



MicroRNAs role in telomere length maintenance and telomerase activity in tumor cells

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Abstract

MiRNAs, a class of non-coding RNA molecules, have emerged as critical modulators of telomere length and telomerase activity by finely tuning the expression of target genes (and not gene targets) within signaling pathways involved in telomere homeostasis. The primary objective of this systematic review was to compile and synthesize the existing body of knowledge on the role, association, and involvement of miRNAs in telomere length. Additionally, the review explored the regulation, function, and activation mechanism of the human telomerase reverse transcriptase (*hTERT*) gene and telomerase activity in tumor cells. A comprehensive analysis of 47 selected articles revealed 40 distinct miRNAs involved in these processes. These miRNAs were shown to exert their function, in both clinical cases and cell line models, either directly or indirectly, regulating *hTERT* and telomerase activity through distinct molecular mechanisms. The regulatory roles of these miRNAs significantly affected major cancer phenotypes, with outcomes largely dependent on the tissue type and the cellular actions within the tumor cells, whereby they functioned as oncogenes or tumor suppressors. These findings strongly support the pivotal role of miRNAs in modulating telomere length and telomerase activity, thereby contributing to the intricate and complex regulation of telomere homeostasis in tumor cells. Moreover, they emphasize the potential of targeting miRNAs and key regulatory genes as therapeutic strategies to disrupt cancer cell growth and promote senescence, offering promising avenues for novel cancer treatments.

Keywords Telomerase · Telomere · Telomere length · *hTERT* · miRNAs · Cancer

Introduction

Telomeres, non-coding repeated sequences of TTA GGG nucleotides, are located at the final portions of the chromosomes and are responsible for maintaining the integrity and

stability of the genome by protecting the ends of eukaryotic chromosomes in a sheltering protein complex [1–3].

In dividing somatic cells, telomeres shorten by about 60–150 bp within each cell division, inducing cell senescence and apoptosis [4–6]. This telomere shortening leads to loss of telomere capping, resulting in chromosomal instability, such as the formation of aberrant chromosomes and end to end fusions [7]. These events trigger DNA damage response (DDR) mechanisms, in which a cascade of cell signaling, mediated by p53, p21 and other cell cycle proteins, controls cell cycle arrest and the fate of cell survival [8, 9].

The synthesis of the TTAGGG repeats at the telomeric ends occurs by the telomerase enzyme, which controls the maintenance of telomere length. This enzyme, a ribonucleoprotein reverse transcriptase, is actively expressed in reproductive and stem cells but not expressed in somatic cells [10, 11]. In most cancer cells, however, telomerase expression is active, enabling the cells to bypass senescence and apoptosis, which results in continuous cell proliferation [12–14].

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The human telomere reverse transcriptase (*hTERT*) gene, located at 5p15.33, codifies the catalytic subunit of the telomerase enzyme and is responsible for regulating telomerase activity [15]. The mechanisms that regulate *hTERT* expression and telomerase activity are extensively studied and can occur via multiple genetic and epigenetic mechanisms, including mutations in the *hTERT* promoter regions, mRNA splicing alterations, amplification, methylation, and/or disruption of telomere position effect (TPE) machinery [16, 17]. In a small fraction of tumors (5–15%), an alternative lengthening of telomeres (ALT) can also occur [18].

MicroRNAs (miRNAs/miRs) are highly conserved, non-coding single strand RNAs of approximately 20 to 22 nucleotides that regulate gene expression post-transcriptionally [19]. These molecules play a crucial role in tumor development and progression by regulating the expression of genes involved in several cancer-associated signaling pathways, including the ones that modulate cell proliferation, survival, and senescence [20–23]. MiRNAs also regulate telomere length and telomerase activity, mediating many aspects of telomere homeostasis, including the sheltering and the telomerase complex [24, 25].

Considering the essential role and function of the telomere length in cancer cells and the role of miRNAs in actively regulating driver oncogenes and tumor suppressors genes in this process, this systematic review main objective was to compile and synthesize the existing knowledge on the role, association and/or involvement of miRNAs in telomere length. Additionally, the review explored the regulation, function, and activation of the *hTERT* and telomerase in tumor cells.

Methods

The review protocol followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [26, 27] and was registered at the International Prospective Register of Systematic Reviews (PROSPERO) database under the identifier CRD42021282906.

