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REVIEW ARTICLE

DNA Methylation Profiling of hTERT Gene Alongside with the Telomere Performance in Gastric Adenocarcinoma

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Abstract

Purpose Epigenetic modification including of DNA methylation, histone acetylation, histone methylation, histon phosphorylation and non-coding RNA can impress the gene expression and genomic stability and cause different types of malignancies and also main human disorder. Conspicuously, the epigenetic alteration special DNA methylation controls telomere length, telomerase activity and also function of different genes particularly hTERT expression. Telomeres are important in increasing the lifespan, health, aging, and the development and progression of some diseases like cancer.

Methods This review provides an assessment of the epigenetic alterations of telomeres, telomerase and repression of its catalytic subunit, hTERT and function of long non-coding RNAs such as telomeric-repeat containing RNA (TERRA) in carcinogenesis and tumorgenesis of gastric cancer.

Results hTERT expression is essential and indispensable in telomerase activation through immortality and malignancies and also plays an important role in maintaining telomere length. Telomeres and telomerase have been implicated in regulating epigenetic factors influencing certain gene expression. Correspondingly, these changes in the sub telomere and telomere regions are affected by the shortening of telomere length and increased telomerase activity and hTERT gene expression have been observed in many cancers, remarkably in gastric cancer.

Conclusion Epigenetic alteration and regulation of hTERT gene expression are critical in controlling telomerase activity and its expression.

Keywords Epigenetic · Telomere · Telomerase · Human telomerase reverse transcriptase (hTERT) · Telomeric repeat-containing RNA (TERRA)

Abbreviations

GC	Gastric cancer
HDGC	Hereditary diffuse gastric cancer
FAP	Familial adenomatous polyposis
DNMTs	DNA methyltransferases
MBDs	Methyl-CpG binding domain proteins
HMT	Histone methyltransferases
HDMs	Histone demethylases
HATs	Histone acetyltransferases
HDACs	Histone deacetylases
HDACI	Histone deacetylation inhibitor
ROS	Reactive oxygen species
TRF	Telomere Restriction Fragment Assay
aTL	Absolute telomere length
ALT	Alternative lengthening of telomeres
TSA	Trichostatin A
TERRA	Telomeric repeat-containing RNA
hTERT	Human telomerase reverse transcriptase
HP1a	Heterochromatin protein 1
siRNA	Small interfering RNA

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Introduction

Molecular Pathogenesis of GC (Gastric Cancer)

GC may continue to have a detrimental effect on global health as a result of aggressive disease. In spite of the general decline in incidence during the last several decades, GC is the fourth most common cancer and the second leading cause of cancer-related death worldwide [1].

From a histological point of view, this cancer is divided into different types: diffuse and intestinal and into cardia and non-cardia cancer according to its anatomic location. The distinction for cardio versus non-cardia disease is essential since there is evidence that both entities have different etiologies and because several reports indicate that gastric cardia cancer and gastro-esophageal junction tumor are increasing in incidence, and there is a similar increased incidence in non-cardia cases in younger western populations. Generally, the less developed nations carry a better GC disease burden than developed countries [2]. Within all afflicted nations, non-cardia GC is more likely to affect persons in lower socioeconomic groups. Similarly, the threat of *H. pylori* infection is related to lower socioeconomic status, overcrowding, and unsanitary conditions. Interestingly, health and education have an inverse relationship with non-cardia tumors but are related to cardiac GC [3]. The impact of environmental factors is further supported by the truth that first-generation migrants originating from the countries of the high incidence in a country of low incidence maintain the chance of the country of origin [4, 5]. GC is classified as a multifactorial disease. If GC diagnosed at an early stage, the chance of treatment is very high. The incidence rate in men is double that of women, and the incidence increases with age. Early GC is restricted to mucosa and submucosa irrespective of participation of the lymph node, and the sole curative therapy is surgical resection. As regards, Symptoms of GC appear too late; identifying and adequately managing the participating risk factors at each carcinogenic phase could decrease GC occurrence [6]. The cornerstone of therapy is surgical resection with adjuvant chemotherapy or chemoradiation in appropriate cases. This kind of approach has resulted in improved survival. Unfortunately, the treatment of advanced or metastatic GC has seen little progress and median overall survival (OS) in this group remains [7]. Infectious, environmental, genetic, and epigenetic factors have been identified as its risk factors. The most important environmental risk factors for GC include the role of Helicobacter pylori infection, lifestyle, and diet. Genetic factors include mutations and polymorphisms which play a key role in this cancer [8]. GC can be sporadic or hereditary. Almost 10% of this cancer is hereditary, but only 1 to 3% of GC are caused by hereditary syndromes such as HDGC, FAP, and Lynch. GC associated with HDGC is an autosomal dominant disease, with approximately 30% of patients having a mutation in one of the Cadherin-E or CDH1 tumor suppressor genes [9].

Epigenetic Mechanisms and their Role in GC

GC is the result of genetic and epigenetic changes in tumor suppressor genes, repaired genes, and cell adhesion molecules. Many proofs proposed that epigenetic alterations act an important role in GC [10]. The word epigenetics was used for the first time in 1942 by Conrad Waddington with the combination of epigenesis and genetics. At that time, the physical nature of the genes and their role in inheritance had not yet been determined. Therefore, Waddington used this term as a model to describe the interaction of genes with environmental factors to produce a phenotype. Epigenetics generally refers to heritable variations in gene expression and changes structure in chromatin that occur without alteration in DNA nucleotides [11, 12]. Robin Holliday describes epigenetics as the study of transient control mechanisms of gene activity through the evolution of the whole organism [13]. A few years later, Arthur Riggs et al. provided a more detailed description. Epigenetics means studying changes in gene function that can be inherited through the dividing of meiosis or mitosis without altering the DNA sequence [14].

