TELOMERASE: MUCH UNDERSTOOD, MUCH MORE REMAINS RIPE FOR DISCOVERY

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Abstract—Telomerase, the reverse transcriptase responsible for extending the ends of linear chromosomes, has been studied in depth for the past few decades. Its importance in processes from cellular lifespan to cancer has been well documented. More recently, it has been exploited as a potential therapeutic target for cancer. With the development of telomerase inhibitors deemed safe for human use, inhibition of telomerase has become a promising adjuvant therapy capable of improving the therapeutic outcomes of other drugs. It is within these studies that we now turn to find a new world of previously under-explored territory for telomerase. Telomerase inhibition has opened the door to new discoveries into how this enzyme functions and what additional effects it might be having in cellular activity and gene regulation. These new avenues of exploration have the potential to advance our understanding of molecular biology, cellular activity, and even cancer development and progression. This review will briefly highlight telomerase inhibitors in cancer therapeutics and the role they might be able to play in defining additional non-telomeric activities of telomerase.

1 Introduction

The replication of linear chromosomes has the problematic consequence of progressive loss of material. In order to ensure that genetic material of significant importance is not lost, the ends are protected by a repeat sequence known as the Telomere. Replication and maintenance of telomeres often falls to Telomerase.

Telomerase, originally discovered by Greider and Blackburn, is a ribonucleoprotein complex containing an RNA component (TERC) and a reverse transcriptase (TERT) (Greider and Blackburn 1985). Several studies have demonstrated that TERT is the rate limiting component as it is the only subunit that changes in proportion to any increases and decreases in telomerase activity (Chang et al. 2002). The activity of telomerase has been shown to be upregulated in 90% of human cancers, with hTERT expression low in normal cells, high in immortal cells, and therefore characteristic of immortalized cells. Given the differential expression of telomerase in human cancers verses non-immortalized cells, telomerase has become an attractive target for cancer therapeutics.

2 Previously Discovered Non-Telomeric Activities of Telomerase

Several activities of telomerase beyond telomere maintenance have been previously identified (Cong and Shay 2008, Jaiswal, Kumar, and Yadava 2013). These activities are not related to the lengthening of telomeres and have significant implications for the effects of telomerase inhibition. Within the nucleus, telomerase can act as a transcriptional modulator of wnt signaling (Park et al. 2009), modify chromatin structure (Masutomi et al. 2005), affect the expression of growth promoting genes (Smith, Coller, and Roberts 2003), and act within DNA damage responses (Ray et al. 2002, Sharma, Gupta, et al. 2003, Sharma, Hwang, et al. 2003). Each of these activities is not necessarily mutually exclusive. For example, wnt signaling regulates several biological processes including cell fate determination. Activated TERT has been shown to upregulate gene expression for genes related to the wnt pathway (Zhou et al. 2014). Furthermore, suppression of the wnt/β-catenin signaling cascade has been implicated in cellular senescence and directly linked to reductions in telomerase activity (Jeoung et al. 2014). It is important to note that at least one study failed to find a link between telomerase activity and wnt signaling implying possible cell type or system specificity for this interaction (Listerman, Gazzaniga, and Blackburn 2014). It is also possible that telomerase is acting through a secondary mediator or indirectly to alter this pathway. Regardless, the potential for activities of telomerase in gene regulation remain worthy of further study.

Additional support for telomerase-mediated gene regulation can be found in the additional activities for telomerase during cellular senescence. Several of these activities were identified as being linked to the gene regulation activities of TERT (Lackner et al. 2014). Cellular senescence clearly involves a multitude of telomerase activities including telomere maintenance. The actions of telomerase during immortalization and oncogenesis also likely involve a combination of activities occurring in conjunction with telomere maintenance (Cao et al. 2014). In addition to the many functions of telomerase within the nucleus, telomerase has also been shown to function outside the nucleus. Current

findings indicate that telomerase has the potential to act within the mitochondria and has been implicated in promoting apoptosis as well as being involved in gene silencing (Maida and Masutomi 2011, Santos et al. 2004). Most of these mitochondria-specific additional activities for telomerase have been linked directly to the TERT subunit.

The ability of telomerase to act outside the traditional functions of telomere maintenance to play a major role in cancer progression and development makes it a valuable target for further study as well as in the development of new cancer therapeutics. In fact, targeting telomerase activity using a variety of mechanisms as a cancer therapeutic has been attempted with mixed results. Understanding the current therapeutic applications of telomerase inhibitors and the unexpected effects of telomerase inhibition on cellular activity provides unique insight into the roles of telomerase in normal cellular function as well as oncogenesis.

