Telomerase: An Exploration toward the End of Cancer

Abstract

Background: The distinguishing feature of cancer cells is their ability to proliferate indefinitely, which is in contrast to the restricted cell multiplication potential for somatic cells. A better understanding of this contrasting behavior was provided in the early 1990s with the discovery of a relationship between telomeres, telomerase, aging, and cancer. Telomeres (tandem repeat DNA sequence TTAGGG) are protective caps at the ends of human chromosomes. Normal human cells experience telomere shortening with each successive cell division. However, in tumor cells, an overexpression of telomerase confers limitless replicative potential to tumor cells by continuous elongation of telomeres. The objective of this review was to systematically assess the data available on telomerase expression in oral cancer, with special reference to its role in diagnosis, prognosis, and treatment. Materials and Methods: A systematic review of studies that investigated the telomerase expression in oral squamous cell carcinoma (OSCC) was registered with PROSPERO. Subsequent to registration, a predetermined search strategy in accordance with PRISMA guidelines was formulated, and a literature search was conducted using online databases along with hand searching. Results: Eighty-nine articles from PubMed, 83 from Scopus, 5 from BioMed Central, 43 from Google Scholar, and 2 from hand search were identified. A total of 21 articles were shortlisted that met strict inclusion and exclusion criteria and quality assessment. Each study was evaluated for the markers under study, type of sample used, study design/methodology, and statistical analysis. The studies were then grouped into three subheads depending on their implications in the diagnosis, prognosis, and treatment of OSCC. Conclusion: This review explains the basic biology and the clinical implications of telomerase-based diagnosis and prognosis, the prospects for its use in anticancer therapy, in the context of oral cancer.

Keywords: hTR, human telomerase reverse transcriptase, human telomerase RNA component, oral cancer, oral squamous cell carcinoma, reverse transcriptase, reverse transcriptase catalytic protein, telomerase, telomerase RNA component

Introduction

The classical hallmark of a malignant cell is its ability to multiply indefinitely.[1] This immortalization of malignant cells has been credited primarily to reactivation of the telomerase enzyme which sustains the telomere length. Telomeres are nucleoprotein structures which cap the ends of eukaryotic chromosomes and show progressive shortening with cellular multiplication. Telomere dysfunction has been known to produce the opposing pathophysiological states of degenerative aging or cancer.[2]

The telomerase enzyme complex consists of two subunits, the reverse transcriptase catalytic protein (telomerase reverse transcriptase [TERT]), and the telomerase RNA component (TERC).

Telomerase helps preserve genome stability as well as replication potential in both embryonic stem cells and proliferating progenitor cells derived from quiescent normal stem cells (e.g., male germline spermatocytes), but it is silent in somatic cells, which make up the vast majority of human tissues.[3] Normal somatic cells thus enter replicative senescence and undergo growth arrest or apoptosis. Sporadically, some cells may evade these cellular checkpoints and continue to grow limitless. These cells are characterized by maintenance of telomere length and telomerase expression.[4] Past research has shown that telomerase is activated as a rule in human malignant tissues but not in adjacent normal tissues.[5] In addition, previous studies have shown that the lack of telomerase activity correlates with critically shortened telomeres and frequent spontaneous cancer remission.[6] Thus, the expression of...
telomerase is important and may be a rate-limiting step for tumor progression.[7] Owing to its selective expression in cancer cells, telomerase targeted cancer therapeutics offer superior specificity, lesser toxicity, and fewer side effects in contrast to conventional chemotherapeutic approaches.[4]

In the past few years, valuable research from various laboratories has provided major insights into telomerase, and telomeres leading to their use as diagnostic and prognostic markers in several types of cancer. This review is an attempt to systematically analyze the data available on telomerase-based diagnosis and prognosis, the prospects for its use in anticancer therapy in the context of oral squamous cell carcinoma (OSCC).

