Telomeres and telomerase as novel drug targets: reflections on the 2009 Nobel Prize in Physiology or Medicine

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In December 2009, the Nobel Prize in Physiology or Medicine will be awarded in Stockholm to three (molecular) biologists: Elizabeth H. Blackburn, her former graduate student Carol W. Greider, and Jack W. Szostak. Their discovery of “how chromosomes are protected by telomeres and the enzyme telomerase” solved a major problem in biology: how chromosomes can be properly copied during cell division and how they are protected from degradation by a highly conserved telomeric DNA sequence [repeats of (TTAGGG)_n nucleotides in human] synthesized by a unique reverse transcriptase called telomerase consisting of a catalyzing protein (hTERT), an RNA template (hTR) [1, 2], and several other accessory proteins [3].

Briefly, during cell cycle, the DNA replication machinery in eukaryotes is not able to completely duplicate the telomere ends of chromosomes. This would lead to a shortening of telomere ends of chromosomes by approximately 50 bp during each cell cycle. In somatic cells of adults, no telomerase is expressed, and therefore these cells will normally age. By contrast, the presence of telomerase prevents this degradation process during embryogenesis as well as in stem cells and germ cells, and consequently, cellular senescence will be delayed. If telomerase activity is high, as in the majority of cancer cells, such cells become immortal [4]. The discovery of these fundamental mechanisms has important medical implications and has stimulated the development of new therapeutic strategies.

A striking association between telomere shortening and a reduction of the replicative life span of cultured human

cells has been observed [5]. In addition, germ-line mutations in the genes encoding hTERT, the RNA subunit, or telomere-binding proteins have been linked to acquired and congenital aplastic anemias [6]. Various forms of congenital dyskeratosis are due to mutations in genes encoding proteins involved in telomere maintenance [7]. In addition, signs of premature ageing such as greying and hair loss, tooth loss, osteoporosis, nail dystrophy, oral leukoplakia, or abnormal skin pigmentation have also been associated with mutations of the telomerase complex [8]. Apparently, genetic defects affecting complicated telomerase maintenance processes can cause premature ageing and certain disorders. Better understanding of those molecular mechanisms will extend our diagnostic tools and may provide novel options for prevention and/or treatment.

Very short or unprotected telomeres induce cellular senescence and cell death, which is an important protective mechanism preventing unlimited cell growth and cancer development. In addition, in contrast to normal somatic cells, tumor cells in about 85% of solid tumors show high telomerase activity [4]. Therefore, telomerase represents a new and promising target for a more selective cancer therapy.

Different strategies have been employed to inhibit telomerase in all kinds of tumor cells [9–11]. Either the hTR or hTERT can be targeted by different molecules. Stabilized antisense oligonucleotides directed against hTR, such as peptide nucleic acids (PNAs) or RNA oligomers with methyl-substituted ribose sugar rings (2'-O-methyl-RNA) prevent telomere elongation by hybridizing to hTR. Telomerase activity can also be inhibited by direct binding of nonnucleosidic compounds to hTERT (e.g. BIBR1532 [12], TELIN [13]) or through stabilizing the so-called G-quadruplex structure of the single-stranded telomeric end by G-quadruplex-interactive compounds (e.g., BRAC19). The new approach of silencing RNA or RNA interference

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(awarded with the Nobel Prize in 2006) has also been applied for suppressing telomerase expression.

A major obstacle with all these potential anticancer agents is their safe and efficient delivery to the tumor cells and their uptake at the site of action. Whereas a covalently bond lipid residue enhances the cellular uptake of the antisense oligonucleotide GRN163L, biodegradable nanoparticles might serve as an efficient carrier system for intracellular delivery of the various telomerase inhibitors [14]. For all these agents, the “proof of principle” has been demonstrated in experiments with tumor cell lines and animal models, and with GRN163L, first clinical trials are ongoing [15].

Furthermore, as hTERT is immunogenic, it represents a suitable target for cancer immunotherapy. Initial clinical trials with multiple vaccine formulations containing peptides directed against hTERT epitopes suggest that an immunoprevention therapy for cancer might be possible [16]. For instance, GV-1001 is a biological telomerase peptide vaccine that is undergoing phase III trials [17]. However, as with most anticancer vaccines, there are some obvious discrepancies between positive immune response and tumor regression, a fact that needs more attention, not only for the development of antitelomerase vaccines.

Telomerase inhibitors do not initially affect tumor cell growth but induce progressive telomere shortening. This will result in reduced proliferation and apoptosis after a certain time delay [18]. Therefore, treatment of cancer patients with telomerase inhibitors will be a long-term treatment. This makes high demands on the profile of side effects of the drugs. Besides tumor cells, stem cells and germ cells also express telomerase activity and might be affected by telomerase inhibitors. However, as these cells have longer telomeres, and proliferation rates are in general lower compared with tumor cells, effects of treatment on these cells should be moderate [19]. In addition, the telomere length in tumor cells is variable, even within one tumor entity. For example, variability is more than three fold in chronic lymphocytic leukemia [20] and approximately nine fold in gastric cancer [21]. From a clinical perspective, it seems necessary to identify telomere length and telomerase activity before treatment with telomerase inhibitors. Preselected patients with very short telomeres in their tumor cells may benefit most from this kind of therapy. Thus, telomere length could be a valuable (predictive) biomarker for “stratified medicine” with these novel agents to ensure high response rates in such subgroups of patients.

Although there is still a long clinical way to go until effective and safe “antitelomerase” agents will be available for a more selective cancer treatment, the discoveries concerning telomerase function and maintenance have paved the way to a new drug target or targets. We still

have to learn more about the involved regulatory mechanisms (e.g., the role of p53; development of drug resistance; specificity). Nevertheless, the scientific discoveries made by the winners of the 2009 Nobel Prize in Physiology or Medicine provide a challenging example of how basic and clinical research, including biology, genetics, and pharmacology, has opened a new avenue of drug development and the potential for therapeutic progresses.

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