Telomerase Reverse Transcriptase in Leukemia

Subjects: Hematology
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Telomerase reverse transcriptase (TERT) has been established to possess diagnostic value in leukemia as most adult cells do not express high levels of telomerase. Indeed, studies have shown that prognosis is not favorable in patients who have leukemias expressing high levels of telomerase. Recent research has indicated that targeting of this gene is able to control the survival of malignant cells and therefore offers a potential treatment for TERT-dependent leukemias.

hTERT leukemia gene regulation cancer hematological malignancy

1. Introduction

The human telomerase reverse transcriptase gene (*hTERT*) spans over a 40 kb DNA region and consists of 16 exons and 15 introns [1]. This gene produces a polypeptide with a length of 1132 amino acids which is then transformed to a 130kD functional TERT protein [2]. The four critical functional domains in TERT comprise the N-terminal regulatory domain, the RNA binding domain, the reverse transcriptase domain, and the C-terminal dimerization domain [3]. Owing to its complexity, *TERT* is regulated at various levels which include transcriptional, post-transcriptional, and post-translational mechanisms [4][5][6].

The primary function of this protein is in the maintenance of telomere length. Telomeres cap the ends of chromosomes with repetitive 5′-TTAGGG-3′ conserved sequences to maintain the integrity of genetic information [7]. With each successive round of cell proliferation, 50 to 200 base pairs of DNA material are dissociated from the ends of the chromosomes due to the loss of RNA primers situated on the lagging strand of Okazaki fragments. This phenomenon leads to the progressive shortening of telomeres which limits the potential of somatic cells to divide [8][9]. Certain cell types, such as stem cells and cancer cells, are able to express telomerase to replenish telomeric ends, thereby permitting continuous self-renewal [10][11][12]. Telomerase exerts its DNA polymerizing function through the formation of a complex consisting of two main subunits: the catalytic protein hTERT and the human telomerase RNA component (hTERC) subunit [13].

2. Transcriptional Regulation of *TERT*

The promoter of TERT is a very dynamic region. It is GC rich and lacks the TATA or CCAAT recognition sequence for the transcription start site (TSS) location by RNA polymerase 11. However, this is compensated for by an initiator like CCTCTCC sequence. It also contains two E boxes for Myc binding, several GC boxes for Sp1 binding,

and a CCAC box [6]. These motifs make the promoter region an excellent hub for the binding of various repressors and activators [5][6].

2.1. Negative Transcriptional Regulators of TERT

Negative transcriptional regulators of *TERT* can either bind directly to the promoter or interact by forming complexes to downregulate *TERT* expression. Direct acting repressors include retinoblastoma (RB), myeloid-specific zinc finger protein (MZF2), activator protein 1 (AP1), Wilms tumor 1 (WT1), and menin [14][15][16]. Transcriptional repressors exert their effect on *TERT* expression by forming complexes and interacting with other proteins including Mad1, the HTLV-1 oncogene TAX, tumor suppressors BRCA1/Nmi and p53, interferon regulatory factor 1 (IRF1), and transforming growth factor β (TGFβ) [17][18][19][20][21]. Mad1 has been identified to form a complex with Max (Mad1/Max) and binds to the E box region located at the promoter to prevent *TERT* expression [17][22][23]. Similarly, it was found that TAX also represses *TERT* expression by E-box binding [18]. Receptor Ck, on the other hand, has been determined to regulate *TERT* via interactions with protein kinase C [24].

2.2. Positive Transcriptional Regulators of TERT

Positive transcriptional regulators also function via direct and indirect interactions with the promoter region to activate *TERT* expression. Positive regulators that act directly include human N-acetyltransferase like protein (hALP), transcriptional elements interacting factor (TEIF), signal transducer and activation of transcription 3 (STAT3), the oncoproteins Ews-ETS, hypoxia inducible factor 1 (HIF1α), and the transcription factor c-Jun [5]. Activators that act indirectly include the c-Myc oncogene, the apoptosis inhibitor survivin, and the viral oncogene E6/NFX1. The oncogene c-Myc activates *TERT* expression by forming a complex with max (c-Myc/Max) and binding to the E-box region whilst survivin acts via SP1/c-Myc binding. The viral oncogene E6/NFX1 on the other hand induces *TERT* expression by p53 degradation, activation of c-Myc, and disruption of upstream stimulatory factor (USF) based repression [25][26][27].