3 Telomerase Inhibition and Its Implications

Table 1. Common Telomerase Inhibitors

General Category	**Name**	**Details**	**References**
G-quadruplex Stabilizing Drugs	Telomestatin and analogs	Inhibit extension of telomeres through direct binding of telomere G-quadruplexes	(Tauchi et al. 2006, Long et al. 2007, Fakhoury, Nimmo, and Autexier 2007, Tsai et al. 2009)
	TMPyP4 (meso-tetra(N-methyl-4-pyridyl) porphine)	Inhibit extension of telomeres through direct binding of telomere G-quadruplexes	(Zhuang and Yao 2013, Yaku et al. 2014, Herz et al. 2014)
	BRACO-19	Inhibit extension of telomeres through direct binding of telomere G-quadruplexes	(Gunaratnam et al. 2007, Harrison et al. 2004, Burger et al. 2005)
	Ruthenium(II) polypyridyl complexes	Induction and stabilization of G-quadruplexes; direct telomere binding	(Yu et al. 2012, Yu et al. 2014, Yu, Liu, Zhang, Yang, et al. 2013, Liao et al. 2014, Liao et al. 2015, Chen, Wu, et al. 2013, Li et al. 2014)

Nucleoside Analog	Azidothymidine (AZT)	Not well defined; direct effect on telomeres; some controversy regarding activity	(Strahl and Blackburn 1996, Gomez, Armando, and Alonso 2012, Sabokrouh et al. 2014, Zhihua et al. 2014)
Catalytic Inhibitor	BIBR1532	Non-nucleotidic small molecule; binds to the active site of hTERT	(Damm et al. 2001, Bashash et al. 2013, Bashash et al. 2012, Nakashima et al. 2013, Wardi et al. 2014)
Oligonucleotides	Imetelstat (GRN163L)	Template antagonist; competitive inhibitor that blocks the binding of chromosomal-telomere substrates to telomerase	(Goldblatt, Erickson, et al. 2009, Goldblatt, Gentry, et al. 2009, Gomez-Millan et al. 2007, Hochreiter et al. 2006, Koziel and Herbert 2015, Marian et al. 2010, Roth, Harley, and Baerlocher 2010, Joseph et al. 2010, Perez et al. 2012, Wu et al. 2012, Mender et al. 2013, Hu et al. 2014, Barszczyk et al. 2014, Brennan et al. 2010)
Other Compounds	Epigallocatechin gallate (EGCG)	A variety of catechin (a polyphenol in Green Tea)	(Chen, Landen, et al. 2013, Zhang et al. 2014)
	Curcumin	Decreases hTERT expression and translocation to the nucleus	(Kazemi-Lomedasht, Rami, and Zarghami 2013, Badrzadeh et al. 2014, Sprouse, Steding, and Herbert 2012)
	Pterostilbene	Dimethyl ether analog of resveratrol; binds active site of telomerase	(Tippani et al. 2014)
	MST-312	Chemically modified derivative of EGCG	(Seimiya et al. 2002, Serrano et al. 2011, Wong et al. 2010, Cohn et al. 2012, Yu, Liu, Zhang, Zhang, et al. 2013, Gurung et al. 2014, Wang et al. 2015)

The targets for telomerase inhibition include all of the major subunits of telomerase itself as well as key regulators of telomerase (Sprouse, Steding, and Herbert 2012). While there have been a variety of compounds developed to inhibit telomerase only a few show significant potential in cancer therapeutics (Table 1). As you can see from the references in the table, many of these compounds remain under investigation. By focusing on a few of the studies of the more promising compounds such as imetelstat (GRN163L), MST-312, and BIBR1532, this review will demonstrate that the potential for additional roles of telomerase in cell activity is clear and worthy of investigation.

3.1 Imetelstat (GRN163L)

The telomerase template antagonist imetelstat (GRN163L) has shown significant potential as both a potent inhibitor of telomerase and a therapeutic agent. It has been shown to alter cell morphology, inhibit cell growth (Hochreiter et al. 2006, Goldblatt, Erickson, et al. 2009, Goldblatt, Gentry, et al. 2009), increase sensitivity to radiation (Gomez-Millan et al. 2007), sensitize resistant cells to treatment with chemotherapeutic agents and other compounds (Goldblatt, Gentry, et al. 2009), and reduce the efficacy of cancer stem cells (Joseph et al. 2010, Koziel and Herbert 2015). These broad ranges of effects correspond with both telomere maintenance and other less defined activities of telomerase. The key differences between the non-telomeric effects of telomerase and those related to telomere shortening can be found in the short-term treatments of cells with the telomerase inhibitors.