Materials and Methods

This systematic review (PROSPERO registration number CRD42016043162) was carried out as a structured search following guidelines suggested by PROSPERO, to identify all reports of investigations that had been undertaken to assess the role of telomerase in OSCC. A search strategy was finalized utilizing MESH terms, Boolean terminology, and free text terms [Annexure 1] with key words Telomerase, hTR, TERT, human telomerase reverse transcriptase (hTERT), TERC, human telomerase RNA component (hTERC), reverse transcriptase catalytic protein, TERC, oral cancer, and OSCC. This search strategy was applied to key databases such as PubMed, Biomed Central, Scopus, and Google scholar and identified articles published from 2006 to 2016 independently by two reviewers, which was cross-checked by the third reviewer. The search was augmented by using the “related articles” link to articles recovered with PubMed. Following this, a search was done of the references cited in these articles to identify additional relevant writings. In addition, hand search of journals was performed for article retrieval. At this stage, all articles were assembled and arranged in reverse chronological order. The articles were screened, and selection of articles to be considered for review was based on stringent inclusion and exclusion criteria. Eighty-nine articles in PubMed, 83 in Scopus, 5 in BioMed Central, 43 in Google Scholar, and 2 from hand searching were retrieved [Figure 1]. Duplicates were separated from the selection, and titles and abstracts of these selected manuscripts were studied, considering exclusion and

![Figure 1: Flowchart depicting the retrieval of studies for review process](image-url)
Inclusion criteria. From the 28 identified articles, a seven had to be excluded (PubMed - 2, Scopus - 1, BioMed Central - 1, and Google Scholar - 3) due to nonavailability of full text. After scrutiny by all reviewers, 21 articles were identified for systematic review [Figure 1].

Inclusion criteria

Participants/population - Human studies, OSCC cell lines, controls, and sample size >15.

Intervention (s), exposure (s) - All original research works done on telomerase/hTERT/hTERC/terminal transferase/TERC in OSCC will be included in the study.

Exclusion criteria

Participants/population - Animal studies, xenograft models, sample size <15, and controls absent.

Intervention (s), exposure (s) - Studies using markers other than telomerase/hTERT/hTERC/terminal transferase/TERC in OSCC. Review articles will not be included.

This was followed by data extraction done independently by two reviewers. The data were recorded in a tabular form based on the following criteria:

1. Participant characteristics: tissue selected, cell line, site of tissue selection, and sample size
2. Study characteristics: marker under study, method used for research, and statistical analysis used.

Quality assessment of the articles included in the review was done based on a quality assessment instrument modified and developed from relevant articles in literature[8,9] given in Annexure 2.

The studies were analyzed and inferences were drawn after grouping of observations from relevant studies to arrive at conclusions with diagnostic, prognostic, and therapeutic implications.

Results

Twenty-one research studies analyzing telomerase activity in OSCC were shortlisted for the systematic review. Sixteen studies focused on assessing the expression of reverse transcriptase catalytic protein (TERT/hTERT) or the TERT gene as a marker for telomerase activity. Two studies used fluorescent in situ hybridization to detect TERC (TERC/hTERC) gene amplifications. Three studies used electrochemical telomerase assay to assess the telomerase activity and one study dealt with an association of glutathione S-transferase M1 (GSTM1) polymorphism with telomerase activity.

Data extraction was done, and the studies were grouped under the following subheads: implications in diagnosis [Table 1], implications in prognosis [Table 2], and therapeutic implications [Table 3].

It was noted that most studies in Tables 1 and 2 were retrospective studies done on tissues from patients. Nine studies compared OSCC with oral premalignant/dysplastic lesions,[10,11,17,18,20‑23,25] whereas five studies compared OSCC with healthy oral mucosa[10,13,17,20,23] Four studies were carried out on exfoliated cells[11,13,15,16] and four others were done on OSCC cell lines.[7,14,19,22] Strikingly, most studies from Table 3 (therapeutic implications) were done on OSCC cell lines[27‑29] and one on tissue microarray of OSCC.[30]

Discussion

Telomeres are the extreme ends of double-stranded eukaryotic chromosomes comprising tandem array of TTAGGG repeats and DNA binding proteins. In humans, it consists of repeats of TTAGGG with a 3’ end overhang that helps in the formation of D-loop and T-loop structures. Telomeres protect the chromosomal ends from degradation by exonucleases and prevent recognition as double-stranded DNA breaks, end-to-end fusions, and ring chromosome formation. Thus, telomeres play a vital role in the regulation of gene expression, functional organization of the chromosome, and in controlling the replicative life of cells and entry into senescence.[56]