Data sources and search strategy

The selected terms “Telomerase” and synonyms (Telomerase **OR** Telomere **OR** *hTERT*), “Cancer” and synonyms (Cancer **OR** Tumor **OR** Carcinoma) and “microRNA” and synonyms (microRNA **OR** miR **OR** miRNA **OR** small non-coding RNA **OR** small ncRNA) in Title/Abstract), were searched in six distinct databases: EBSCO, EMBASE, Lilacs, Pubmed, Scielo, and Scopus. The searches were independently carried by two reviewers, using Rayyan Web App. Duplicate articles were excluded and the remaining articles were screened based on the title and abstract. The full text of

the selected articles was then assessed for relevance and eligibility. The end of the search date was October 2nd, 2023.

Study selection and eligibility criteria

Two reviewers evaluated the studies independently, according to the established inclusion and exclusion criteria. Inclusion criteria: (1) articles describing the interaction of miRNAs and the telomerase enzyme activation/function for telomere length maintenance in human tumor cells and cell lines; (2) articles that have been peer-reviewed and written in English. Exclusion criteria: (1) articles on telomerase enzyme activation/function that did not report on the role, association, and/or involvement of miRNAs; (2) articles on miRNAs analysis performed in animals; (3) articles on clinical intervention and/or other types of clinical trials; (4) non-original (review) articles, letter from reviewers, book chapters, unpublished studies; (5) articles for which the full-text was not available; (6) articles considered with low-quality risk of bias.

Data extraction

Two reviewers independently extracted the following information from the selected articles: name of the first author, year of publication, methodology (miRNA related and other relevant methods), sample source (biological material (patient’s samples and/or cell lines), miRNAs analyzed, miRNA target genes, and main results and conclusions.

Quality and bias evaluation

The Quality in Prognosis Studies (QUIPS) tool was used to assess the quality of the studies and the risk of bias. This tool evaluates studies based on the following categories: study participation and attrition, prognostic factor and outcome measurements, study confounding, and statistical analysis, and reporting [28]. Based on these categories, the article quality and risk of bias were classified into high quality (+): with little or no risk of bias, acceptable (\pm): with moderate risk of bias, and low quality (-): with high risk of bias, and unsure (?). According to this classification, the articles received a general evaluation as low, moderate, or high risk of bias.

Results and discussion

Search results

The search of the selected terms in the six selected databases resulted in 741 articles. After removing duplicate entries, 380 articles were screened for relevance and compliance

based on the established inclusion and exclusion criteria. Forty-seven full-text articles were identified and further proceeded for qualitative synthesis analysis. The entire search process, comprising four distinct strategy steps, along with the respective number of articles is presented in Fig. 1.

Risk of bias and quality of the studies

The assessment of the risk of bias in the six established categories by the QUIPS tools, resulted in 47 studies. From these, 22 presented a low risk of bias and 25 a moderate risk of bias (Supplementary Table 1). Following these analyses 47 studies were included for final qualitative synthesis.

Selected final articles

The 47 original articles were further subjected to qualitative synthesis based on the established inclusion and exclusion criteria. All the articles met the criteria and therefore, their findings were detailed described in this systematic review. The most frequent types of cancer studied were cervical (six studies), melanoma and colorectal (five studies each), followed by bladder, breast and gastric (three studies each), colon, glioblastoma, glioma, hepatocellular, ovarian, and thyroid cancer (two studies each), and osteosarcoma, lymphoma, lung, pancreatic, oropharyngeal, mesothelioma, head and neck squamous cells, laryngeal, and non-small-cell lung cancer (NSCLC) (one study each). In addition, one study was conducted in 56 different human cell lines, one

in the Isogenic Telomerase Positive (TEP), and one in Alternative Length of Telomeres (ALT) cancer cells (Table 1).

Altogether 40 different miRNAs were described as potential regulators of gene and telomerase expression, and activity in these studies (Table 1). The most frequent miRNAs described in the 47 articles were: miR-138-5p (nine articles), miR-1182 and miR-34a-5p (three articles), and miR-195-5p, miR-21, miR-296-5p, miR-29a-3p, miR-346, miR-491-5p, and miR-512-5p (two articles). The other 30 miRNAs were described in one article each. Nine miRNAs were described regulating telomere length and function: miRNA-34 (two articles), miR-155, miR-182-3p, miR-185, miR-193b-3p, miR-29a, miR-376, miR-490, and miR-708 (one article each).

MiRNAs that directly regulate the *hTERT* gene

Among the 47 articles selected, 27 of them reported 24 miRNAs that directly regulate the *hTERT* gene (Supplementary Table 2). In most of the studies the miRNA-mRNA (*hTERT*) interactions were experimentally validated by luciferase assay [study references 30–32, 34–40, 45, 48, 51–53, 55, 57–59, 69, 70, 74], RT-qPCR [30–40, 44, 53, 55, 57–60, 69, 70], and Western Blot [29, 31, 32, 34–37, 39, 40, 44, 45, 51–55, 57–59, 69]. In five studies [39, 54, 55, 59, 74] the miRNA regulation of telomerase activity was described by the Telomerase Repeated Amplification Protocol (TRAP) assay.

