

**TELOMERASE- A BIOMARKER IN ORAL CANCER CELL PROLIFERATION.**

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**ABSTRACT**

As percent of cancer populations is increasing sharply, the incidence of oral squamous cell carcinoma (OSCC) has also been expected to increase as well. Cancer prevention is more important than treatment for overcoming increased cancer death in the future. Oral cancer is the most common cancer worldwide which continues to be the most prevalent cancer resulting from the consumption of tobacco, alcohol and other carcinogenic products. A large part of cancer load in parts of India is formed from Oral cancer. Oral cancer is categorized into precancerous and cancerous stages. Precancerous stage includes Leukoplakia, Erythroplakia and Lichen Planus, while cancerous or malignant stage is Squamous Cell Carcinoma. Oral cancer

development is a multistep process which arises from pre- existing malignant lesions. Oral carcinogenesis is a highly complex, multistep process which involves accumulation of genetic alterations that lead to the induction of proteins promoting cell growth (encoded by oncogenes), increased enzymatic (telomerase) activity promoting cell proliferation. Maintenance of telomeres plays an essential role during transformation of cancer to malignant stage. Mammalian telomeres, a specialized nucleoprotein structures are composed of large conctamers of the guanine-rich sequence 5\_-TTAGGG-3\_. The roles of telomeres in regulating both stability of genome and replicative immortality seems to contribute in

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essential ways in cancer initiation and progression. Telomeres are maintained by a multisubunit enzyme called telomerase, comprised of an RNA component, hTR and a protein reverse transcriptase component, hTERT.

**KEYWORDS:** Oral cancer, pre-cancer, telomere, telomerase activity, biomarker.

## INTRODUCTION

International Agency for Research on Cancer published the World Cancer Report and the World Health Organization projects 20 million new cancer patients globally in 2025, compared to 14 million cases in 2012.<sup>[1]</sup> As cancer populations is increasing sharply, the incidence of oral squamous cell carcinoma (OSCC) has also been expected to increase as well. Cancer prevention is more important than cancer treatment for overcoming increased cancer death in the future. The immortalization of normal epithelial cells is the initial step of cancer progression for which telomerase activity is required, which maintains telomere length by adding TTAGGG hexamers at the chromosomal end and inhibiting cellular senescence, eventually resulting in persistent epithelial proliferation.<sup>[2,3]</sup> Human telomerase reverse transcriptase (hTERT) has also active roles in tumorigenesis by preventing apoptosis<sup>[8,9]</sup> and by enhancing motility and invasiveness .

Oral cancer continues to be the most prevalent cancer resulting from the consumption of tobacco, alcohol and other carcinogenic products. In nature more than 95% of the carcinomas of the oral cavity represents squamous cell type.

A large part of cancer load in parts of India is formed from Oral cancer.<sup>[8]</sup> They constitute a major health problem in developing countries including India, representing an increase in mortality rate leading to the cause of death. The survival index continues to be small (50%), as compared to the progress in treatment and diagnosis of other malignant tumors. According to World Health Organization, in developing countries, oral carcinoma in males is the sixth commonest cancer after lung, prostate, colorectal, stomach and bladder cancer, while in females, it is the tenth commonest site of cancer after breast, colorectal, lung, stomach, uterus, cervix, ovary, bladder and liver.<sup>[9]</sup>

Oral cancer is categorized into precancerous and cancerous levels. Precancerous stage includes Leukoplakia, Erythroplakia and Lichen Planus, while cancerous or malignant stage is Squamous Cell Carcinoma. Oral cancer development is a multistep process which arises

from pre-existing malignant lesions. 85% of precancer represents lesions of Leukoplakia=<sup>[10]</sup> and 95% of oral cancers are squamous cell carcinomas.<sup>[11]</sup> In India the vast majority of oral squamous cell carcinomas arises from pre- cancer, Leukoplakia.<sup>[12]</sup>

Oral carcinogenesis is a highly complex, multistep process which involves accumulation of genetic alterations that lead to the induction of proteins promoting cell growth (encoded by oncogenes), increased enzymatic (telomerase) activity promoting cell proliferation as well as the loss of proteins which restrain cell proliferation (encoded by tumor suppressor genes).<sup>[13]</sup> The molecules (telomerase) involved in these processes may therefore provide markers for the early detection of malignant transformation.