Progression to malignancy requires that cells overcome senescence and switch to an immortal phenotype. Mammalian cells have an intrinsic program, the Hayflick limit,[31] that limits their multiplication to about 60–70 doublings, at which point they reach a stage of senescence. The cell division limit can be overcome, allowing them to continue doubling until they reach a critical stage (M2 or crisis). At this point, chromosomal instability arises due to end-to-end fusions and/or chromosome breakage. DNA damage checkpoints are activated along with apoptosis. Unless the cell develops a mechanism through which to stabilize telomere length, it will not survive. Cells that escape crisis and become immortalized generally achieve telomeric stability through the reactivation of telomerase.[32]

Telomerase is a ribonucleoprotein that acts to elongate telomeres in cells that possess its activity.[33] This enzyme is expressed during embryonic development, loses its expression during differentiation of somatic cells, and is almost undetectable in most normal human somatic cells.[34] By contrast, telomerase is expressed in ~85% of human cancers.[11,35] There are a few types of cells that normally express telomerase including germline cells, stem cells, hematopoietic cells, cells lining the intestine, and other rapidly proliferating cells. The widespread expression of telomerase in a variety of human cancers, while being almost undetectable in most normal cells, makes it a very attractive drug target.[32]

Diagnostic potential

The hTERT, the catalytic subunit of telomerase, is strongly associated with telomerase activity implicated in cellular immortalization and tumorigenesis.[33] Most
Table 1: Diagnostic implications

| Author/year                  | Marker                  | Type of sample                                      | Method/technique                                      | Statistical analysis                           | Findings                                                                 |
|------------------------------|-------------------------|-----------------------------------------------------|-------------------------------------------------------|-------------------------------------------------|--------------------------------------------------------------------------|
| Raghunandan et al., 2016(10) | hTERT                   | Archival biopsy tissues. OED (n=21), OSCC (n=20) and NOM (n=10) | IHC                                                   | Pearson’s Chi-square test, ANOVA using SPSS version 16 | Intense expression of hTERT in potentially malignant lesions and OSCC suggests telomerase activity is involved in the development of dysplastic epithelium leading to multistage oral carcinogenesis. |
| Hayakawa et al., 2016(11)    | hTERT and telomerase activity | EOCs and tissues of oral cancer (n=30), mucosa-associated disease (n=30) and healthy volunteers (n=30) | hTERT mRNA expression levels by RT-PCR and telomerase activity with ECTA | Kruskal-Wallis tests and the Steel-Dwass method using Microsoft excel 2010 | hTERT expression, telomerase activity highest in patients with oral cancer and lowest in healthy volunteers. EOCs and tissues from the oral cavity could be used for diagnosis of oral cancer by ECTA. |
| Vinothkumar et al., 2016(12) | TERT                    | 181 primary tumors of the uterine cervix and oral cavity (140 uterine cervix and 41 oral cavities) | PCR amplification and sequencing of TERT gene Promoter, screening for high-risk HPV-16 and HPV-18 by real time PCR | Statistical analyses with Fisher’s exact test were performed using GraphPad Prism version 6 | High frequency of TERT hotspot mutations in both cervical (21.4% [30/140]) and oral (31.7% [13/41]) SCCs TERT reactivation through promoter mutation - important role in the carcinogenesis of cervical and oral cancers No significant differences in sensitivity and specificity associated with age, size of tumor, site of lesion, or degree of malignancy. ECTA, therefore, seems to be a promising assay for screening for oral cancer miR-31 collaborates with hTERT to immortalize NOKs and that this may contribute to early stage oral carcinogenesis. The targeting of downstream factors by miR-31 may further advance the neoplastic progression of immortalized NOKs, allowing them to become malignant SOX, and TERC gene amplifications are common in all SCCs, and their detection in early stages could be crucial for early detection and more accurate prognosis of OSCC |
| Hayakawa et al., 2016(13)    | Telomerase              | Exfoliated cells from the whole oral cavity, exfoliated cells from local lesions, and tissue from the lesion itself from 44 oral cancer patients and 26 healthy volunteers | ECTA                                                   | Mann-Whitney U-test using SPSS software (version 11, SPSS, Chicago, IL, USA) | The Mann-Whitney test, analysis of variance test and linear regression analysis |
| Hung et al., 2014(14)        | miR-31 and hTERT         | NOKs and OSCC cell lines - SAS and OECM-1           | In situ hybridization, viral infection for exogenous gene expression, quantitative RT-PCR analysis, p53 mutation, Western blot analysis, IHC | The Mann-Whitney test, analysis of variance test and linear regression analysis | The Mann-Whitney test, analysis of variance test and linear regression analysis |
| Kokalj Vokac et al., 2014(15)| TERC-specific DNA probe and a SOX, DNA specific probe | Brush biopsies from 71 patients (exophytic and exulcerated oral and oropharyngeal lesions) and 22 healthy controls | Interphase FISH with a chromosome enumeration double-color DNA probe | ORs and the Fisher’s exact test was used to compare groups | The Mann-Whitney test, analysis of variance test and linear regression analysis |

