and highly conserved motifs that form the active site for RNA-dependent DNA polymerization. The tertiary structure of TERT, similar to HIV RT, consists of the finger, palm, and thumb domains (Lingner et al., 1997; Peng et al., 2001). The finger domain is composed of motifs 1 and 2, believed to bind incoming nucleotides. The palm domain is composed of motif A–E and forms the catalytic site. Between them there is motif 3, unique to TERT, closely related to the property of the enzyme (Xie, Podlevsky, Qi, Bley, & Chen, 2010). Mutational analysis of the Asp residues from motifs A and C is evidence that the telomerase employs acidic metal-coordination by the aspartic acid for DNA polymerization, which is common to conventional RTs (Haering, Nakamura, Baumann, & Cech, 2000; Lingner et al., 1997). The CTE in TERT binds to the RNA template/DNA primer duplex, sharing functionality with the HIV RT-C terminus referred to as the thumb domain (Peng et al., 2001). The TEN and TRBD domains are telomerase specific and unique to TERT protein. The TEN domain contains anchor sites that bind to single-strand telomeric DNA. Mutational analysis has identified it as related to the repeat addition processivity, which is the characteristic of telomerase, but not to nucleotide addition processivity (Sealey et al., 2010; Zaug, Podell, & Cech, 2008). According to the recently resolved, the co-crystal structure of the TRBD with conserved regions 4 and 5 (CR4/5) of TR in teleost fish *Oryzias latipes*, as well as crosslink data, TRBD contains RNA interacting domain which has a high affinity binding site for TR (Bley et al., 2011; Huang et al., 2014). The structure of TERT protein from *Tribolium castaneum* was solved in 2008 by Emmanuel Skordelakes et al (Gillis, Schuller, & Skordalakes, 2008). The challenge to get the crystal structure of human TERT (hTERT) is to determine how to purify highly concentrated protein. The crystal structure of hTERT could offer insight into research on the enzyme function, which provides a novel opportunity for rational drug design.

Unlike conventional reverse transcriptase, telomerase contains its own template provided by the integral RNA component within the telomerase catalytic core (Feng et al., 1995). The size, structure, and sequence of TR are quite diverse among different species; however they all contain two conserved domains: the template/pseudoknot domain and CR4/5 domain (or three way junction/stem-terminus elements) (Fig. 1B). These two domains are essential for telomerase activity. In fact, an active telomerase enzyme reconstituted *in vitro* only requires the TERT protein and these two excised elements from TR (Mitchell & Collins, 2000). The secondary structure of TR has implications for telomerase function (Chen, Blasco, & Greider, 2000). The template/pseudoknot domain contains template boundary element (TBE), the template region, and pseudoknot.

TBE that is conserved across eukaryotes defines the template boundary physically. The hTR template region promotes base pairing with telomeric DNA, primer to each cycle of the DNA synthesis as well as contains pause signal for each round of repeats by a recent study (Brown et al., 2014). The pseudoknot with a triple helix and part of it binds to