Fig. 1 Flow diagram of the search strategy steps of the selected articles

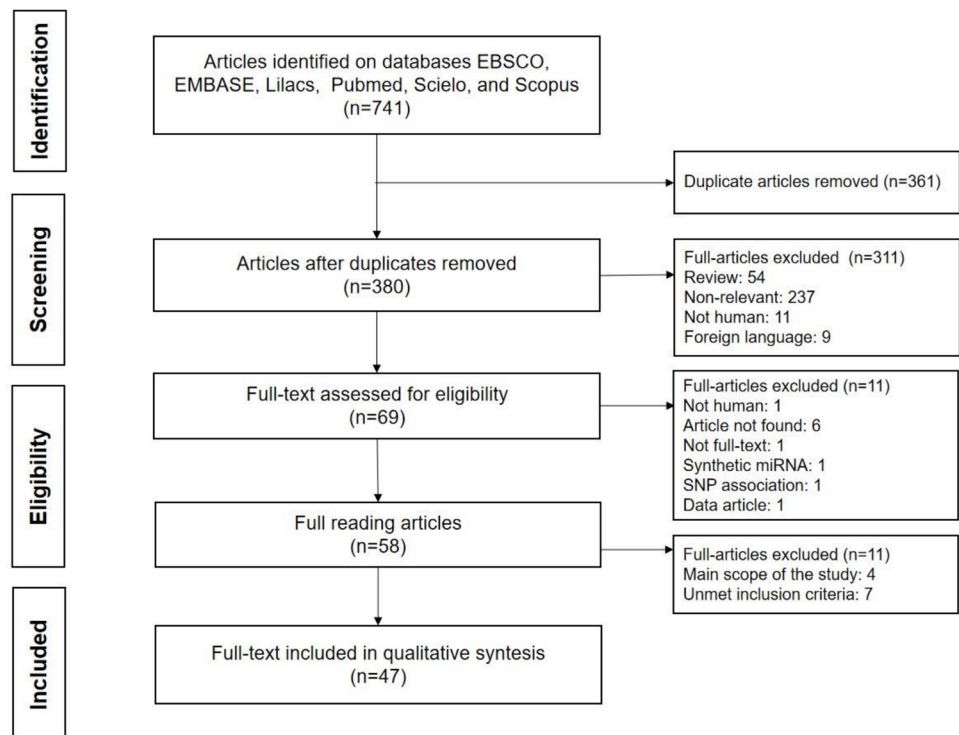


Table 1 MiRNAs described as regulators of the *hTERT* gene and telomerase expression, and activity, and respective cancer types (presented by the number of cited articles)