Contd...
### Table 1: Contd...

| Author/year | Marker | Type of sample | Method/technique | Statistical analysis | Findings |
|-------------|--------|----------------|------------------|----------------------|----------|
| Mori et al., [16] | Telomerase | In oral cancer patients and 10 healthy volunteers, clinical samples were EOCs and tissue. In 17 healthy volunteers, only EOCs were collected | ECTA using FND as the probe. Real-time RT-PCR, and TRAP assay | Electrochemical data in the presence and absence of oral cancer - derived cultured cells and on clinical samples was evaluated by the Student’s t-test | The ECTA yielded high hit rates for cancerous and normal cells, especially in EOCs, results indicating that this minimally invasive test is suitable for oral cancer diagnosis |
| Palani et al., 2011[17] | hTERT | OSCC (n=30), leukoplakia (n=15), OSF (n=15) and NOM (n=10) | IHC | Pearson’s χ² test, Kappa statistics, ANOVA, Student’s t-test | Increased expression of hTERT protein in OSCC and leukoplakia samples when compared to NOM. hTERT immunostain parameters (cellular localization, nuclear labelling indices and nuclear LSs) in OSF were significantly different from OSCC and leukoplakia |
| Abrahao et al., 2011[18] | p53 and p16 and hTERT | 15 PMD and 30 OSCC and 5 OEH | IHC | Pearson correlation, Fisher’s exact test, Kruskal-Wallis and Mann-Whitney tests using Graph Pad Prism 5.00, USA | The intense hTERT expression in OEH, PMD and OSCC suggests that telomerase activity is involved in the development of hyperplastic and dysplastic oral epithelium. No correlation with the grade of dysplasia in PMD or with the differentiation degree of OSCC |
| Kang et al., 2009[19] | hTERT | Cell lines: Primary NHOK and NHOF Human OSCC cell lines (SCC4, SCC9, SCC15, HEP-2, FaDu, BaP-T, and 1483) | Promoter magnetic precipitation assay, Western blotting, 2DGE, mass spectrometry, RT-PCR, protein function analysis, telomerase assay | - | MSH2, the hnRNPs and GRHL2 as novel hTERT promoter-binding proteins. Since these proteins were necessary for the intact expression of the hTERT promoter activity in OSCC cells, we speculate that they are in part responsible for the elevated hTERT expression and telomerase activation during oral carcinogenesis |
| Kim et al., 2008[7] | Hsp90 and hTERT | HOK-Bmi-1/E6 Immortalized cell Population, HNOK, OSCC cell lines | Western blotting, TRAP assay, semiquantitative RT-PCR, analysis of the hTERT promoter activity, ChIP assay | - | Physical interaction between Hsp90 and the hTERT promoter occurs in telomerase-positive cells but not in normal human cells. Hsp90 association with the hTERT promoter complex may, in part, be responsible for telomerase activation during cellular immortalization |