2.3. Conditional Transcriptional Regulators of TERT

There are some regulators, such as transcription factors E2F and specificity protein 1 (Sp1), upstream stimulatory factors (USF1/2), and tumor suppressor p73, which act as both repressors and activators of *TERT* expression depending on the condition. E2F acts as a *TERT* repressor in cancer cells; however, the condition is reversed in normal cells where it activates the expression of *TERT*. This transcription factor controls *TERT* regulation by binding to the GC box region on the promoter [28][29]. USF1/2 on the other hand acts as a repressor in normal cells and activator in cancer cells. This is achieved by E box binding on the promoter [30][31].

3. Post Transcriptional Regulation of *TERT*

TERT Regulation by microRNAs

The mechanisms associated with miRNA biogenesis and its influence on the *hTERT* gene have made substantial progress as evidenced by recently published studies. It has been reported that miRNAs can post-transcriptionally alter *hTERT* transcripts directly or indirectly [13][32]. MiRNAs can directly bind to conserved *hTERT* complementary sites located in the 3′-untranslated region (3′UTR) to restrict *hTERT* expression by negatively regulating their translation [33][34][35]. Specifically, expression of specific miRNAs have been shown to exert changes in tumor cell proliferation, cell cycle control, apoptosis, metastatic invasion, and protein expression levels [36][37]. Each miRNA has the potential to mediate the expression of their target genes. miR-1182 and miR-491-5p are two miRNAs that directly target the transcript of *hTERT* [38][39]. These interactions have been confirmed via luciferase assay. Further, it has been elucidated that miR-491-5p is involved in the regulation of the *PI3K*/*AKT* signaling pathway. Therefore, together these epigenetic gene regulators influence hTERT gene expression in cancers. In contrast, miRNAs may also indirectly target the transcription factors involved in *hTERT* regulation [13]; however, the precise molecular mechanisms that underlie post-transcriptional repression are still unclear.

4. Post Translational Regulation of *TERT*

Post-translational modifications of hTERT could modify protein stability, subcellular localization, and ultimately, enzyme activity [36]. To date, studies have identified that post-translational regulation of hTERT mainly involve phosphorylation and ubiquitination by kinases and ligases respectively [4][36][40]. Both phosphorylation and ubiquitination give rise to prominent effects by positively and negatively regulating hTERT activity [41].

In phosphorylation, kinases such as protein phosphatase 2A (PP2A) interact with c-Abl tyrosine kinase protein, leading to a threefold reduction of telomerase activity by dephosphorylation [36][42][43]. On the contrary, protein kinase B (an AKT kinase) increases telomerase activity through TERT phosphorylation-dependent activation of the PI3K/Akt/mTOR pathway [42][43]. Similar to protein kinase C, they phosphorylate hTERT by PKC isoenzymes α, β, δ, ε, and ζ to enhance telomerase activity, which is increased along with nuclear accumulation of hTERT [33][40][43][44][45]. In addition, kinases including kinase interacting protein (KIP), c-jun, and mitogen activated protein kinase (MAPK) also regulate post-translational modifications of hTERT. KIP regulation involves interactions with the upstream kinase domain of DNA–PKcs to improve telomerase activity in human cells, while, MAPK upregulate hTERT in a serum and pH-dependent manner within the hypoxic environment of solid tumors [5][46].

5. TERT Dysregulation in Leukemias

Studies on the various forms of leukemia have disclosed the dysregulation of *TERT* gravely affects the prognosis of the disease and is known to exert its mechanism of action via a plethora of modifications including epigenetics, mutations, amplifications, structural variants, and influences on oncogenes. The prevalence of *TERT* dysregulation remains to be determined in each subtype of leukemia and is also highly dependent on the population and recruitment criteria of each study. An early study has indicated that it affects approximately half of AML (53.3%) patients [47], while another report indicated that *TERT* dysregulation is present in all acute promyelocytic leukemia (APL, AML-M3) patients in their cohort [48]. Other studies have shown that *hTERT* dysregulation was only observed

in limited cases of relapsed childhood ALL [49], while a study in Saudi Arabia disclosed that *hTERT* mutations are not linked to predisposition to childhood acute leukemia [50]. In terms of relevance in disease pathogenesis, studies have shown that in adult T-cell leukemia/lymphoma (ATLL), the activation of telomerase is required for the disease development and progression, while in AML and CML it is only required for maintenance and not initiation [51][52][53].