| MiRNAs | #Cited articles | Cancer type | # Supplementary Table with article description |
|------------------------------|-----------------|--|--|
| miR-138-5p (miR-138) | 9 | Bladder cancer [29], Cervical cancer [30, 31], Colorectal cancer [32, 33], Melanoma [34–36], Thyroid cancer [37] | 2 |
| miR-1182 | 3 | Bladder cancer [38], Gastric cancer [39], Ovarian cancer [40] | 2 |
| miR-34a-5p (miR-34, miR-34a) | 3 | Gallbladder cancer [41], Glioma [42], Hepatocellular cancer [43] | 3, 4 |
| miR-195-5p | 2 | Melanoma [44], Thyroid cancer [45] | 2 |
| miR-21-5p (miR-21) | 2 | Colorectal cancer [46], Glioblastoma [47] | 3 |
| miR-296-5p (miR-296) | 2 | Breast cancer [48], Breast Cancer, Osteosarcoma, Fibroblasts [49] | 2, 3 |
| miR-29a-3p (miR-29) | 2 | Colon cancer [50], Gastric cancer [51] | 2, 4 |
| miR-346 | 2 | Astrocytic gliomas [52], Cervical cancer [30] | 2 |
| miR-491-5p | 2 | Cervical cancer [53, 54] | 2 |
| miR-512-5p | 2 | Breast cancer [48], Head and neck cancer [55] | 2 |
| let-7 g* (let-7 g*) | 1 | Cervical cancer [54] | 2 |
| miR-103a-3p (miR-103) | 1 | Hepatocellular cancer [56] | 3 |
| miR-1207-5p | 1 | Gastric cancer [57] | 2 |
| miR-1255b-5p (miR-1255b) | 1 | Colorectal cancer [58] | 2 |
| miR-1266 | 1 | Gastric cancer [57] | 2 |
| miR-128 | 1 | Cervical cancer [59] | 2 |
| miR-133a | 1 | Cervical cancer [54] | 2 |
| miR-135a | 1 | Non-small-cell lung cancer [60] | 2 |
| miR-150-5p (miR-150) | 1 | Malignant lymphoma [61] | 3 |
| miR-155-5p (miR-155) | 1 | Breast cancer [62] | 4 |
| miR-182-3p | 1 | Breast cancer [63] | 4 |
| miR-185-5p (miR-185) | 1 | Lung cancer [64] | 4 |
| miR-193b-3p | 1 | Colorectal, and colon cancer [65] | 4 |
| miR-19b-3p (miR-19b) | 1 | Melanoma [66] | 3 |
| miR-202-3p (miR-202) | 1 | Pancreatic cancer [67] | 3 |
| miR-22-3p (miR-22) | 1 | Cervical cancer [68] | 3 |
| miR-299-3p | 1 | Laryngeal cancer [69] | 2 |
| miR-3064 | 1 | Ovarian cancer [70] | 2 |
| miR-342-5p | 1 | Cervical cancer [54] | 2 |
| miR-375 | 1 | HPV-positive cancer (Oropharyngeal tissue and Cervical and Tongue cell lines) [71] | 3 |
| miR-376a-3p | 1 | Colon cancer [50] | 4 |
| miR-380-5p | 1 | Malignant peritoneal mesothelioma [72] | 3 |
| miR-422a | 1 | Colorectal cancer [33] | 2 |
| miR-455-3p | 1 | Melanoma [44] | 2 |
| miR-490-3p (miR-490) | 1 | Glioblastoma [73] | 4 |
| miR-497-5p | 1 | Melanoma [44] | 2 |
| miR-532-5p (miR-532) | 1 | Ovarian cancer [70] | 2 |
| miR-541-3p | 1 | Cervical cancer [54] | 2 |
| miR-615-3p | 1 | 56 human cell lines [74] | 2 |
| miR-708 | 1 | TEP and ALT cancer cells [75] | 4 |

ALT Alternative Length of Telomeres, HPV Human Papillomavirus, TEP Isogenic Telomerase Positive

Among the miRNAs identified directly targeting the *hTERT* gene, the miR-138 was described in a higher number of studies (nine studies). The downregulation of miR-138 in clinical cancer cases compared to normal tissue was reported

in six studies, in the following types of cancer: cervical [30], colorectal [32, 33], anaplastic and papillary thyroid [37], bladder cancer [29] and melanoma [35]. In the cell line models of most of these cancers, the downregulation

of miR-138 led to the up-regulation of the *hTERT* expression and induced cell growth. However, in another study in melanoma, overexpression of miR-138 was observed in the tumor tissue when compared to adjacent non-tumor tissue [34]. In the cell line models its overexpression was observed in five studies in the three different types of cancer: cervical [31], colorectal [32] and melanoma [34–36]. In these studies, the overexpression of miR-138 was shown to reduce the expression of *hTERT* and inhibit cell proliferation, migration, invasion, and metastasis. These last studies suggested that miR-138 acts in the described cancer types as a tumor suppressor, decreasing the expression of *hTERT* and reducing tumorigenicity.

MiR-1182 was investigated in three studies [38–40]. In the only study performed in clinical cases (ovarian tumors), miR-1182 was observed down-regulated in the tumor tissues compared to the adjacent normal tissues [40]. In cancer cell lines, overexpression of miR-1182 was described in bladder [38], and ovarian [40] cells. In these cells, the ectopic expression of miR-1182 suppressed *hTERT* expression and inhibited cell proliferation, invasion, metastasis development, and induced chemosensitivity of the cells to cisplatin [38]. Conversely, in gastric cancer cell lines [39], downregulation of miR-1182 increased *hTERT* expression, promoting cell proliferation and conferring metastatic potential to the cancer cells. These studies pointed out to the tumor suppressor action of miR-1182.

The expression of miR-195-5p was observed downregulated in two studies in clinical cases [44, 45]. In the study of Liu et al. (2021) [45], miR-195-5p was observed down-regulated in thyroid tumor tissues when compared to non-tumor tissues, and in the study of Chai et al. (2018) [44], the same was observed in melanoma samples. Conversely its upregulation in the cell line models of these tumors, showed reduced expression of *hTERT*, and the inhibition of cell proliferation and invasion, metastasis, and induction of apoptosis [44, 45]. The results of both studies suggested that this miRNA presents a tumor suppressor role in these cancer cells.

