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Review article

# Modulation of telomerase expression and function by miRNAs: Anti-cancer potential

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ABSTRACT

Telomerase is a nucleoprotein reverse transcriptase that maintains the telomere, a protective structure at the ends of the chromosome, and is active in cancer cells, stem cells, and fetal cells. Telomerase immortalizes cancer cells and induces unlimited cell division by preventing telomere shortening. Immortalized cancer cells have unlimited proliferative potential due to telomerase activity that causes tumorigenesis and malignancy. Therefore, telomerase can be a lucrative anti-cancer target. The regulation of catalytic subunit of telomerase (TERT) determines the extent of telomerase activity. miRNAs, as an endogenous regulator of gene expression, can control telomerase activity by targeting TERT mRNA. miRNAs that have a decreasing effect on TERT translation mediate modulation of telomerase activity in cancer cells by binding to TERT mRNA and regulating TERT translation. In this review, we provide an update on miRNAs that influence telomerase activity by regulation of TERT translation.

## 1. Introduction

MicroRNAs (miRNAs) are minuscule biological regulators that were first discovered in nematodes nearly 26 years ago, and their regulatory role in gene expression has been elucidated. miRNAs are an abundant class of non-protein-coding and single-stranded ribonucleotide acids with a length of almost 22 nucleotides [1,2]. Gene expression regulation occurs in the post-transcriptional level by miRNAs, and miRNAs mostly interact with 3′_untranslated region (3′UTR) of the target mRNA. miRNAs can play an important role in the activation or blocking of different cellular processes like proliferation, differentiation, aging, immune responses and growth of cell by controlling the large number of genes which involved in different biological signaling pathways [3–5]. miRNAs also affect most aspects of cancer biology like apoptosis, metastasis, and angiogenesis by regulating cancer-related genes, such as oncogenes or tumor-suppressive genes, as a result, cause initiation, progression, and even suppression of cancer [6–8]. miRNAs expression levels can be different between healthy and unhealthy cells, and aberration in their expression shows the presence of a malignancy or pathological condition. miRNAs are considered important diagnostic markers in body fluids, and their use as biomarkers has improved the diagnosis and treatment of many diseases [9–11].

## 2. MicroRNAs biogenesis

The main biogenesis pathway of miRNAs, in that most miRNAs are produced, consisting of two main processing steps depending on the

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specific type of proteins. Initially, RNA polymerase II mostly and RNA polymerase III in some limited clusters transcribe primary-miRNAs (pri-miRNA) [12,13]. MiRNAs transcription occurs mostly from intergenic coding regions. pri-miRNA transcripts possess thousands of nucleotides in length. pri-miRNAs are similar to protein-coding transcripts, due to having the cap at the 5′- end and the poly-A tail at the 3′- end in their structure [14–16]. The primary processing in the nucleus is done by the microprocessor complex, which contains a double-strand binding protein DGCR8 (DiGeorge Syndrome Critical Region 8) and ribonuclease III, Drosha. DGCR8 subunit of microprocessor complex binds to the N6-methyladenylated GGAC region in pri-miRNA, and Drosha cleaves the 3′ and 5′ strands of stem-loop to releasing hairpin-shaped pre-miRNA [17–19]. Precursor miRNA (pre-miRNAs) are approximately 70 nucleotides in length. Pre-miRNA, which is the product of the initial processing step, is exported from the nucleus to the cytoplasm by Exportin 5/RanGTP, to complete the second stage of processing in the cytoplasm [20,21]. In the secondary processing, mature miRNA is produced by the RNase III endonuclease Dicer. Dicer excises the terminal loop and produces 18–24 base pair duplex strand miRNA [22,23]; this miRNA contains guide and passenger strands. Two strands of duplex miRNA are separated by the RNA helicase. The passenger strand is degenerated, and the guide strand is loaded into the Argonaut (AGO) family of proteins, and this leads to the forming of the miRNA-induced silencing complex (miRISC) [24–26]. In the event the miRNA-RISC complex complemented/paired with the 3′ untranslated region of the target mRNA, either mRNA will be degraded, or the mRNA translation will be suppressed [27–29]. The non-main miRNA biogenesis pathway, in which only certain types of miRNAs are synthesized, is not dependent on the presence of Drosha, dicer, exportin5, and Argonaut proteins. Mirtrons and $m^7G$-capped pre-miRNAs are produced within the non-main biogenesis pathway. The mirtrons are products of the mRNA splicing process [14,30,31] (Fig. 1).