5.1. Chronic Myeloid Leukemia (CML)

Mechanism of *TERT* dysregulation was reported to occur via *TERT* promoter methylation. However, contrary to canonical research, DNA methylation would increase TERT activity, as methylation occurs on the genomic region bordering the *TERT* promoter. This results in the inaccessibility of repressor on this site, which is proximal to the *TERT* promoter, thereby increasing the affinity of *TERT* promoter binding of crucial activators leading to *TERT* hyper-expression (**Figure 1**A).

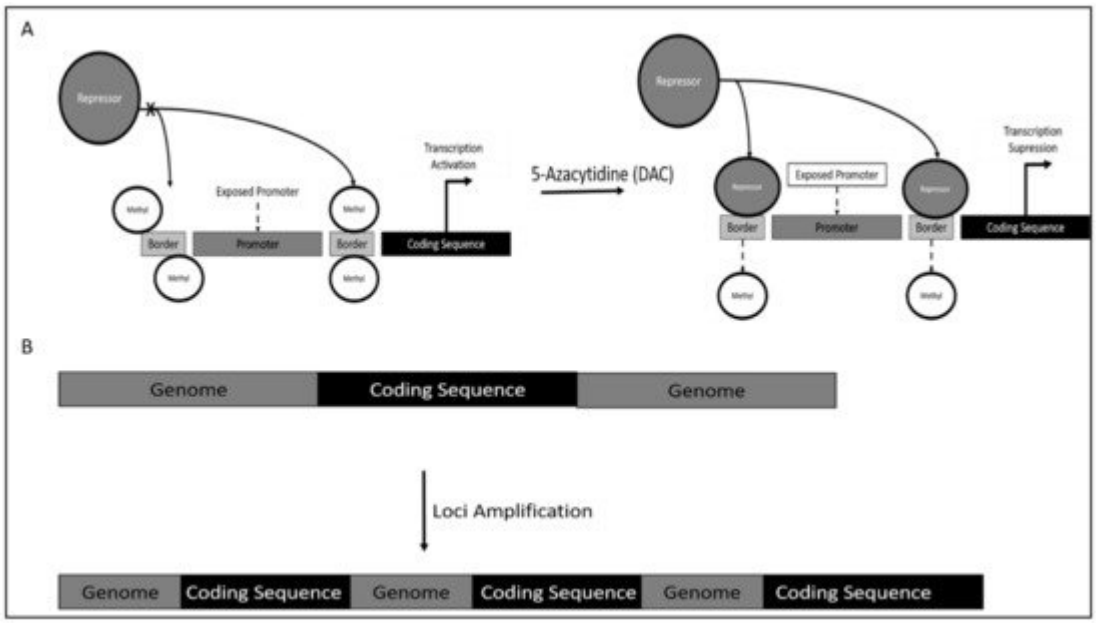

Figure 1. (**A**) Epigenetic regulation of *TERT* via promoter border region methylation. (**B**) Loci amplification of *TERT* gene resulting in an increase in copy number.

Dysregulation of *TERT* also occur via gene amplification, which results in an increase in gene copy number (GCP). This phenomenon translates to increased gene expression levels due to relatively higher abundance of mRNA resulting from amplification (**Figure 1**B). It was observed that TERT catalytic components were amplified in patients with CML. Interestingly, TERC amplification does not correlate with resistance towards imatinib mesylate, although the copy number was calculated to be above 3 [54].

The CML fusion oncogene *BCR-ABL* was also found to contribute to the overall activity of TERT in aiding disease progression.

TERT was targeted indirectly through upstream factors crucial for *TERT* activation via inhibition of kinase activity. Imatinib mesylate (IM) is currently one of the major drugs used in CML treatment. However, treatment of CML

using IM was found to potentiate CML towards leukemogenesis as it was reported that it enhances expression of *hTERT* via phosphorylation of STAT5 [55] (**Figure 2**).