MiR-346 was observed down-regulated and up-regulated in gliomas [52] and cervical [30] tumor tissue compared to normal tissues, respectively. In the cell line models, overexpression of miR-346 increased the expression of *hTERT* and promoted cell growth [44, 45].

The regulation of *hTERT* expression by miR-491-5p was described in two studies in cervical cancer [53, 54]. In the Zhao et al. study [53], the overexpression of miR-491-5p decreased the expression of *hTERT*, inhibiting the PI3K/AKT signaling pathway and resulting in cell growth inhibition. Additionally, the authors demonstrated that the expression of miR-491-5p in cervical tumor clinical cases was lower when compared to adjacent normal tissue, supporting its tumor suppressive action [53]. In the other study, the overexpression of miR-491-5p reduced telomerase activity

in HeLa cells leading to the reduction of cell proliferation [54].

MiR-512-5p was observed directly regulating *hTERT* in one study in breast cancer [48] and one in head and neck squamous cell carcinoma [55]. In breast cancer, miR-512-5p was downregulated in the tumor tissue when compared to non-tumor tissues [48]. In the functional analysis both in breast cancer and head and neck cancer cell lines, the overexpression of miR-512-5p led to *hTERT* reduced expression and decreased tumorigenesis [48, 55].

Seventeen miRNAs (let-7g*, miR-1207-5p, miR-1255b-5p, miR-1266, miR-128, miR-133a, miR-135a, miR-296-5p, miR-299-3p, miR-29a, miR-3064, miR-342-5p, miR-455-3p, miR-497-5p, miR-532, miR-541-3p, and miR-615-3p) that also directly regulate the *hTERT* gene, were described (only once each) in eight different types of cancers: breast [48], cervical [54, 59], colorectal [58], gastric [51, 57], laryngeal [69], melanoma [44], non-small lung cancer [60], and ovarian [70] cancer.

Chen et al. (2014) [57] showed in gastric cancer cells that miR-1207-5p and miR-1266 interacted with the 3' UTR of *hTERT*, and their overexpression inhibited tumor growth. In these studies, both miRNAs were downregulated in the gastric tumor cases compared to non-tumor tissues. Zhang et al. (2020) [58] described the interaction between miR-1255b-5p and the *hTERT* and *BRG1* genes in colorectal cancer. The downregulation of miR-1255b-5p increased the expression of *hTERT* and *BRG1* promoting the epithelial-mesenchymal transition.

Guzman et al. (2018) [59] described that miR-128 significantly reduced the mRNA and protein levels of *hTERT* in HeLa cells. In another study, also in HeLa cell lines, [54] the overexpression of let-7g*, miR-133a, miR-342-5p, and miR-541-3p was shown to reduced telomerase activity, affecting cell proliferation. The miR-135a was associated with *hTERT* gene expression regulation in Choi et al. (2023) study [60]. The expression of miR-135a was observed downregulated in tumor tissues and cell lines of non-small-cells lung cancer, leading to the upregulation of the *hTERT* gene.

In gastric cancer, miR-29a was described directly regulating the *hTERT* gene in only one study [51]. In gastric tumor tissues, the lower expression of miR-29a was observed compared to precancerous gastric tissues. The same authors showed that in gastric cancer cell line models *hTERT* enhanced ITGB1 protein levels via the downregulation of miR-29a expression, leading to the invasion and metastasis [51].

Dinami et al. (2017) [48] described the downregulation of miR-296-5p expression in breast cancer compared to healthy controls. In the breast cancer cell line models, overexpression of miR-296-5p reduced *hTERT* expression, suppressing tumor progression, metastasis, and neovascularization. In addition to *hTERT*, this miRNA targets *HMGAI*, *IKBKE*,

MAP2K3, *MMP1*, *PUMA* and *SCRIB* genes. Li et al. (2015) [69] described in the Hep-2 laryngeal cancer cell line that the overexpression of miR-299-3p was associated with the downregulation of *hTERT* mRNA and protein expression resulting in the inhibition of cell growth.

Qin et al. (2015) [33] described in colorectal cancer cells that miR-422a expression was downregulated and inhibited *hTERT* expression. In melanoma cells, Chai et al. (2018) [44] showed the downregulation of miR-455-3p and miR-497-5p in tumor tissue compared to matched nevi tissues. The tumor suppressor action of these miRNAs by downregulating *hTERT* expression, led to the inhibition of cell proliferation, migration, invasion, and to the promotion of apoptosis. In ovarian cancer, Bai et al. (2017) [70] described the downregulation of miR-532 and miR-3064 in ovarian tumor tissue compared to normal tissues. In the cell lines, the ectopic expression of these miRNAs downregulated *hTERT* expression levels by directly targeting its 3'-UTR regions, inhibiting cell proliferation and invasion of the ovarian cells.