DNA Methylation

DNA methylation usually occurs at the CPG islands, which are located within or near the gene promoter and cause the genes and non-coding genomic regions to be silenced. More than 70% of the genes have these islands. The CpG islands are called CG rich nucleotide sequences of between 200 and 500 bp in length. DNA methylation is one of the most important epigenetic mechanisms involved in developmental processes including transcription, inactivation of X-chromosome, embryonic development, genomic imprinting, germline and somatic cell development, and transposable element silencing [15]. Methylation is usually carried out by DNMTs enzymes. Significant increases in the expression of DNMTs seem to be a common feature of all cancers [16]. There are different main types of DNMTs: DNMT1, DNMT3A, DNMT3B, and DNMT3L. DNMT1 is responsible for preserving methylation after DNA replication, whereas DNMT3A and DNMT3B are required in methylation [17]. DNMT3L is an inactive regulatory protein for methyl transfer that is associated with gene suppression independent of DNA methylation [18]. Methylation patterns appear during the embryonic and are inherited through mitosis. DNA hypomethylation and hypermethylation are both responsible for the progress of cancer. Hypermethylation of CPG islands through methyltransferase enzyme induces gene silencing and inhibits transcription. Under regular conditions, CPG islands are usually unmethylated in normal cells. However, in tumoral cells, these islands are hypermethylated. This change occurs with the silencing of tumor suppressor genes, thus the pattern of abnormal methylation at the promoter site is associated with the process of malignant cells [19, 20]. Previous studies have shown that

fluctuations in methyltransferase expression and activity are involved in many diseases, especially cancer, whereas hypermethylation has been observed in all cancers. In the development of cancer, three mechanisms of DNA hypomethylation including increased genomic instability, reactivation of transposable elements, and loss of imprinting were proposed. Hypermethylation of CPG island in tumor suppressor genes, genes involved in the cell cycle, DNA repair, carcinogen metabolism, intercellular interactions, apoptosis, and angiogenesis promote cancer progression [21]. Methylation of DNA through various mechanisms can inhibit gene expression, including MBDs proteins, the members of the MBD family also assembling histone, and chromatin-modifying complexes at the methylation sites. DNA methylation can also directly inhibit gene expression by preventing the bind of specific proteins to their target sites [22].

Histone Modification

Along with DNA structural changes, histone modification plays an important role in epigenetic regulation. Histone modification is a post-translation modification that occurs mainly on the N-terminal tails of the histones which include methylation, acetylation, phosphorylation, carbonylation, ribosylation, glycosylation, sumoylation, and ubiquitination [23, 24]. The most significant histone modification related to activation of gene expression is acetylation and methylation [25]. Histone modification is accomplished by histone methyltransferases (HMT), histone demethylases (HDMs), histone acetyltransferases (HATs), and histone deacetylases (HDACs). The eukaryotic chromosome is consists of approximately 146 bp DNA bound in a histone octamer, composed of two of each protein H2A, H2B, H3, and H4 histones known as the nucleosome [26]. In many cancers, the genomic alterations in the patterns of CpG methylation result in patterns of histone modification. However, the alterations in patterns of histone modification directly related to the progression of cancer [21]. lymphovascular invasion and tumor of GC interact favorably with H3K9 trimethylation. Histone acetylation happens by activation of transcription at the lysine residues in the N-terminal. Accordingly, diffuse histology or differentiated was consistent of histone acetylation [27].

Histone Acetylation and Deacetylation Regulation of histone acetylation performed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) [28]. Histone acetylation modulates DNA function in two main ways: first, acetylation of lysines and subsequent neutralizing the positive charge of the histone reduces the interaction between histones and DNA and result in altered chromatin remodeling, and second, lysine modification acts as anchors for binding proteins, thus leading to the application of transcription factors and chromatin-modifying proteins [29]. HDAC inhibitors induce differentiation, cell cycle relaxation, and apoptosis in malignant cells through the production of ROS. These inhibitors activate both the intrinsic and extrinsic pathways of apoptosis; regulate tumor suppressor activity of p53 and p73, which are important in the induction of apoptosis, also target DNA repair enzymes [30]. By neutralizing the positive charge of chromosome components, HATs promote gene expression. HDACs promote condensation of chromatin and silencing the gene expression [31]. Overexpression of HDACs is observed in blood malignancies and solid tumors such as breast, prostate, and colorectal cancer. Increased expression of HDAC1 and HDAC2 genes has also been observed in GC. The promoters of tumor suppressor genes enhanced with H3K4me3 and H4 acetylation, whereas suppressive marker, such as H3K27me3, H3K9me2, and H4K16Ac, are in normal cells, whereas tumor cells lose all acetylation and H3K27me3 and H3K9me. Inhibition of some genes expression such as p16, MLH1, and E-cadherin (CDH1) by histone modification suggests that histone acetylation plays an important role in GC [32]. Histone acetylation is correlated with TNM stage, lymph node metastasis, and invasion of GC [33].