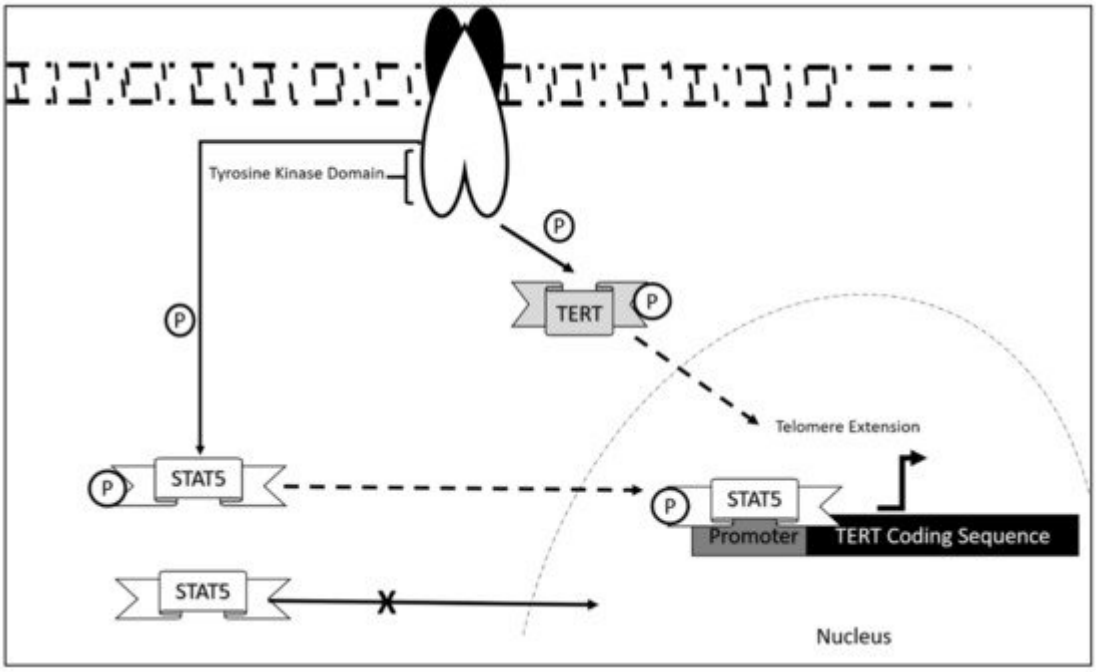

Figure 2. BCR-ABL control of TERT activity and expression generally targeted via Imatinib Mesylate or Tyrosine Kinase Inhibitor treatment.

5.2. Chronic Lymphoid Leukemia (CLL)

The mechanism of dysregulation of *TERT* in CLL is still largely unexplored. However, correlation studies report that telomeric length serves as an initial prognostic marker in CLL disease progression [56]. This was also associated with telomerase/telomere associated proteins expression, which also contribute as markers in the diagnosis of CLL onset. Research showed that significant decrease in telomeric length was observed in chromosomes 13p, 12, 11p, and 17p in which hypomethylation was also present. These aberrations were significant in CLL patients as compared to normal controls. Upregulation of telomerase/telomere-associated proteins was also observed in patients at early disease onset [57]. As in the case of most leukemia, *TERT* activation occurs via STAT5 mediated binding and its phosphorylation state [58][59][60]. This mechanism, although not studied extensively in CLL, may be a contributing mechanism as to how CLL progress in addition to the upregulated telomerase/telomere-associated proteins and telomeric length dysregulation.

Furthermore, in terms of DNA methylation status it was found that methylation of *TERT* promoter was crucial in different stages of leukemia development as it confers different functional advantages.

Dysregulation of *TERT* in CLL was also observed via single nucleotide polymorphisms (SNPs). Different SNPS led to different potential in *TERT* induced self-renewal [61].

Additionally, variation within the *TERT* and *TERT C* gene also contributed to an increase in telomeric ends. It was found that *TERTC* rs10936599 SNP with C allele had a significant association with CLL development as high allele frequency was observed in CLL patients.