## 3. Cancer and telomerase

The healthy somatic cells do not have the unlimited replicative capacity, and they cannot continuously divide. In the normal mortal cells, cell cycle arrest, senescence, or apoptosis occur after successive cell divisions. The shortening of telomere is one of the important factors in cell mortality and prevention of unlimited division. The telomere is a protective nucleoprotein structure at the end of eukaryotic linear chromosomes that includes thousands of G-rich tandem repetitive sequences of 5′-TTAGGG-3′ and protein complexes [32–35]. Telomere enhances chromosome integrity and genomic stability by preserving of chromosomes end against degradation. Also, telomere acts as a protector against the occurrence of rearrangement and end to end fusion. The telomere is a non-coding, double-stranded structure located at the end of the chromosome, but in the 3′ terminal, it becomes a single-stranded overhang which does not have a complementary DNA strand [36,37]. The telomeres associated with shelterin protein complexes through protein binding sites and this leads to the formation of D-loop (displacement loop) and T-loop (telomeric loop) structures to further protect telomeric structures against exonuclease activities. Shelterin complex plays a basic role in the regulation of telomere length and protection of chromosome ends from inappropriate DNA repair, and it is consists of telomere-repeat binding factors 1 and 2, TRF1, and TRF2, Protection of telomeres 1 (POT1), Tripeptidyl-peptidase 1 (TPP1, also known as adrenocortical dysplasia protein; ACD), Repressor/activator protein 1 (RAP1), and TRF1- and TRF2-Interacting Nuclear Protein 2 (TIN2) [38,39].

TRF1 by connecting to the double-stranded TTAGGG region controls the replication of telomeric DNA and with recruit helicases and interacts with Tankyrases facilitates the telomere lengthening process. TRF2 is involved in prevents the activation of DNA damage responses and the formation of T-loops.RAP1 binds toTRF2, and POT1 binds to 3′single-stranded TTAGGG repeats in associated with TPP1. TIN2 inter-