Finally, Yan et al. (2018) [74] in the analysis of diverse types of cell lines, demonstrated in rectal cancer cells (RKO cells), that the overexpression of miR-615-3p reduced the expression of *hTERT*, affecting cell proliferation.

Altogether, these studies demonstrated the miRNAs' ability to directly regulate the expression of *hTERT* gene by the exogenous modulation of their own expression levels. The functional consequences of these expression modulations in the cancer phenotypes largely depend on the type of tissue and the cellular action of the miRNAs within the tumor cells, where they can function either as oncogenes or tumor suppressors. Among the miRNAs directly targeting the *hTERT* gene, miR-138 stands out exhibiting both tumor-suppressive and oncogenic effects across different cancer types. Similarly, miR-1182 has been shown to exhibit diverse roles, acting as tumor suppressors in some cancer types while promoting tumorigenesis in others.

MiRNAs that indirectly regulate the *hTERT* gene and telomerase expression

A total of 12 articles cited the association of miRNAs that indirectly regulate *hTERT* expression and/or telomerase function by modulating the expression of genes involved in the telomere/telomerase signaling pathways (Supplementary Table 3). In these articles, ten different miRNAs were described: miR-103 [56], miR-150 [61], miR-19b [66], miR-202 [67], miR-21 [46, 47], miR-22 [68], miR-296 [49], miR-34a [42, 43], miR-375 [71], and miR-380-5p [72]. The most frequent type of cancer studied (in two articles) was hepatocellular carcinoma [43, 56], followed by breast [49], cervical [68], colorectal [46], glioma [42], glioblastoma [47], HPV positive (including oropharyngeal primary tissue, cervical

and tongue cell lines) [49], lymphoma [61], melanoma [66], malignant peritoneal mesothelioma cells [72], osteosarcoma [49], and pancreatic cancer [67].

MiR-34a was cited in two studies, one in gliomas [42] and one in hepatocellular carcinoma [43]. Li et al. (2019) [42] showed in glioma cell lines that the overexpression of miR-34a, by exogenous expression from mesenchymal stem cells, regulated telomerase activity by *SIRT1* downregulation (an anti-senescence factor) and caused the expression of senescence-related genes *TP53*, *CDKN1A*, and *CDKN2C* resulting in cellular senescence. In the study of Xu et al. (2015) [43], overexpression of miR-34a in SMMC-7721 and HHCC cell lines, inhibited telomerase activity and telomere length in liver cancer cells by negatively regulating the *hTERT* activators, *FOXMI* and *c-MYC*. These genes are transcription factors that regulate several biological processes with relevance to tumorigenesis, including cell cycle, cell proliferation, differentiation, renewal, survival, and senescence [76–78]. In human HCC tissues, miR-34a was down expressed and was correlated with tumor malignant features and poor prognosis [43]. The authors of these two papers point out that miR-34a may offer a potential strategy for the treatment of the cancers studied [42, 43].

Other miRNAs which expression alterations were associated with the *hTERT* expression were miR-19b, miR-202, miR-21, and miR-22. The overexpression of miR-19b was shown to increase *hTERT* expression in melanoma cell lines, by the downregulation of *PITXI*, a homeobox gene that acts as a *hTERT* suppressor gene [79]. Lower expression of miR-202, mediated by the Adamantyl Retinoid-Related (ARR) molecule 3-CI-AHPC, induced the increased expression of *MXDI* (*MAD1*) gene, a transcription factor that mediates cellular proliferation, differentiation and apoptosis [80]. The *MDXI* increased expression inhibited *MYC* and *hTERT* expression and promoted apoptosis in pancreatic carcinoma cell lines [67]. The up regulation of the *MXDI* and its family members, directly bind to the *hTERT* promoter, inhibiting its expression. Yang et al. (2015) [46] described the regulation of *hTERT* expression by miR-21 via PTEN/PI3K/AKT signaling pathway, in colorectal tumors. This miRNA was also observed affecting *hTERT* expression in HCT166 cell line, inhibiting cell proliferation through inactivation of *hTERT* and *ERK1/2* genes [46]. The PTEN/PI3K/AKT signaling pathway is one of the most affected signaling pathways in cancer cells, that regulates the signaling of multiple biological processes, such as apoptosis, metabolism, cell proliferation, and growth [81, 82]. AKT is a central protein of this pathway and was shown to be essential for telomerase activation, telomere protection, and maintaining telomere length [83]. MiR-21 was also described affecting *hTERT* in glioblastoma cell lines, where the repression inhibited *hTERT* mRNA expression by modulating the expression

of *STAT3*, a signal transducer gene that targets critical cell signaling genes, such as *CYCLIN D1* and *c-MYC* [84] and inhibited the cell proliferation and induced cell cycle G0/G1 phase arrest and cell apoptosis [47]. On the other hand, miR-22 was associated with the reduction of the *hTERT* expression in cervical cancer cells by inhibiting its direct target *MYCBP*, a MYC binding protein [85]. This reduction led to an increase in the radiosensitivity of the cells and indicated the potential use of miR-22 as a novel radiotherapy approach in cervical cancer cells [68].