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Table 1: Contd...

| Author/year | Marker | Type of sample | Method/technique | Statistical analysis | Findings |
|-------------|--------|----------------|------------------|----------------------|---------|
| Chen et al., 2007[20] | hTERT | 82 specimens of OSCC, 116 specimens of OED, and 21 specimens of NOM | IHC | ANOVA, Student’s t-test, Chi-square test and log-rank test with the statistic program (StatSoft Inc., USA) | Increased expression of hTERT protein is an early event in oral carcinogenesis and hTERT may be a biomarker for OSCCs. Measuring the amount of cytoplasmic or nuclear expression of hTERT in OSCC samples may predict the oral cancer progression, recurrence, and prognosis |

Table 2: Prognostic implications

| Author/year | Marker | Tissue/site/sample | Method/technique | Statistical analysis | Findings |
|-------------|--------|--------------------|------------------|----------------------|---------|
| Raghunandan et al. 2016[10] | hTERT | Archival biopsy tissues. OED (n=21), OSCC (n=20) and NOM (n=10) | IHC | Pearson’s Chi-square test, ANOVA using SPSS software version 16.0 | Increase in the mean percentage of cells showing hTERT expression from NOM to OED to OSCC |
| Dorji et al., 2015[21] | hTERC | 30 OPMLs | Dual-color interphase FISH | Kaplan-Meier analysis using MedCalc® version 14.8.1 Fisher’s exact test using GraphPad QuickCalcs on-line tool | Precise morphological evaluation together with FISH assessment for hTERC gain might pave the way to stratify OPMLs into high-risk and low-risk categories and could be helpful in selecting the most appropriate treatment |
| Miyazaki et al., 2015[22] | hTERT, telomerase activity | 46 cases of epithelial dysplasia (including carcinoma in situ) and 15 cases of OSCC The human gingival SCC-derived cell line, Ca9-22, and the human tongue SCC-derived cell lines HSC-3 and HSC-4 | IHC, PCR-ELISA | Mann-Whitney U-test | Chronic inflammation, progressive epithelial dysplasia and long-term exposure to inflammatory cytokines lead to telomerase expression. This in turn leads to malignant transformation and regulates the invasion of certain types of oral cancer cells |
| Zhao et al., 2015[23] | hTERT | Paraffin embedded 37 OSCC, 15 OED samples and 10 matched adjacent NOM | Primary cultures of Human OSCC, lentiviral vector constructs, siRNA synthesis, IHC analysis, confocal immunofluorescence microscopy, wound healing assay, Western blot analyses | The mean hTERT LS and clinicopathological characteristics for OSCC, OED, and NOM samples were compared by ANOVA and Student’s t-test | The expression of hTERT increases from NOM to OED and OSCC. hTERT is over expressed in OED and OSCC tissues and correlates with clinical aggressiveness of OSCC patients |

IHC=Immuno-histochemistry, ANOVA=Analysis of variance, hTERT=Human telomerase reverse transcriptase, OSCC=Oral squamous cell carcinoma, EOCs=Exfoliated oral cells, RT-PCR=Reverse transcription polymerase chain reaction, ECTA=Electrochemical telomerase assay, HPV=Human papillomavirus, SCCs=Squamous cell carcinomas, NOKs=Normal oral keratinocytes, FISH=Fluorescence in situ hybridization, ORs=Odds ratios, hTERC=Human telomerase RNA component, FND=Ferrocenylnaphthalene diimide, TRAP=Telomerase repeat amplification protocol, PMD=Potentially malignant disorders, OEH=Oral epithelial hyperplasia, 2DGE=Two-dimensional gel electrophoresis, Hsp=Heat shock protein, ChiP=Chromatin immunoprecipitation, OED=Oral epithelial dysplasia, NOM=Normal oral mucosa, LSs=Labelling scores, OSF=Oral submucous fibrosis
studies in our review employed hTERT to study telomerase activity in OSCC. Immunohistochemical labeling for hTERT, telomerase repeat amplification protocol (TRAP), polymerase chain reaction amplification, and sequencing of TERT gene, ECTA, and interphase FISH were the main methods for the selected studies. Archival biopsy tissues of diagnosed OSCC patients and potentially malignant disorders, exfoliated oral cells, OSCC cell lines, and normal oral mucosal biopsies were used as study samples. The following inferences were drawn from the studies under consideration:

a. Telomerase activity was highest in patients with OSCC and lowest in normal mucosa. The increased expression of hTERT protein is an early event in oral carcinogenesis and hTERT may be a biomarker for OSCCs.