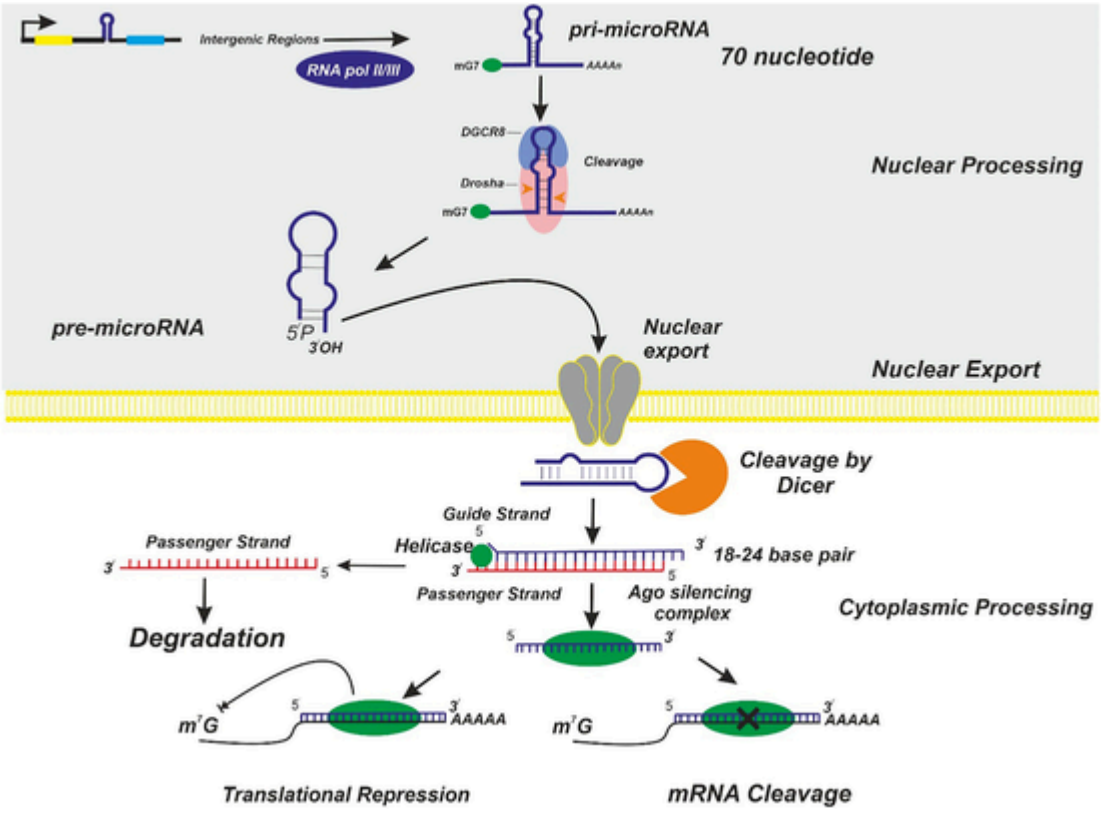


**Fig. 1.** The schematic diagram provided shows the microRNAs biogenesis.

act with both TRF1 and TRF2, and then it connects to TPP1, which in turn binds to POT and improves complex stabilization [40–42].

Since DNA polymerase is unable to synthesize the latest Okazaki fragments and cannot wholly replicate the 3′end of the chromosome, which is known as “end replication problem” and exonuclease activity degrades the end of the chromosome, for these reasons, the length of the telomere becomes shorter with each replication [43–45]. The end replication problem causes the telomere to act as an internal clock that determines the number of cell divisions [46,47]. Apart from the protective roles, the telomere position effect can suppress the expression of genes near telomere. The reducing telomere length decreases its inhibitory effect and increases the expression of adjacent genes. Shortening the telomere length and losing its protective roles acts as a tumor suppressor; consequently, Cells enter the senescence and crisis phase, arrest the cell cycle and cause cell death. Conversely, telomerase can alter the cell fate and leads cells to immortality and cause malignancy by telomere synthesis [48–52] (Fig. 2).

Telomerase is a reverse transcriptase which responsible for expanding telomeric DNA sequences at the 3′ end of chromosomes and compensates for shortened telomeres in each cell division, thereby prevents cell death and induces unlimited cell division and cell proliferation [53,54]. The functional structure of telomerase consists of 3 subunits, the catalytic portion responsible for the synthesis of telomeric DNA called telomerase reverse transcriptase (TERT), The functional RNA component that acts as the template for the enzymatic part called telomerase RNA component (TERC), telomerase associated protein 1 (TEP1), Dyskerin Pseudouridine Synthase 1 (DKC1), NHP2, Nop10, Pontin and Reptin. These components are assembled to the formation of a ribonucleoprotein enzyme complex [55,56]. In the S phase of the cell cycle, telomeric sequences can duplicate [57]. Telomerase activity depends on the regulation of TERT component expression; its regulation is performed at transcriptional and post-transcription levels, and during the alternative splicing process [58–62]. In addition to maintaining telomere length, telomerase can affect the Regulation of genes involved in the progression of cancer, cell proliferation, cell death, NF-kB, and WNT/βcatenin signaling pathway, and also can control glycolysis in cancer cells [63–65]. Telomerase function in cancer cells is significantly increased due to genetic and epigenetic changes in TERT expression. Genetic mechanisms include amplification, promotor mutation, and structural variants in TERT [62,66,67]. Hypermethylation of the TERT promoter-specific region, also known as, TERT hypermethylated oncologic region (THOR) is one of the epigenetic changes that can increase its expression and lead to tumor progression [66].