The miRNAs shown to regulate the telomerase activity, included: miR-103 [56], miR-150 [61], miR-296 [49], miR-375 [71], and miR-380-5p [72] (one study each). In the study of Xia et al. (2016) [56], the overexpression of miR-103 increased telomerase activity by AKAP12 downregulation, a scaffold kinase protein [86] that functions as tumor suppressor in hepatocellular cells, and increasing PKC α activity in HCC cell lines, another kinase protein. The downregulation of miR-150 was shown in the study of Watanabe et al. (2011) [61] in lymphoma cell lines to activate telomerase expression via AKT2 and DKC1, critical components of telomerase complex [83, 87]. The authors pointed out miR-150 function as a tumor suppressor that could potentially be used as therapeutic target in NK/T-cell lymphoma. Yoon et al. (2011) [49] were unable to demonstrate a significant impact of miR-296 on telomerase activity, by the regulation of p-21, WAF1 and p53 protein levels. However, the overexpression of this miRNA was observed in immortalized cells that presented with telomerase activation. In the study of Jung et al. (2014) [71], the reduction of TERT transcription by the ectopic expression of miR-375 was observed. This regulation occurred via CIP2A, E6, and/or E6AP, HPV associated proteins, in SiHa, HEK293, and HeLa, increasing p53, p21, and RB activities resulting in cell cycle arrest and cell proliferation inhibition. Finally, the study of Cimino-Reale et al. (2017) [72] reported the repression of telomerase activity by the overexpression of miR-380-5p, that directly targets the telomerase associated protein 1 (*TEP1*) and the testis-specific protein, Y-encoded-like 5 (*TSPYL5*) genes, resulting in the induction of apoptosis. These studies are described in Supplementary Table 3.

The findings from these studies underscore the pivotal role of miRNAs in modulating *hTERT* gene expression by targeting key genes involved in biological processes and signaling pathways within cancer cells, particularly those associated with telomere maintenance. Moreover, the intricate interplay between several of these genes further contributes to the modulation of telomerase activity and can ultimately dictate the senescence or survival fate of the cancer cells. Among the genes implicated in these processes, *MYC*, *AKT2*, *TP53*, and *p21* were frequently cited as pivotal regulators capable of modulating telomerase activity.

MiRNAs role in telomere length maintenance and protection

Nine studies described nine microRNAs that affected the telomere length and protection (Supplementary Table 4). MiR-155 [62], miR-182-3p [63], miR-185 [64], miR-193b-3p [65], miR-29a-3p [50], miR-34a (cited by two studies [41, 42]), miR-376a-3p [50], miR-490 [73], and miR-708 [75]. The most frequent type of cancer studied was colon cancer (cited by two studies) [50, 65], followed by breast [62], colorectal [65] and gallbladder [41] cancer, glioma [42], and glioblastoma [73].

MiR-34a was shown to be associated with telomere length in gallbladder cancer [41] and glioma cell lines [42]. In gallbladder tumor tissues, the overexpression of miR-34a, decreased telomere length by downregulating the expression of *PNUTS*, a regulator of the Protein Phosphatase 1 [88], which resulted in tumor growth inhibition [41]. In glioma cell lines, miR-34a overexpression delivered by human mesenchymal cells (hMSCs) was associated with the downregulation of the autophagy and apoptosis regulator *SIRT1* gene, which led to the higher expression of p53, decreased telomere length, and cellular senescence [42]. In both articles miR-34a was demonstrated to act as a tumor suppressor, opening new possibilities for its use as an effective therapy for these tumors.