Very few studies have attempted to identify or link the non-telomeric effects of telomerase inhibition to the activity of the telomerase inhibitor. A study on imetelstat specifically found that short-term treatment with imetelstat affected clonogenic growth in multiple myeloma (Brennan et al. 2010). In the multiple myeloma study, imetelstat was found to induce differentiation of cells and reduce the cancer stem cell population.

Both of these activities have been previously identified as potential non-telomeric activities of telomerase. Agreeing with that idea, very recently, a study in breast cancer demonstrated a similar reduction in cancer stem cell activity following treatment with imetelstat (Koziel and Herbert 2015). In their entirety, these effects were most likely the result of the combination of telomeric and non-telomeric activities of telomerase not solely the consequence of the drug. It is, however, important to note that another study indicated off-target effects of imetelstat on the cytoskeleton (Mender et al. 2013). Further investigation into whether other inhibitors of telomerase activity also affect the cytoskeleton need to be completed to declare this a true off-target effect of the drug (Mender et al. 2013). Overall, these finding implicate imetelstat as an important tool for identifying additional non-telomeric activities for telomerase in both short-term and long-term studies.

3.2 MST-312

MST-312 is a chemically modified derivative of epigallocatechin gallate (EGCG). It has been shown to have potent anti-telomerase activity in both short term and long-term assays (Seimiya et al. 2002). The short-term assays were consistent with similar

assays for imetelstat and demonstrated potent effects long before telomere shortening had reached a critical juncture. For example, MST-312 was determined to act within DNA damage pathways through ATM/pH2AX, generate double strand breaks, and induce cell cycle arrest all preceding telomere shortening (Serrano et al. 2011, Gurung et al. 2014). As discussed in the previous section for non-telomeric activities of telomerase, the role for telomerase in activities such a DNA damage response is potentially important to cellular immortalization and cancer development. Additionally, in line with other telomerase inhibitors, MST-312 was also found to sensitize cells to X-ray radiation (Wang et al. 2015). This potential to exhibit short-term and long-term effects indicates that this telomerase inhibitor has significant promise for use as a molecular tool for studying and defining non-telomeric activities of telomerase.

3.3 BIBR1532

The small, non-nucleotidic molecule, BIBR1532, non-competitively binds to the active site of hTERT successfully blocking telomerase activity. This drug acts to impair DNA elongation rather than basic template copying although the overall activity remains ill-defined (Ruden and Puri 2013). This particular drug has been shown to require longer time frames to obtain critically short telomeres, although some short-term effects have been observed (Ruden and Puri 2013). This drug did demonstrate the same benefits of combination therapy and sensitization to other therapeutic agents observed with imetelstat and MST-312 (Ward and Autexier 2005). Given the fact that some short-term benefits were observed in a drug requiring significantly longer time frames to induce critically short telomeres, this drug has perhaps the most potential as an agent for elucidating additional non-telomeric activities of telomerase. Specific short-term, cyto-toxic effects for BIBR1532 have been shown to involve p21 activity and could imply a role for telomerase in the gene regulation of these molecules (Bashash et al. 2012). Relevant to the applications of BIBR1532 in defining telomerase, one study did identify possible off-target activities for the drug (Wardi et al. 2014); however, it remains probable that those activities are, in fact, the result of telomerase inhibition.

4 Concluding Remarks

The short term effects of telomerase inhibitors provide valuable insights into the more complicated features of telomerase within both normal and immortalized cell populations. Although many of the potential activities of telomerase not related to telomere maintenance have been explored, much remains to be elucidated regarding these known activities. These studies collectively demonstrate that the anti-cancer effects of telomerase inhibition are relatively consistent across multiple inhibitors and likely involve a combination of telomeric and non-telomeric activities of telomerase. Studies better linking the known non-telomeric effects of telomerase with the potent anti-cancer effects of telomerase inhibition are required to fully understand the complicated relationship between telomerase activity and oncogenesis. New roles and functions for telomerase in cells may also be found from observing the actions of telomerase inhibitors in the variety of cancer types currently under investigation. Further investigation including comparative analysis of the effects of the inhibitors is warranted to fully define where drug

activities and telomerase activities begin and end.

5 Acknowledgments

The authors would like to acknowledge The Center for Genomic Advocacy and the Unbounded Possibilities Initiative at Indiana State University for its support. They would also like to acknowledge Dr. Brittney-Shea Herbert at Indiana University School of Medicine for her contributions to student training and professional development.

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