## 4. Direct regulation

Direct regulation occurs with direct interaction between the miRNA and the 3′ UTR region of TERT mRNA. The protein and mRNA levels of TERT affect telomerase activity positively or negatively (Table 1). Regulation of TERT by interaction with miR-138 is evaluated in human cervical cancer cells, anaplastic thyroid carcinoma cell lines, and colorectal cancer. MiR-138, as a tumor suppressor, with a similar function, has an inhibitory effect on TERT expression and telomerase activity. In the ATC cell line of thyroid carcinoma, downregulation of miR-138 expression increases the protein level of TERT and, ultimately, telomerase activity, which contributes to cancer progression. Following induction of miR-138 expression in the ATC cell line, luciferase assay revealed that miR-138 inhibited TERT mRNA translation by targeting the 3′ UTR of TERT mRNA, and dysregulation of miR-138 may contribute to cancer progression [68]. Two miRNAs can simultaneously have the opposite functional effects on TERT expression. In HeLa and C33A human cervical cancer cell lines, miR-138 and miR-346 exert contrasting effects on telomerase activity. MiR-138 and miR-346 have an identical binding site on the 3′ UTR region of TERT mRNA that competes with each other to interact with this site. MiR-138 has a repressive effect on TERT mRNA translation and telomerase activity, which reduces TERT and active telomerase levels. Inhibitory action of miR-138 is dependent on AGO (argonaut), and downregulation of TERT by mir-138 is an AGO-dependent manner. Conversely, miR-346 has an increasing effect on TERT expression, which results in increased active telomerase assembly and growth of human cervical cancer cells. Upregulation of TERT mRNA translation is dependent on G-rich RNA sequence binding factor 1 (GRSF1), which binds to the middle sequence motif (CCGCAU) of miR-346 and promotes TERT translation in AGO-independent manner [69]. MiR-1182 is a negative regulator of TERT in the bladder and gastric cancers. The levels of miR-1182 and TERT in the bladder tumor cell lines and gastric cancer cell lines are inversely correlated. By comparison, the expression of miR-1182 as a tumor suppressor is low, and TERT expression is high, but, the upregulation of miR-1182 reduces the translation level to TERT. In both bladder and gastric cancer, miR-1182 targets TERT mRNA. In gastric cancer, the miR-1182 interacts with the