Chromosomal aberrations were also found to contribute to *TERT* dysregulation in CLL. This includes the *TERT/CLPTM1L* locus in chromosome 5p15 and t(5,14) translocation, which resulted in the fusion of *TERT* to the *IGH* gene [62]. The *IGH* locus, which contains strong promoters, may have enhanced *TERT* activation as a significant increase in *TERT* expression was observed in CLL harboring this translocation [63]. Besides that, it was also found that deletion on chromosome 13 at locus q14 (13q14del) also contributed to better prognosis in patients. This locus contains the coding sequence for miR-15a/16-1 cluster which target *TERT* suppressor p53 [64][65]. The mechanism is illustrated in **Figure 3**.

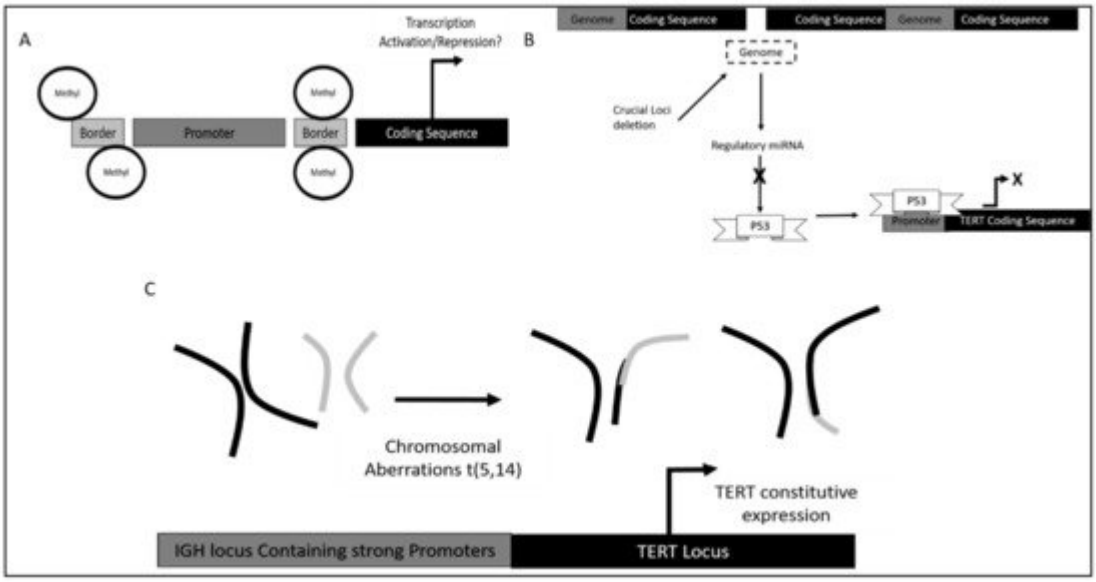

Figure 3. Control of TERT activity and expression occurring in CLL. (**A**) Promoter methylation status; (**B**) chromosomal deletion containing loci encoding for transcription factor suppressors; (**C**) chromosomal aberrations resulting in fusion of critical cis-regulatory elements with *TERT* coding sequence.

5.3. TERT Dysregulation and Clinical Implications in Acute Leukemias

Acute leukemia refers to an aggressive form of leukemia that typically progresses rapidly over a short period of time. It can be further subdivided into acute myeloid (AML) and acute lymphoid leukemia (ALL) [66]. *TERT* dysregulation that has been identified as one of the contributors to the pathogenesis of leukemia and has been reported in both AML and ALL.

5.3.1. *TERT* Dysregulation and Clinical Implications in Acute Myeloid Leukemia

Epigenetic regulation of *TERT* has been the focus of many studies as it is believed to aid in the malignant transformation of cells [32]. Hypermethylation of the *TERT* promoter results in TERT transcription activation that subsequently leads to higher telomerase activity [67]. According to a study by Zhao et al. (2016), *TERT* proximal

promoter and a partial exon 1 (TERTpro/Ex1) region exhibits CpG site hypermethylation in AML cell lines and primary blasts [68]. This distinct methylation profile could serve as a prognostic factor in AML as it is associated with shorter overall survival and drug resistance. Apart from the above regulation, *TERT* is also regulated by oncogenic signaling FLT3-ITD (FMS-Like Tyrosine Kinase 3 Internal Tandem Duplication). FLT3, a type of receptor tyrosine kinase is often mutated in AML with internal tandem duplications within the juxtamembrane domain. Compelling evidence has implicated the role of FLT3-ITD mutation in leukemogenesis especially in the case of AML [69]. Recently, it has come to light that FLT3-ITD mutation could induce the expression of *TERT*, resulting in increased telomerase activity that contributes to AML pathogenesis [70]. Another method of telomeric elongation identified in promyelocytic leukemia (PML) (a form of AML) is alternative lengthening of telomeres (ALT) [71].