b. ECTA was shown in three studies to be a promising assay in comparison with TRAP for screening for oral cancer.

c. Physical interaction between heat shock proteins and the hTERT promoter occurs in telomerase-positive cells but not in normal human cells. Heat shock protein association with the hTERT promoter complex may, in part, be responsible for telomerase activation during cellular immortalization.

d. SOX2 and TERC gene amplifications are common in all squamous cell carcinomas and their detection in early stages could be crucial for early detection and more accurate prognosis of OSCC.

Prognostic potential

Telomerase activity as shown by the mean hTERT expression in cells showed a steady increase from normal oral mucosa to oral epithelial dysplasia to OSCC. The selected studies help us draw the following inferences. Oral premalignant lesions could be classified into high-risk and low-risk categories by morphological evaluation together with FISH assessment for hTERC gain. Telomere expression has been linked to Chronic inflammation, progressive epithelial dysplasia, and long-term exposure to inflammatory cytokines which in turn pave the way to malignant transformation and regulates the invasion

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**Table 2: Contd...**

| Author/year | Marker | Tissue/site/sample | Method/technique | Statistical analysis | Findings |
|-------------|--------|--------------------|------------------|---------------------|----------|
| Sainger et al., 2009<sup>[24]</sup> | GSTM1 polymorphism with telomere length and telomerase activity | Tissues specimens from 100 patients with confirmed diagnosis of oral cancer | Telomerase activity by TRAP assay, telomere length changes by Southern hybridization method, GSTM1 polymorphism by PCR | Student’s t-test, Chi-square test, Mann-Whitney U-test, multivariate analysis and Pearson’s correlation coefficients | Possible link between the absence of GSTM1 gene and telomere length alterations. Implicates the usefulness of GSTM1 polymorphism analysis and its role in determining individual susceptibility towards cancer and telomere associated changes |
| Pannone et al., 2007<sup>[25]</sup> | hTERT gene expression | Tissue specimens of oral dysplasia (15) and OSCC (42). Metastatic lymph nodes also collected. | Real-time RT-PCR and IHC protein analyses | ANOVA, student-Newman-Keuls test, Chi-square test and Kaplan-Meier’s methods | No significant relationship between hTERT expression and classical clinicopathological parameters |
| Sainger et al., 2007<sup>[26]</sup> | Telomere length, telomerase activity, TRF-1 and TRF-2 | Tissues specimens from 100 patients with confirmed diagnosis of oral cancer | Southern hybridisation method, Western blot method and telomeric repeat amplification protocol | Unpaired and paired t-tests, ANOVA, multivariate tests, Kaplan-Meier survival curves and log rank statistics | Significant clinical usefulness of telomere length, TRF and telomerase activation in the prognosis of oral cancer patients |
| Chen 2007<sup>[20]</sup> | hTERT | Specimens of OSCC (82), OED (116), and NOM (21) | IHC | ANOVA, Student’s t-test, Chi-square test and log-rank test | Significantly higher recurrence rate in OSCC patients with nuclear hTERT LSs >100% than in OSCC patients with nuclear hTERT LSs ≤100% |

hTERT=Human telomerase reverse transcriptase, OSCC=Oral squamous cell carcinoma, NOM=Normal oral mucosa, IHC=Immunohistochemistry, ANOVA=Analysis of variance, OPMLs=Oral potentially malignant lesions, FISH=Fluorescence in situ hybridization, hTERC=Human telomerase RNA component, SCC=Squamous cell carcinoma, RT-PCR=Reverse transcription polymerase chain reaction, ELISA=Enzyme-linked immunosorbent assay, OED=Oral epithelial dysplasia, LS=Labelling scores, GSTM1=Glutathione S-transferase M1, TRAP=Telomerase repeat amplification protocol, TRF=Telomeric repeat binding factor
Table 3: Therapeutic implications