Histone Methylation The other histone modification involves the methylation of histone lysine and arginine residues. Histone methyltransferase (HMT) and histone-modifying enzymes catalyze this methylation [30]. Transcriptional results will be different depending on the position and type of histone. For example, methylation of H3K9, H3K27, and H4K20 is involved in heterochromatin formation and transcriptional repression, whereas H3K4, H3K36, and H3K79 methylation is associated with transcriptionally active regions. H3K9 methylation has been identified as a distinct prognostic factor for GC. The acetylation of lys-9 residues on H3 also provides to inhibit the tumor suppressor gene in GC [34, 35].

Chromatin Remodeling

Chromatin remodeling performance as an epigenetic marker in many cancers depends on ATP-dependent chromatin-remodeling enzyme, which contains SWI/SNF, ISWI, CHD, INO80, and SWR1 families. Gene expression, chromosome separation, replication, and repair of DNA, processes of development, apoptosis, and pluripotency are the essential roles of chromatin remodeling [36]. HLTF methylation as SWI/SNF homolog has been recognized in many GC patients. Consequently, these potential epigenetic alterations may be useful biomarkers for early GC diagnosis and prevention [37].

Telomere Length in GC

Telomeres are repeated nucleotide (TTAGGG) sequences that protect the end of chromosomes. Since telomere's length is gradually shortened in each cell division, telomeres are considered to be a key factor in cell aging. In humans, the length of telomeres is approximately 5 to 15 kb [38]. Human

telomeres bind to a protein complex called "shelterin", which contains telomere repeat binding factor 1 (TRF1) and telomere repeat binding factor 2 (TRF2) that can directly bind to double-stranded telomeric repeats, repressor/activator protein 1 (RAP1) bind to TRF2, TRF1-interacting nuclear protein 2 (TIN2) bind to TRF1, TRF2, and TPP1-POT1 complex, TIN2-interacting protein 1 (TPP1) bind to POT1, and protection of telomeres 1 (POT1) interact as a heterodimer with the single-stranded 3′overhang [39–42]. The performance of the shelterin gene mutations, like upregulation of TRF1 and TRF2, has been proven in GC [43].

Epigenetic alterations are a significant mechanism for exactly controlling telomere length at the telomeric level of chromatin. Environmental changes through telomerase activity and stability affect telomere length maintenance. Epigenetics can influence the deregulation of telomere length, indicating that epigenetic elements are related to telomere length maintenance and age-related diseases [44].

Any defects in telomere capping lead to cell cycle arrest and destruction of chromosome ends. Telomere shortening contributes to chromosome instability and inhibition of cellular senescence, which may lead to cancer progression. The risk of cancer is high in patients who have germline problems in telomere biology. Therefore, people with short telomeres are biologically possible to have an increased cancer risk compared with persons having longer telomeres. Some reports suggest that telomere shortening has been implicated in some cancers, including gastric, esophageal, renal, head, and neck, ovarian cancer. Study findings in non-Hodgkin's lymphoma, colorectal, lung, and breast cancer were inconsistent. However, single reports on prostate, endometrial, and skin cancers found no associations [45, 46].

The interesting discovery is telomere shortening in relation to several health problems and may be altered in response to environmental exposures and has underscored the requirement for methods to correctly and regularly quantify telomere length. Polymerase chain reaction-based techniques (qPCR) is one of these methods used in many studies. Cawthon's initial qPCR method is the method applied most regularly by investigators. The total amount of the telomere amplification product (T) to that of a single-copy gene (S) with amplification of the telomere and single gene process in distinct wells telomere length is measured. The T/S frequency is then determined to produce an average telomere length associated value. Nevertheless, measurement accuracy may be reduced due to unavoidable limitations in measurement. In order to improve this problem, monochrome multiplex quantitative PCR was used to amplify the telomeric and single-copy DNA areas from the same tube [47]. Nonetheless, another adaptation of the essential qPCR-based method is aTL qPCR method, which is completed using a process comparable to that of the first qPCR assay but has the adaptation of using a standard curve of identified telomere length [48].

A method called TRF can indirectly measure the telomere length. This method by using the terminal restriction fragment lengths measured the telomere length heterogeneity in cell culture. Just like PCR-based methods, the TRF assay demands DNA with high quality. PCR-based methods are less expensive and convenience for high-performance testing than other methods. PCR-based methods have become a technique for estimating telomere length as a result of relatively low price, amenability for high-throughput testing, and general ease of investigators access to the required equipment used in the assay [49, 50].

Epigenetic of Telomeres

Due to end-replication problem, the ends of chromosomes are not suitable to be completely replicated during replication, and RNA polymerase is unable to synthesize parts of the chromosome end such as the okazaki fragment because of removing the RNA primer from the end and lead to telomere shortening after each replication in somatic cells [51]. There are two mechanisms involved in the maintenance of telomeres length. After each cell division, telomerase adds telomeric repeats at the end of chromosomes and in this way prevents the end-replication problem. ALT is another way used to elongation of telomeres through homologous recombination [52]. In addition, much evidence suggests that genetic and epigenetic changes play an important role in telomere length regulation [53–55]. Epigenetic regulatory mechanisms are contained histone modification, DNA methylation, histone acetylation noncoding RNA, and more [56].