MiR-155, miR-193b-3p, miR-29a-3p, miR-376a-3p, and miR-490 were also described affecting telomere length by the interaction mostly with shelterin complex proteins that directly regulate telomere length. The overexpression of miR-155 reduced the expression of the *TRF1* gene, a member of the shelterin complex which protects chromosome ends, regulates telomere length, recombination, and DNA damage checkpoints [89], and decreased telomere length in breast cancer cell lines. In clinical cases, the overexpression of this miRNA was associated with poor clinical outcomes in patients with the luminal subtype of breast cancer [62]. In the Dinami et al. (2022) study [65] it was shown that the *TRF2* gene, another member of the shelterin complex that protects telomere length [90], cooperated with *CTCF*, a chromatin organization factor [91], and led to the overexpression of miR-193b-3p. This upregulation resulted in the decrease of the expression of the *SUV39H1* gene, a histone methyltransferase, and increased cell growth. Liu et al. (2021) [50] described in colon cancer, that miR-29a-3p and miR-376a caused dysregulation in telomere length by binding to the *CTC1* gene, a member of the heterotrimeric CTC1-STN1-TEN1 (CST) complex which is involved in the repair of replication errors to facilitate telomeric DNA and genomic DNA replication [92, 93]. Re-expression of *CTC1* in the colon cancer cells restored the telomere length. These two miRNAs however, acted in the colon cancer cells distinctly: miR-29a-3p expression alterations led to the increase in the

frequency of telomere's signals and induction of telomere replication stress, while alterations in miR-376a expression led to replicative telomere damage, resulting in cancer progression. In glioblastoma cell lines, the overexpression of miR-490 decreased telomere length by repressing *TERF2*, *TNKS2* and *SMG1* genes, causing activation of p53 pathway, suppression of tumorigenicity and DNA damage [73].

Finally, other three miRNAs, miR-182-3p, miR-185, and miR-708, were described associated with DNA damage. MiR-182-3p [63] caused DNA damage by regulating *TRF2* expression and affecting the telomeric and pericentromeric regions in breast cancer; miR-185 overexpression led to downregulation of the *POT1* (Protection of telomere 1) gene, and increased of the ATR (Ataxia Telangiectasia and Rad3-related kinase) signaling pathway [94] causing telomere DNA damage and promoting senescence in lung cancer cells [64]; and miR-708 overexpression caused downregulation of the *MRE11A*- and *BRCA1* genes (involved in DNA damage response) [95, 96], expression and resulted in DNA damage, suppression of cell migration, invasion, and angiogenesis and telomere dysfunction [75]. In TEP cells the overexpression of miR-708 caused DNA damage by affecting genes of the CARF-p53 pathway, a tumor suppression pathway that acts as a molecular sensor and regulator of cellular stress, senescence, and immortalization [75].

In this section, a notable trend observed across most studies (detailed in Supplementary Table 4) was the involvement of miRNAs in regulating genes associated with the telomere shelterin complex, particularly the TRF family of genes. This observation is not unexpected, given the fundamental role of the shelterin complex in protecting chromosome ends, regulating telomere length, facilitating telomere replication, and coordinating DNA damage responses. By targeting genes within the shelterin complex, miRNAs exert regulatory control over these critical aspects of telomere biology, thereby influencing telomere maintenance pathways and impacting cellular senescence. This emphasis on shelterin complex regulation also highlights the significance of miRNA-mediated mechanisms in fine-tuning telomere dynamics and underscores their importance in maintaining genomic stability and cellular homeostasis.

Conclusions

In this comprehensive systematic review, we have identified 47 articles that collectively described the involvement of 40 different miRNAs in modulating *hTERT* expression, telomerase activity, and telomere length and function. These miRNAs exert their influence through distinct molecular mechanisms, acting both directly and indirectly and functioning both as oncogenes and tumor suppressors across several types of cancer cells. The scope of the

selected studies encompassed clinical samples and cell line models, including the isogenic telomerase positive (TEP) and alternative mechanism of lengthening of telomeres (ALT) cell lines. It is important to acknowledge that the diverse methodologies employed, and the wide variety of tumor cell models utilized across these studies introduce inherent variability and complexity. In addition, the intricate interplay between miRNAs and telomere biology is influenced by numerous biological factors, including cell type, genetic background, and tumor microenvironment, which can lead to distinct experimental outcomes. Therefore, reaching a definitive conclusion regarding the precise role of the cited miRNAs may be difficult. However, despite these challenges, the findings from these studies strongly support the role of miRNAs in telomere biology. The intricate regulatory mechanisms of miRNAs, involving the expression regulation of target genes within critical cancer associated signaling pathways, highlights the complex regulatory networks underlying telomere maintenance. Moreover, these findings emphasize the potential of targeting miRNAs and key regulatory genes as a strategy to disrupt cancer cell growth and promote senescence. By directly or indirectly blocking *hTERT* gene expression and telomerase activity, tumorigenesis can be inhibited, and cancer progression contained. Overall, understanding the intricate interactions between miRNAs, target genes, and cellular signaling pathways represents a crucial step toward the development of novel target therapies directed to the manipulation of telomerase activity and induction of cellular senescence in cancer.

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Declarations

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