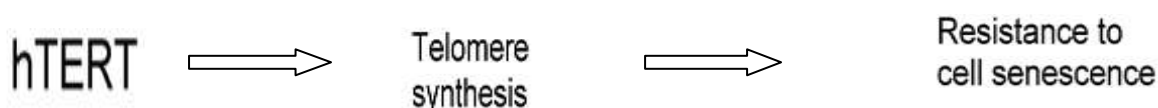
Maintenance of telomeres plays an essential role during transformation of cancer to malignant stage. Mammalian telomeres, specialized nucleoprotein structures are composed of large conctamers of the guanine-rich sequence 5\_-TTAGGG-3\_, constitute the ends of eukaryotic chromosomes which serves to protect linear chromosomes from damage and also promote genomic stability to the chromosomes . In addition to this protective function, the regulation of telomere length as an important factor in regulating cellular life span. The roles of telomeres in regulating both stability of genome and replicative immortality seems to contribute in essential ways in cancer initiation and progression. Telomeres are maintained by a multisubunit enzyme telomerase, comprised of an RNA component, hTR (template for the synthesis of telomeric repeats) and a protein reverse transcriptase component, hTERT, it catalyzes the synthesis reaction.

Activity of telomerase, is generally absent in normal tissues. It is known to be induced upon immortalization or malignant transformation of human cells such as in oral cancer cells. Telomerase activity is present in highly proliferative normal tissues in the oral mucosa.<sup>[14]</sup> Telomerase activity therefore, supports the use of telomerase a biomarker for detection of tumors.

**Telomere:** “Telomeres”, the specialized structures containing unique simple repetitive sequences (TTAGGG in humans) are present at the ends of eukaryotic chromosomes. Telomeres help in maintaining the integrity of human chromosome. Genetic instability are caused by the dysfunction in telomeres which causes increased incidence of cancer cell proliferation. Telomeres main function is to prevent the chromosomal fusions, chromosomal instability (CIS), and the activation of DNA-damage responses by capping the chromosomal

ends.<sup>[15,16]</sup> During each round of cell division telomeres shorten due to the “end-replication inefficiency” of enzyme DNA polymerase.<sup>[17]</sup>

Function of Telomeres is to prevent the loss of DNA sequences that occurs as a consequence of the incomplete replication of linear DNA at chromosome ends. They are replicated by telomerase, which is inactive in most somatic cells, and thus at each generation the ends of the DNA shorten by 40–50 bp. When telomeres shorten to a critical length, cellular senescence is induced and normal cells cease proliferating. In most tumors, however, cells have acquired the capability to maintain telomere length through reactivation of telomerase.



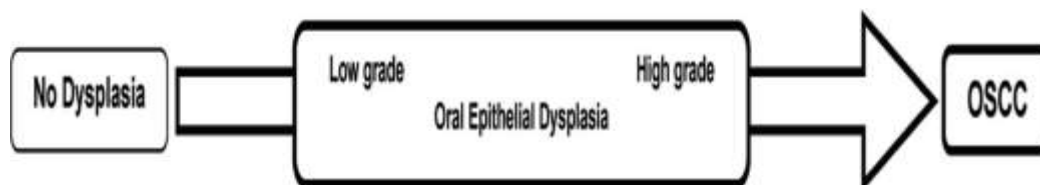
The telomerase, a holoenzyme compensates for DNA polymerase’s inability by synthesizing telomere repeats *de novo*. Telomerase, consist of RNA component (TERC), which serves as a template for the synthesis of telomere sequence, and the reverse transcriptase (TERT), the catalytic component of the holoenzyme. The proliferative capacity of primary human cells is maintained by telomere shortening. Telomeres reach a critical short length and lose capping function at the senescence stage. Telomere shortening apparently has a dual role in development and progression of tumor. Activation of telomerase enzyme is the predominant mechanism for stabilization of telomere in oral tumor cells .In humans TERC is ubiquitously expressed, whereas expression of TERT is suppressed and acts as a rate-limiting factor for telomerase activity in most human cells and tissues.<sup>[18]</sup>