Histone modifications are associated with the role of telomere and subdomain chromatin in maintaining telomere length. On the other hand, the results of chromosome segregation failures eventually lead to tumor progression [56, 57]. Obviously, the absence of histone modifications in heterochromatin include HMTases (SUV39H1 and SUV39H2) that show reduce tri-methylated H3K9 levels and HMTases (SUV39H1 and SUV39H2) with three members of retinoblastoma (RB) family that show reduce of H4K20 trimethylation levels associated with elongation of telomere length [58, 59]. Subtelomeric and telomeric are identified by low levels of acetylated H3 (AcH3) and H4 (AcH4) as other histone modifications [60].

DNA methylation is a significant chromatin modification in mammals and plays an important function in transcriptional regulation and telomere length. DNA methylation happens in recurrent genomic regions of CpG that are methylation-susceptible of DNMT (such as DNMT1, DNMT3a, and DNMT3b). DNA methylation directly through the binding of transcription factors and indirectly through methyl-CpG-binding proteins leads to suppression of transcription (Fig. 1, Table 1) [61].

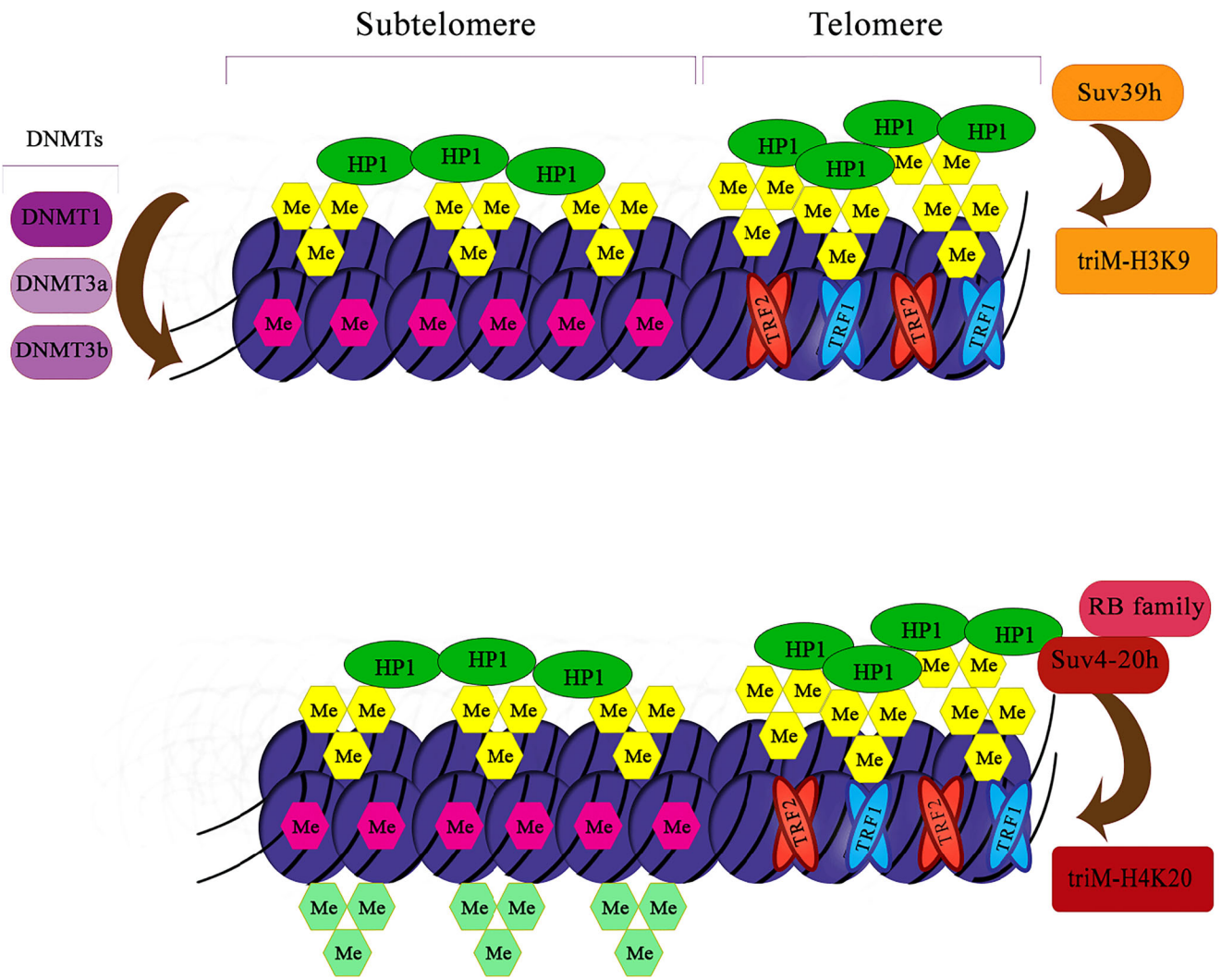

Fig. 1 Epigenetic modifications at telomeric and subtelomeric regions

Hypomethylation and CpG island hypermethylation change through aging to have their influence on cell senescence over the effect on telomere attrition and telomerase activity. Mammalian telomere repeats cannot be methylated because of the lack of CpG sequences, which are the substrates for DNA methyltransferases. Nevertheless, highly repetitive regions including pericentric chromatin are gradually methylated which has been proposed to be important in preventing the high level of homologous recombination that would otherwise be expected to take place at these domains [62]. Reduction in DNA methylation, particularly at subtelomeric regions, is accompanied by dramatically elongated telomeres, even when there is no loss of heterochromatic histone methylation results. These results imply that DNA methylation represents an additional way to control telomere length, independently of histone methylation. Notwithstanding the fact that histone methylation at telomeres and subtelomeres seems to be independent of DNA methylation, and cells that lack SUV39H HMTases or the retinoblastoma family of proteins, which show defective histone methylation at telomeres, also show a global decrease in DNA methylation. Furthermore, loss of DNA methylation may also indirectly induce telomere length through effects on the expression of other telomere length regulators or proteins included in the recombination process [63].