| Author/year | Marker | Tissue/site/sample | Method/technique | Statistical analysis | Findings |
|-------------|--------|--------------------|------------------|----------------------|----------|
| Tian *et al.*, 2015[^27] | hTERT | The CAPAN-2 human pancreatic cancer cell line (HTB-80) and the CAL-27 human oral cancer cell line (CRL-2095) | Cell Viability Test, Annexin V fluorescein staining assay, Comet assay for DNA damage, telomerase activity assay, design and transfection of small interfering RNA, real-time PCR, Western blotting for hTERT | Two-tailed Student’s t-test or post hoc Bonferroni’s test using GraphPad Prism 5 software | Bufalin down regulates hTERT expression via the activation of the JNK/p38 pathway, providing new insights into the mechanisms underlying bufalin’s anti-cancer activity |
| Zhao *et al.*, 2015[^23] | hTERT | Paraffin embedded 37 OSCC, 15 OED samples and 10 matched adjacent NOM | Primary cultures of human OSCC, lentiviral vector constructs, siRNA synthesis, IHC analysis, confocal immunofluorescence microscopy, wound healing assay, Western blot analyses | The mean hTERT LS and clinicopathological characteristics for OSCC, OED, and NOM samples were compared by ANOVA and Student’s t-test | hTERT provides an explanation for the aggressive nature of human tumors and the possibly mechanism that links hTERT to EMT property, which may partially by targeting activation of the Wnt/β-catenin pathway. Thus, hTERT represents a possible therapeutic target in highly metastatic cancers |
| Liu *et al.*, 2011[^26] | hTERT | PAMAM dendrimers, generations (G), Human oral cancer cell lines (Tca8113 and SCC-9) | Real-time RT-PCR analysis, Western blot analysis, telomerase activity assay, IHC, cell apoptosis and proliferation assay | One-way ANOVA, two-tailed Student’s t-test and Mann-Whitney U-test. (Statview 4.01, Abacus Concepts) | Dendrimer-mediated shRNA efficiently silenced the hTERT gene in vitro, resulting in cell growth inhibition and apoptosis. RNAi - mediated hTERT gene silencing, coupled with dendrimer delivery, may provide a promising approach for the treatment of oral cancer |
| McCaul *et al.*, 2008[^29] | hTERT, telomerase activity | OSCC cell lines | Telomere function, as measured by the ABI, was tested as a predictor of radio-resistance in a panel of OSCC lines | Mann-Whitney U-test and regression analysis using the SPSS software package (SPSS Chicago, Illinois) | Telomerase inhibitors could sensitise a subset of oral SCCs with short telomeres to radiotherapy and for the first time demonstrate that the tumour ABI may assist the selection of cancers that would be suitable for such sensitisation therapy |
| Freier *et al.*, 2007[^30] | TERT gene and hTERT | TMA sections including 247 OSCC and 105 PSCC/LSCC | FISH for TERT and IHC for hTERT | Fisher’s exact test, Kaplan-Meier analysis, log-rank tests | High hTERT expression is a frequent finding in OSCC. It might be a promising target for the development of specific anti-neoplastic therapy approaches |

[^27]: Human telomerase reverse transcriptase, RT-PCR=Reverse transcription polymerase chain reaction, OSCC=Oral squamous cell carcinoma, OED=Oral epithelial dysplasia, NOM=Normal oral mucosa, IHC=Immu-histochemistry, LSs=Labelling scores, ANOVA=Analysis of variance, EMT=Epithelial mesenchymal transition, SCC=Squamous cell carcinoma, ABI=Anaphase Bridge Index, TMA=Tissue microarray, PSCCs=Pharyngeal squamous cell carcinomas, LSCCs=Laryngeal squamous cell carcinomas, FISH=Fluorescence in situ hybridization

of certain types of oral cancer cells[^22]. Telomere length, telomeric repeat binding factor, and telomerase activation have been strongly linked to the prognosis of oral cancer patients.[^26] A high recurrence rate in OSCC patients has been associated with high hTERT labeling indices. On the contrary, work done by Pannone *et al.* showed that telomere activity could not be linked to classical clinicopathological parameters, as there was no significant relationship between hTERT expression and several clinicopathological parameters such as tumor stage, size, and histological grade.[^23] GSTM1 polymorphism has also been linked to determine individual susceptibility toward cancer and telomere-associated changes.[^24]