Conventional chemotherapy is one of the therapeutic approaches used to treat AML patients, whereby it is common that a combination of chemotherapeutic drugs is used. Each of the chemotherapeutic drug aims to target and inhibit the cancer cells via various mechanisms. One of the mechanisms targeted by a commonly used drug for AML, 5-azacytidine (5-AZA) is DNA methylation. 5-AZA, a DNA methyltransferase inhibitor (DNMTI), has been shown to downregulate *TERT* expression and decrease telomerase activity in AML patient samples and cell lines [72].

TERT-targeted immunotherapy has also become the subject of many cancer therapeutic studies. A study by Sandri et al. (2017) demonstrated that TERT865-873-specific, TCR-engineered T-cell lymphocytes were able to halt AML progression in vivo [73]. The main idea behind this therapy, known as adoptive T-cell therapy (ACT), is to increase the affinity and specificity of T-cell receptor (TCR) of T cells to tumor associated antigen (TAA). It is rather interesting to note that TERT865-873-specific, TCR-engineered T-cell lymphocytes were able to distinguish between AML blasts and peripheral blood mononuclear cells (PBMC), indicating its high cell recognition specificity.

5.3.2. *TERT* Dysregulation and Clinical Implications in Acute Lymphoblastic Leukemia

TERT-targeted therapies are quickly gaining popularity as a therapeutic option due to the role of TERT as a universal tumor associated antigen (TAA). BIBR1532, a small biological molecule specific for telomerase inhibition, has been studied by Bashash et al. (2017) for its application in pre-B ALL [74]. BIBR1532 was able to suppress *TERT* expression and telomerase activity in vitro in a dose-dependent manner. In addition, BIBR1532 suppressed the expression of c-Myc that acts as a transcriptional activator of *TERT*. Doxorubicin, a commonly used chemotherapeutic drug, was found to exert the same inhibitory action as BIBR1532 on *TERT* and *c-Myc*. This study also reported that the combination of doxorubicin together with BIBR1532 showed a greater inhibition of *TERT* and *c-Myc* transcription as compared to its individual counterparts. Thus, this combination therapy can be an effective therapeutic approach for pre-B ALL.

6. Conclusions and Future Perspectives

In summary, many preclinical and clinical studies on leukemia have concluded that TERT plays a pivotal role in disease progression and deteriorating prognosis of patients. Studies aimed at determining the factors influencing

TERT expression during leukemogenesis and the stage where this gene is upregulated has contributed immensely to the understanding of the fundamental roles of TERT in various forms of leukemias. Additionally, it has also been proven that different mechanisms are involved in the expression of this gene including transcriptional, genetic, and epigenetic regulators. These mechanisms of activation and dysregulation of *TERT* in onco-hematological diseases are progressively being elucidated and in recent years, researchers have deciphered many novel variants, modifications, pathways, and mediators leading to its overexpression. The latest findings have identified mutations in the *hTERT* gene, methylation of its promoter, and microRNAs targeting its transcript as leading areas of fundamental studies and highly promising for therapeutic development. Indeed, progress in clinical trials have determined that chemical drugs and biomolecules targeting various regulators of *TERT* have immense potential in controlling the proliferation and metastasis of leukemias, primarily via arrest of cellular division. Noteworthy, in recent years there have been renewed interests in targeting hTERT as a therapeutic strategy due to developments in several areas including immunotherapies, drug discovery, gene therapy, and radiotherapy. Combination of agents specific to TERT and other anti-cancer drugs have also been widely explored with many studies indicating synergistic effects, reduced dose requirements and decreased side effects. The survival outcomes of leukemic patients have improved in recent years, and it is expected that therapies targeting TERT and their combinations with other anti-cancer agents could further improve the prognosis of patients especially those with TERT-dependent leukemias.

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