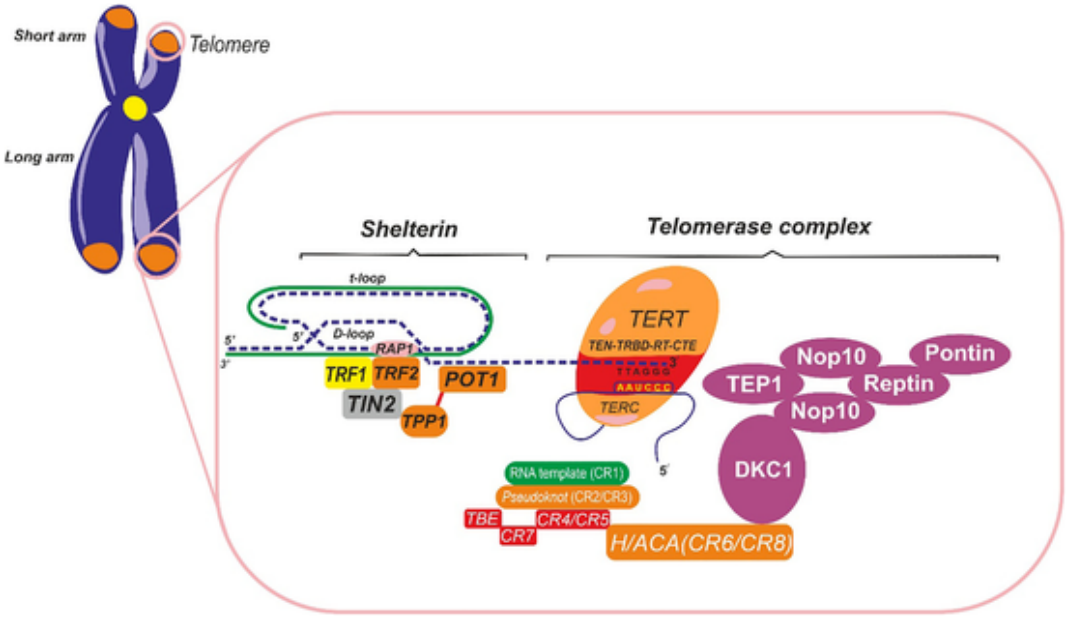


**Fig. 2.** The schematic diagram provided reveals the telomerase structure and function.

**Table 1**
miRNAs involved in direct regulation of TERT.

| miRNAs | Target | Cancer | Reference |
|---|---|---|---|
| Mir-138 | 3′ UTR of TERT mRNA | Thyroid human anaplastic carcinoma cell line | [68] |
| Mir-346, MiR-138 | 3′ UTR of TERT mRNA | Cervical cancer | [69] |
| Mir-138-5p | 3′ UTR of TERT mRNA | Colorectal cancer | [82] |
| MiR-1182 | 3′ UTR of TERT mRNA | Bladder cancer | [71] |
| MiR-1182 | ORF of TERT mRNA | Gastric cancer | [70] |
| MiR-299-3p | 3′ UTR of TERT mRNA | Laryngeal cancer cell | [83] |
| MiR-491-5p | 3′ UTR of TERT mRNA | Cervical cancer | [84] |
| MiR-128 | Coding region of TERT mRNA | Hela cells and teratoma cells | [85] |
| MiR-497-5p, miR-195-5p, miR-455-3P | 3′ UTR of TERT mRNA | Melanoma A375 | [86] |
| MiR-512-5p | 3′ UTR of TERT mRNA | Head and neck squamous cell carcinoma | [87] |
| MiR-532, miR-3064 | 3′ UTR of TERT mRNA | Ovarian cancer | [88] |
| MiR-1182 | 3′ UTR of TERT mRNA | Ovarian cancer | [89] |
| MiR-1207-5p, miR-1266 | 3′ UTR of TERT mRNA | Gastric | [90] |
| MiR-133a, miR-138, miR491 | 3′ UTR of TERT mRNA | Cervical cancer | [91] |
| MiR-661 | 3′ UTR of TERT mRNA | Glioma | [92] |
| MiR-615-3p | 3′ UTR of TERT mRNA | Human cancer | [93] |
| MiR-138-5p, miR-422a | Potentially inhibit TERT | Colorectal cancer | [94] |
| MiR-498 | 3′ UTR of TERT mRNA | Ovarian cancer | [74] |
| miR-296-5p, miR-512-5p | 3′ UTR of TERT mRNA | Breast cancer | [95] |

open reading frame (ORF) of TERT mRNA, consequently, reduces translation of TERT and prompts apoptosis and the cell cycle arrest. This inverse correlation between miR-1182 and hTERT protein levels also was founded in tissues from 42 gastric cancer patients [70]. However, in bladder cancer, miR-1182 binds to 3′ UTR of TERT mRNA and attenuates TERT translation and proliferative potential of bladder tumor cells *in vivo* [71].

In telomerase-positive cells, in diffuse malignant peritoneal mesothelioma (DMPM), ectopic expression of miR-380-5P interferes with telomerase activity and impairs cell growth, and promote apoptosis by targeting open reading frame of telomerase associated protein 1 (TEP1) [72]. In acute promyelocytic leukemia (APL) cell line, H19 as a primary miRNA transcript that generates miR-675-3p and miR-675-5p, decreases the hTR association with hTERT component and thereby impairs the functions of telomerase [73]. MiR-498 is a potential target gene for 1,25-dihydroxy vitamin D3 in ovarian cancer cells that enhances miR-498 expression. MiR-498 has a decreasing effect on the stability and translation of TERT mRNA in malignant human ovarian tumor tissues as well as human ovarian cancer cell lines. It also inhibits cell growth by suppressing TERT expression [74].