Epigenetic Modification of Telomerase and hTERT

Epigenetic regulation of telomere structure is essential for telomere length control. Telomerase activity is important in maintaining telomere length and preventing DNA damage. On the other hand, telomerase plays an essential role in the development and progression of many cancers, especially GC, by inducing its catalytic subunit, hTERT [64], while telomeres shorten in subtelomeric transcription conducted by reduced DNA methylation and enhanced histone acetylation in inhibition of telomerase mouse cells,which is intended to improve telomerase recruitment [60, 65]. TERRA expression as

Table 1 Hypermethylation and hypomethylation genes in GC

Gene	Function	Correlation with clinical outcomes
Hypermethylation genes in GC		
BNIP3	Apoptosis pathway gene	Association with poor prognosis
CDH1	Adhesion and invasion	Association with poor prognosis, *H. pylori* infection, and EBV infection
p15	Cell cycle regulation	Association with EBV infection
p16	Cell cycle regulation	Association with poor prognosis, H. pylori infection and EBV infection
CACNA2D3	Apoptosis pathway gene	Correlation with lymph node metastasis
DAPK	Advanced stage and poor survival	Correlation with cell differentiation, lymph node metastasis
GPX3	Apoptosis pathway gene	Correlation with lymph node metastasis
MGMT	Impair biological effects of O6-methylguanine and O4-methylthymine, DNA damage repair	Association with poor prognosis
hMLH1	Involve in mismatch repair system	Association with poor prognosis
RASSF1A	Required for death receptor-dependent apoptosis, cell cycle regulation	Association with poor prognosis
RASSF2	KRAS effector protein; promote apoptosis and cell cycle arrest	Association with poor prognosis
RUNX3	Binds to various enhancers and promoters including of IL-3 and GM-CSF	Correlation with TNM stage and H. pylori infection
Hypomethylation genes in GC		
LINE-1	Prognostic biomarker	Association with poor prognosis and H. pylori infection
ASCL2	Cell growth, differentiation	Associated with poor prognosis
MAGE	Transcriptional regulation	Associated with poor prognosis
SURF	Prognostic and metastasis predictive markers	Association with poor prognosis
SNCG	Activates MAPK and Elk-1 pathway	Correlation with lymph node metastasis

telomerase suppressor can control the subtelomeric methylation [66]. The compact methylation structure reported in the telomere-positive cells. In this way, cells with telomerase activation involved in controlling telomere length by decreasing of TERRA in the subtelomere [67]. Many transcription factors and the epigenetic state of the hTERT promoter are identified to be important for control of hTERT in normal tissues; however, the molecular mechanisms affecting hTERT reactivation in cancer are not well explained. Increased hTERT expression in cancer is associated with different genetic and epigenetic factors, such as hTERT promoter mutations, transcriptional amplification, splice variants, and epigenetic alterations via hTERT promoter (Fig. 2) [68]. In telomerase activation, hTERT is necessary by immortality, invasion, proliferation, differentiation, and malignancies and also plays an important role in maintaining telomere length (Fig. 3), while repression of hTERT gene expression or inhibition of telomerase activity leads to induction of apoptosis and restraint of cell proliferation [69]. The main epigenetic alterations for the expression of hTERT are DNA methylation, histone acetylation, deacetylation, and methylation and non-coding RNA [70].

hTERT promoter methylation has a specific role in the development of cancer. High levels of hTERT hypermethylation have been reported in GC. Consequently, determining the patterns of hTERT hypermethylation is essential in distinguishing patients at high risk of GC. The association between methylation and expression of hTERT in GC is beneficial and may influence the anti-telomerase strategy in the treatment of cancer [71]. Long telomeres play a role in suppressing the chromatin and DNA methylation of hTERT while change in chromatin is associated with short telomeres [72]. The unrestricted growth of GC cells is connected with hTERT. Further, hTERT is correlated with lymphatic metastasis and distant metastasis [73]. Increased DNMT3a and DNMT3b expression which involved in hTERT methylation are observed in GC [74].

One study reported only a few cases of methylation of the hTERT promoter in GC. As a result, there is a weak relationship between the expression of hTERT protein and methylation in GC. But because of the significant differences in hTERT expression and methylation in tumoral and non- tumoral tissues, it has been introduced as a treatment strategy for cancer [71]. In addition to hTERT promoter methylation in GC samples compared with normal samples, hTERT promoter hypermethylation is correlated with metastasis, differentiation, and stage N and T-stage. hTERT hypermethylation in cancer progression was identified as a diagnostic biomarker of GC. hTERT methylation can be used to classify patients