**Enhanced telomerase activity:** Telomerase is an enzyme with polymerase activity formed from a protein-RNA complex. It is produced in embryonic germline cells and its function is to lengthen the telomeres by copying the TTAGGG sequence. It is suppressed by mature somatic cells after birth, allowing telomere shortening after each cell division .Telomerase plays an important role in the formation, maintenance, and renovation of telomeres, preventing cell apoptosis. Activity of telomerase, is generally absent in normal tissues. It is known to be induced upon immortalization or malignant transformation of human cells such as in oral cancer cells. Telomerase activity has been detected in human immortalized cell lines and tumor tissues .<sup>[19]</sup> Telomerase enhanced activity has been demonstrated in a high percent of extracts from most tumor types.<sup>[20]</sup> For example, telomerase has been demonstrated

in oral carcinomas.<sup>[14]</sup> lung cancers<sup>[21]</sup>, prostate cancers<sup>[22]</sup>, liver cancers<sup>[23]</sup>, breast cancers<sup>[24]</sup>, neuroblastomas<sup>[25]</sup>, colorectal cancers<sup>[26]</sup>, and bladder cancers.<sup>[27, 28]</sup>

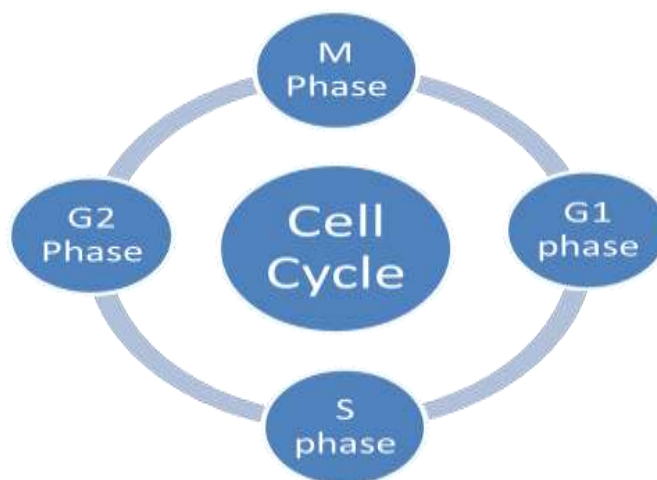
- oral carcinomas-75%
- lung cancers- 80%
- prostate cancers-84%
- neuroblastomas- 94%
- colorectal cancers- 95%
- bladder cancers- 98%

**Telomerase as a molecular marker:** Telomerase activity has also been assessed in many normal tissue types. Normal somatic cells are telomerase-negative, whereas stem cells such as in the germ-line and hemopoietic tissues are telomerase-positive.<sup>[29,30]</sup> An inhibitor of telomerase, possibly on chromosome 3 is present in normal cells, whose deletion or inactivation is required for immortalization and tumorigenic transformation. Activity of telomerase has also been demonstrated in normal oral mucosa, a highly proliferative tissue.<sup>[14]</sup> During tumorigenic transformation activation of telomerase occurs. In humans, activity of telomerase is absent in most of the normal cells but present in the majority of tumors.<sup>[31]</sup> Normal cells do have the capability to express telomerase activity given proliferative conditions. Telomerase activity is present in highly proliferative normal tissues in the oral mucosa.<sup>[14]</sup> Telomerase activity therefore, supports the use of telomerase as a biomarker for detection of tumors. Overexpression of leukoplakia, shows increased telomerase activity, an initial process of oral carcinogenesis, a precancerous stage.<sup>[32]</sup>