## 5. Indirect regulation

The level of miR-34a, which acts as a tumor suppressor, is reduced in hepatocellular carcinoma (HCC). The reduced expression level of miR-34a is effective in promoting malignancy and producing immortalized cells. Induction of miR-34a expression in liver cancer cell lines by transfection of synthetic miR-34a or by blocking anti-miR-34a function inhibits telomerase activity and shortens telomere length *in vitro*. The mechanism by which telomerase activity decreases is the inhibition of related transcription factors expression that increases the TERT level. MiR-34 directly targets c-MYC and foxM1 that are transactivators of the TERT expression and binds to 3′ UTR of c-MYC and foxM1 and suppresses c-MYC and foxM1 expression. They also provided an inverse correlation between the miR-34a level and telomere indices *in vivo* by the investigation of tumor tissues of 75 HCC patients [75]. MiRNAs can indirectly have an additive effect on telomerase activity and increase transcription of TERT component. MiR-19b plays a significant role in enhancing malignancy through increased expression of TERT in a telomerase-dependent pathway. Overexpressed miR-19b cells block paired-like homeodomain1 (PITX1) by Interaction with 3′ UTR of PITX1 mRNA, PITX1, by binding to the promoter region of TERT, inhibits its expression and ultimately decreases telomerase activity. MiR-19b by suppressing PITX1 eliminates its inhibitory effect on TERT, likewise positively affects telomerase activity [76]. MiR-21 in colorectal cancer (CRC) tissues and cell lines through PTEN/PI3K/AKT pathway promotes telomerase activity. MiR-21 is a potential oncogenic regulator in CRC that directly targets phosphatase and tensin homolog (PTEN), a tumor suppressor. Overexpression of miR-21 significantly reduces protein and mRNA levels of PTEN. The reduction of PTEN tumor suppressor increases TERT expression [77], and consequently, telomerase activity is elevated [78]. In human cervical cancer cell lines, miR-375 indirectly reduces approximately 60% TERT transcription and 35% telomerase activity. MiR-375 negatively regulates TERT transcriptional activator (HPV E6, MYC), thereby reduces TERT transcription. Besides, miR-375 increases the level of p53, RB, and p21 tumor suppressors, by inhibiting target genes like HPV E7, E6AP, and CIP2A, by this means, reduces TERT expression and telomerase activity. MiR-375 also blocks TERT nuclear localization by suppressing 14-3-3ζ translation, so it reduces telomerase activity. Thus, miR-375 contributes to telomerase inactivation and inhibition of cell proliferation [79]. Table 2 summarized the miRNAs involved in indirectly TERT regulation. The miRNAs involved in direct and indirect telomerase activity regulation are summarized in Fig. 3.

## 6. Targeting telomerase by miRNAs: therapeutic implication in cancer

One of the common approaches to inhibiting telomerase is to prevent its catalytic subunit TERT activity since studies have shown that TERT is widely expressed in most types of cells as well as in telomerase-negative cells like differentiated somatic cells, Conversely, transcription of TERT is tightly controlled during differentiation and in most somatic cells TERT is either not expressed or expressed in the de-

**Table 2**
miRNAs involved in indirectly TERT regulation.

| miRNA | Target gene | Cancer | Regulation | Reference |
|---|---|---|---|---|
| MiR-34a | c-MYC, foxM1 | Hepatocellular carcinoma | Downregulation of TERT | [75] |
| MiR-19b | PITX1 | Melanoma | Upregulation of TERT | [76] |
| MiR-202 | MXD1-MYC/MAX | pancreatic cancer | Upregulation of TERT | [96] |
| MiR-21 | PTEN | Colorectal cancer | Upregulation of TERT | [78] |
| MiR-21 | STAT3 | Glioblastoma | Downregulation of TERT | [97] |
| MiR-103 | *AKAP12* | Hepatocellular carcinoma | Upregulation of TERT | [98] |