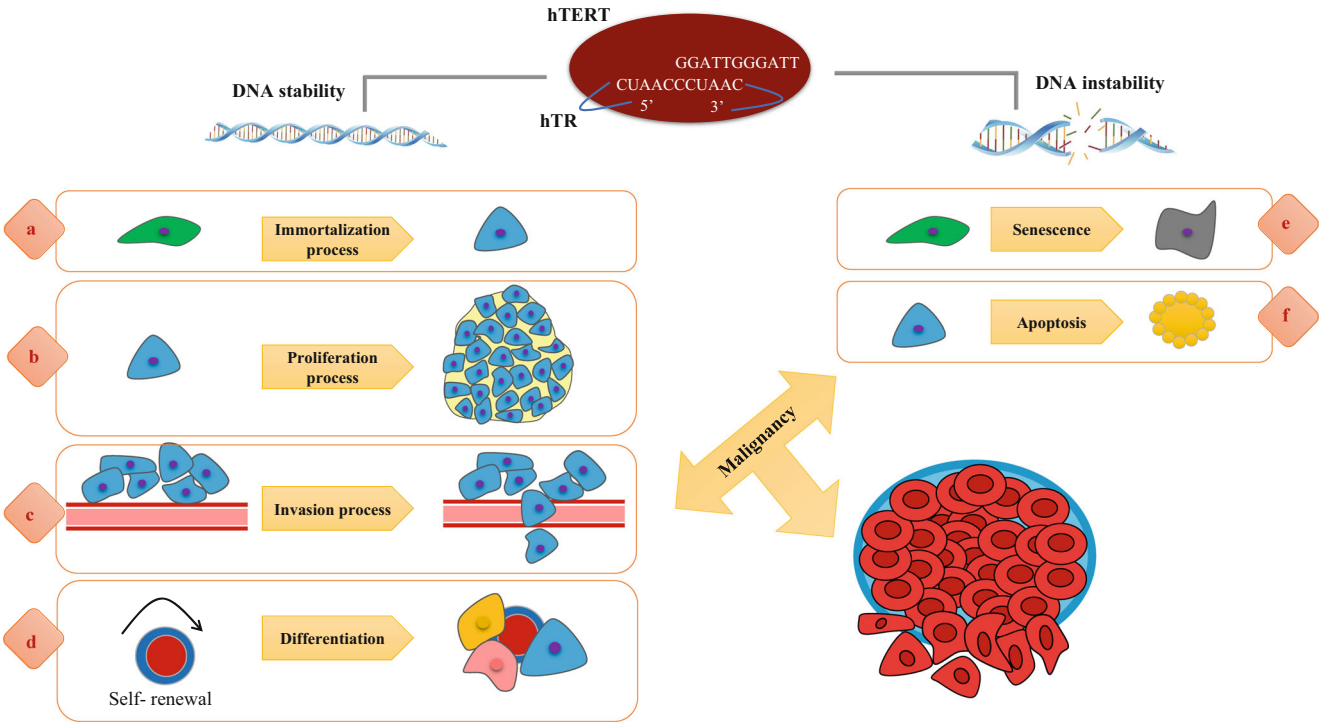

Fig. 2 Altered situation of hTERT gene including apoptosis, DNA stabilization which is lead to malignancy

with high-risk GC; however, further studies are required [75]. Many reports suggest that hypermethylation of specific regions of the gene may suppress the expression of that gene. In cases with the expression of the hTERT like GC, hTERT promoter methylation was determined. Methylation of DNA prevents binding to DNA by altering the structures of transcriptional repressors. Accordingly, changes in gene expression are possible. Consequently, methylation of hTERT is capable of affecting telomerase activity and hTERT expression [76]. Many studies also have shown the role of the hTERT promoter in GC. However, the causes of the different fluctuations of the hTERT promoter mutation are not fully

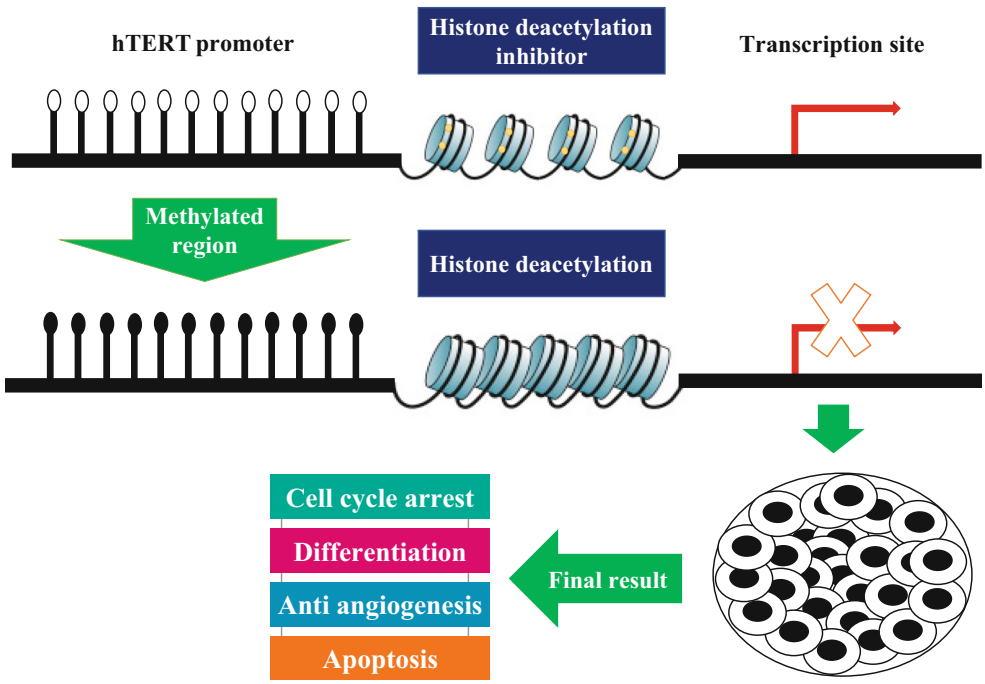

Fig. 3 Role of methylation of hTERT gene fluctuation

understood [77]. The repressive role of the hTERT promoter in telomerase activity and cancer needs further investigation.