**Therapeutic potential**

Several telomerase-based immunotherapy strategies have been developed and many are in advanced clinical trials, making this a rapidly progressing field of antitelomerase cancer therapy.[^37-40] Telomerase is an attractive target antigen for cancer immunotherapy because it is expressed
almost universally in human cancers and is functionally required to sustain malignant tumor long-term growth.\textsuperscript{[41]}

While telomerase is expressed in some normal tissues,\textsuperscript{[42-44]} no patients have exhibited serious adverse effects (such as autoimmune disease or bone marrow depletion) indicative of an immune response against normal cells. One explanation is that normal cells express very low levels of hTERT,\textsuperscript{[44]} making them poor targets relative to tumor cells with high levels of hTERT expression.

In OSCC patients, hTERT has been linked to epithelial mesenchymal transition, providing an explanation for the aggressive nature of human tumors and partially explained by activation of the Wnt/β-catenin pathway.\textsuperscript{[23]} Telomerase inhibitors could also be used to sensititize a subset of OSCCs with short telomeres to radiotherapy and for the first time demonstrate that the tumor Anaphase Bridge Index may assist the selection of cancers that would be suitable for such sensitization therapy.\textsuperscript{[29]} Thus, hTERT represents a possible therapeutic target in highly metastatic cancers.\textsuperscript{[23]}

**Conclusion**

This systematic review is focused on the association of OSCC and telomerase activity with special emphasis on telomerase-based diagnosis, prognosis, and the prospects for its use in anticancer therapy. The literature search and critical review suggested a positive link between increased expression of hTERT protein and oral carcinogenesis. Increased telomerase activity/hTERT expression correlates with poorer prognosis and a high recurrence rate for OSCC. However, there is a need for studies exploring its plausible role as a biomarker in diagnostic immunopathology. The telomere hypothesis of cancer cell immortalization remains an attractive yet not fully understood concept.

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**Conflicts of interest**

There are no conflicts of interest.

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Annexures

Annexure 1: Search strategy ([telomerase] or [telomerase reverse transcriptase] or [human TERT gene] or [reverse transcriptase catalytic protein] or [TRAP] or [terminal transferase] or [telomerase RNA component] or [telomerase RNA] or [hTR]) and ([oral cancer] or [oral squamous cell carcinoma] or [head and neck cancer])

Annexure 2: Quality assessment instrument

I. Study design
1. Objective – objective clearly formulated (Y), (No), (Unclear)
2. Sample size – considered adequate (Y), (No), (Unclear)
3. Spectrum of patients/sample representative of patients receiving the test in practice (Y), (No), (Unclear)
4. Was an appropriate sample size calculation performed and were sufficient patients included in the study (Y), (No), (Unclear)
5. Ethical clearance mentioned (Y), (No), (Unclear)
6. Selection criteria-clearly described (Y), (No), (Unclear)
7. Randomization – stated (Y), (No), (Unclear)
8. Baseline characteristics-clearly defined (Y), (No), (Unclear)
9. Control-clearly defined (Y), (No), (Unclear)
10. Were withdrawals from the study explained (Y), (No), (Unclear).

II. Statistical analysis
1. Dropouts – dropouts included in data analysis (Y), (No), (Unclear)
2. Statistical analysis – appropriate for data (Y), (No), (Unclear)
3. Statistical significance level – P value stated (Y), (No), (Unclear)
4. Confidence intervals provided (Y), (No), (Unclear).

III. Study results and conclusions
1. Conclusions-specific (Y), (No), (Unclear).