**Effect on cell cycle:** The cell cycle is divided into four phases: G1 (first gap), S (DNA synthesis), G2 (second gap), and M (mitosis). In each round of cell cycle telomere ends shortens. During cell divisions telomeres are lost, the chromosomal ends are no longer protected, which leads to the fusion of the chromosomes and karyotypic abnormalities, that eventually results in cell death. In tumor the cells continue to proliferation with such short telomeres that the ends of the chromatids were susceptible to fusion and subsequent abnormal cell division occurs leading to cancer.<sup>[33,34]</sup> Telomerase a ribonucleo protein becomes active

during uncontrolled cell cycle leading to the cause of tumor. It extends the telomeric repeat sequences at the chromosomal ends; is active in a majority (90%) of human neoplasia, but inactive in most normal cells.<sup>[35]</sup> Increased activity of telomerase was observed in oral precancer and OSCC patients.<sup>[36]</sup>



**Figure: Cell cycle.**

### HPV

Human papillomavirus (HPV), is also a risk factor which is also closely associated with benign and malignant oral lesions. It is detected in condylomas, focal epithelial hyperplasia, squamous cell papilloma and malignant oral lesions. HPV positivity is higher in case of oral cavity (59%) than from pharynx (43%) and larynx (33%).<sup>[37]</sup> Only a small fraction of HPV-infected lesions rarely proceed to malignant transformation, specially subtypes of HPV-16,18.<sup>[38-41]</sup>

The overall incidence of OSCC related to high risk human papilloma virus (HPV) infection is gradually increasing. In India, the prevalence of HPV-16 infection in OSCC reaches the range of 20-50%.<sup>[42]</sup> Several keys are also questioning with regard to HPV-associated with OSCC, such as unclear information about HPV-infection to oral precancerous and cancerous lesions and unproven multi-step progression of infection from precancer to cancer, restrict the opportunities for the development of preventive and therapeutic drugs.<sup>[43]</sup> Stepwise carcinogenesis by HPV-16/18 infection from immortalization to invasive carcinoma is required for prevention of and improved survival outcomes for OSCC. The down regulation of hTERT can inhibit cancer progression in HPV-16-infected OSCC.<sup>[44]</sup> Thus targeting of hTERT against OSCC will prove to be a novel therapeutic approach for cancer treatment.

## CONCLUSION

The global increase in frequency and mortality, as well as the poor prognosis of oral squamous cell carcinoma, has intensified current research efforts in the field of prevention and early detection of this disease. The advances in the understanding of the molecular basis of oral cancer should help in the identification of new markers. The study of the carcinogenic process of the oral cancer, including continued analysis of new genetic alterations, along with their temporal sequencing during initiation, promotion and progression, will allow us to identify new diagnostic and prognostic factors, which will provide a promising basis for the application of more rational and efficient treatments.

Telomerase activity has been readily found in most cancer biopsies, in premalignant lesions or in normal tissue samples with a few exceptions that include germ cells and hemopoietic stem cells its activity is absent. It is concluded that activity of telomerase can be used as a biomarker for diagnosis of malignant oral cancer and a target for inactivation in chemotherapy or gene therapy. Its expression will also prove to be an important diagnostic tool as well as a novel target for cancer therapy. The activation of telomerase may be an important step in tumorigenesis which can be controlled by inactivating its activity during chemotherapy therapy.

The expression and activity of telomerase are indispensable for cancer formation.<sup>[34]</sup> In light of the role of hTERT in carcinogenesis, targeting hTERT can be a promising tool to inhibit cancer initiation and progression.

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