DNA methylation is a sign of cancer progression. In most cancer with upregulation of hTERT and telomerase activation, most of hTERT promoter regions are consist of hypermethylated CPG sites. The TERT promoter is correlated with hypoacetyled core histones. Furthermore, histone and CpG are involved in the regulation of hTERT. HDAC is involved in suppressing hTERT gene expression and hTERT promoter activity through transcriptional inhibition. However, HDACI leads to telomerase activation and increased hTERT gene expression (Fig. 4) [78]. In cells without telomerase activity, TSA as HDAC inhibitors affects the hyperacetylation of the hTERT promoter which decreases hTERT expression and begins to apoptosis [79].

siRNA is a good tool to suppress genes. RNA molecules including siRNA and miRNA via silencing cancer-related genes or the control of pathways involved in the development and progression of malignancies have a great impression on the treatment of some diseases, especially cancer [80]. Notably, it is important to select the appropriate gene, such as hTERT, which is expressed only in cancer cells. Previous studies have reported the overexpression of the hTERT gene in AGS cells of GC. Consequently, a significant decrease in hTERT gene expression by specific siRNAs induces apoptosis of cancer cells and inhibits cell growth and proliferation [69, 81]. The function of the hTERT gene may be influenced by epigenetic mechanisms including DNA methylation and histone modifications in the hTERT gene promoter. The epigenetic silencing of the hTERT has been documented to repress telomerase activity which leads to the shortening of the telomere [60, 82].

Terra

TERRA is transcribed from telomeric and subtelomeric regions in the chromosome ends and consists of G-rich telomeric repeats that carry replicates of UUAGGG and range in size between 100 and 9 kb [83].

Many results indicate a single TERRA that can bind and control multiple telomerase at the ends of the chromosome [84]. Telomeric repeat-containing RNA (TERRA) contacts to hTERT through complementary base pairing against RNA template which determines that TERRA is a natural ligand and negative human telomerase regulator [85]. In vitro, TERRA is a highly effective suppressor of telomerase [86]. Several pathways, consisting of the state of development, telomere epigenetic state, and cellular stress may control the TERRA expression.

TERRA transcripts and the chromosome termini thus identifying telomeric RNA as a novel functional component of telomeric chromatin. TERRA transcripts have the capacity to influence telomeres and telomeric chromatin epigenetically. Furthermore, telomere elongation inhibited TERRA transcription by an increased frequency of H3K9me3 in the telomeric chromatin and by HP1a [87]. Epigenetics of aging and its

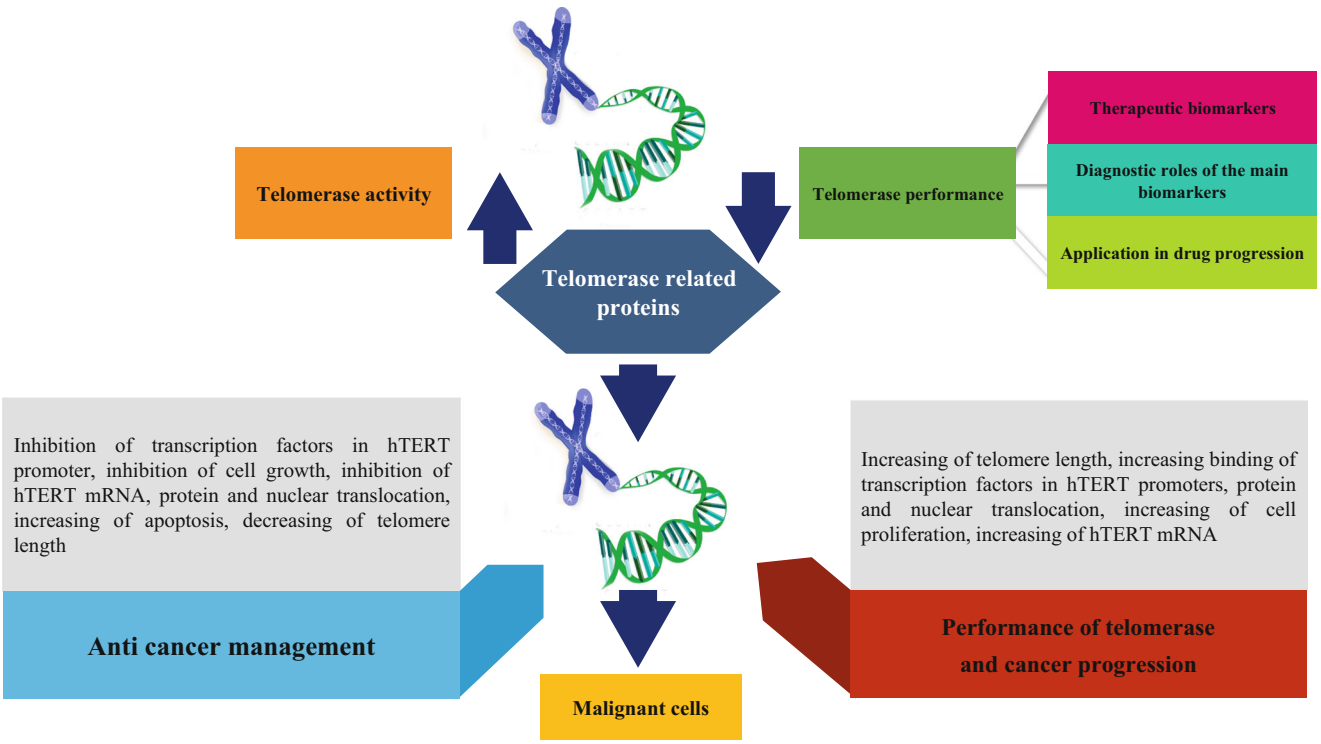

Fig. 4 Role of the hTERT gene and also the activity of telomeres and telomerase in cancer progression