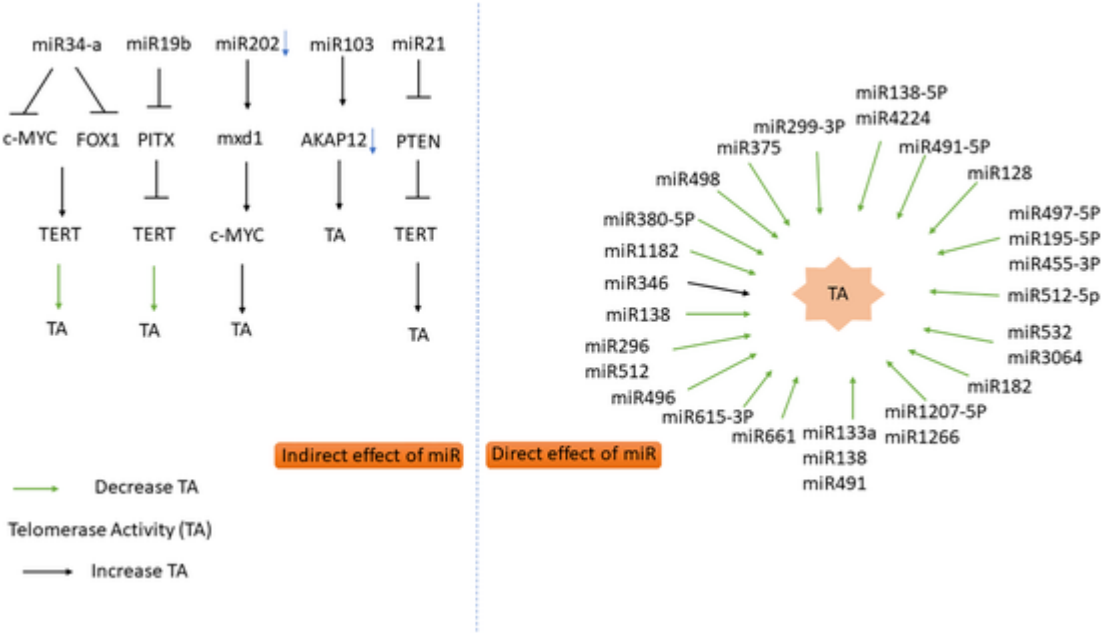


**Fig. 3.** The miRNAs involved in direct and indirect telomerase activity regulation.

ficient level [80,81]. MiRNAs can serve as oligonucleotide regulators of TERT and affect its expression directly by binding to the 3′ UTR region of TERT mRNA, then can completely degenerate mRNA of TERT or prevent mRNA translation. Also, the miRNAs can control and increase or decrease telomerase activity indirectly by regulating the expression of transcription factors or upstream regulators involved in the transcription of the telomerase main subunit TERT.

## 7. Conclusion

TERT aberrant expression, which is the main subunit in the regulation of telomerase activity, is one of the most important determinants of cell fate and cellular processes like cell division, cell proliferation, senescence, and apoptosis. The expression profile of TERT can control the initiation and progression of cancer and can play a role in malignancy, tumorigenesis, carcinogenesis, and metastasis. Understanding the causes of abnormal TERT expression and then regulation of the TERT expression at different regulatory levels contribute to finding regulators and inhibitors for regulated expression of TERT. At the transcriptional level, promoter mutation and epigenetic alterations, such as methylation and acetylation as well as changes and mutations of transcription factors that are involved in transcription of TERT mRNA can cause abnormal expression of TERT. At the post-transcriptional level, alternative splicing and non-coding RNAs like miRNAs can decrease or increase TERT mRNA translation. MiRNAs, as endogenous positive and negative regulators, can have an increasing and decreasing effect on TERT mRNA translation. In this review, we classify miRNAs that can, directly and indirectly, control TERT mRNA levels and thereby influence telomerase activity. A detailed understanding of the type of effect and mechanism of action of miRNAs can provide information through which we can modify the expression of TERT for cancer treatment in different malignancies. TERT-based cancer diagnosis and miRNA-based cancer treatment can be used as a practical and new approach alongside current cancer treatments.

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## Declaration of competing interest

The authors declare no conflict of interest related to this study.

## Ethical approval

This article does not contain any studies with human participants.

## Data availability statement

Research data not shared.

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