relevance to telomere length 129 in cell lines is derived from humans. Previous studies have shown that a mixture of siRNAs directed against telomerase caused a near-complete silencing of its activity along with a destruction of the protein. This inactivation was correlated with a marked decrease in telomere length. Further, different in vitro studies in humans explained the inhibition of telomerase by TERRA. However, other knowledge in vivo explained that telomerase activity is independent of TERRA level in human cancer cells [88].

Regulation of telomerase is very complicated as many factors can influence hTERT expression and telomerase activity. Telomerase may be regulated by such established methods as transcriptional regulation, post-transcriptional regulation, localization within the cell, assembly of the subunits, epigenetic regulation, and by telomeric proteins and RNAs [89].

Regulation of hTERT transcription commonly takes place at the promoter region which has been determined to contain an excess of CpG sites, obvious targets for methylation. DNA methylation and chromatin remodeling are common regulators of gene activity that alter the binding of transcription factors to gene promoters. The field of epigenetics has become very important in the study of telomerase regulation, and several recent studies have begun on some of the factors required [90].

Methylation of the promoter region is regularly associated with gene silencing. Some researchers have shown no significant relationship between hTERT expression and methylation. So, it is possible that hTERT expression and methylation are reliant on the cell type (Fig. 2) [91].

Some studies have investigated the epigenetic regulation of telomerase in all types of cancer. Methylation PCR and promoter bisulfite sequencing investigated maintained at least one allele with less methylation maintained throughout the start site of transcription in breast, lung, and colon cancer. Although many of the hTERT promoters are methylated in cancer cells, some of them remain unmethylated around the transcriptional region which are involved in gene silencing [92].

However, the mechanisms of action of telomeric noncoding RNAs remain mainly to be elucidated. It is now significantly obvious that TERRA transcripts actively participate in the different features of telomeres and in telomere stability. Besides a function for TERRA in tumor cells, telomere dysfunction also happens throughout replicative senescence, indicating that TERRA may possibly play a role in aging and age-related diseases. TERRA is overexpressed in Immunodeficiency, Centromeric region instability, Facial anomalies syndrome (ICF) patients, probably because of the hypomethylated state of their subtelomeric promoters. Other diseases, like telomeropathies, might be related to TERRA misregulation [93]. The well-established process to successfully deplete total TERRA in cells remains to be developed. Recent strategies, using RNAi or antisense oligonucleotides, just partly decrease TERRA levels. As a function for TERRA as a scaffold molecule involved in the recruitment and organization of enzymatic activities at telomeres is emerging, a significant challenge will be to determine how these numerous activities are organized by TERRA based on the state of a telomere [94].

TERRA can serve as a telomerase regulator and telomeric R-loops formation in telomere length homeostasis. TERRA can be used as a therapeutic target by disrupting telomerase activity. Some results observed an increase in TERRA expression from three subsets of chromosomes (1q-21q, 5p, and 9p-15q-Xq-Yq) when telomeres were short compared with long. TERRA can serve as a telomerase regulator [95–97].

Conclusion

Epigenetic fluctuation has an impressive role in many different types of cancer, particularly gastric one. The most important element of epigenetic which is the DNA methylation always shows a central key role in carcinogenesis and tumorigenesis. In this way, histone methylation, DNA methylation, and histone acetylation are the most primitive epigenetic regulations included in the expression of hTERT. Correspondingly, noncoding RNA also accomplishes the main formation of epigenetic control of hTERT gene. This regulation of hTERT on the base of epigenetic is really essential in using a mechanism for reversibility of hTERT control in different biological situations. These comprise embryonic down-regulation of hTERT subsidizing to aging and also the upregulation of hTERT which has the main role in more than 90% of malignancies.

Many forms of TERRA promoters are related to CpG islands installed in repetitive DNA tracts. Conspicuously, cytosines in these subtelomeric CpG islands have the frequent methylated situation in telomerase-positive GC cells, and demethylation induced by reduction of DNA methyltransferases is related with upregulation of TERRA levels. Meaningly, many evidence, and findings include this remarkable mechanism regulating TERRA expression through subtelomeric CpG islands methylation.

On the other hand, telomerase is expressed in early developmental process of human and then becomes silenced in most normal tissues automatically. Considerably, about 90% of early human tumors express telomerase and principally keep them very short, and telomerase is carefully regulated, especially in all mammalians.

Conclusively, aberrant expression of hTERT is mainly related with carcinogenesis and tumorigenesis, metastasis, apoptosis inhibition, cancer cell stemness maintaining, senescence evasion, and cell proliferation. The molecular basis of hTERT regulation is highly elaborated and contain many different layers. Exact and deep understanding of the regulatory mechanisms of hTERT has a strong potential in understanding the pathogenesis processes in therapeutic strategies for gastric cancer.

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