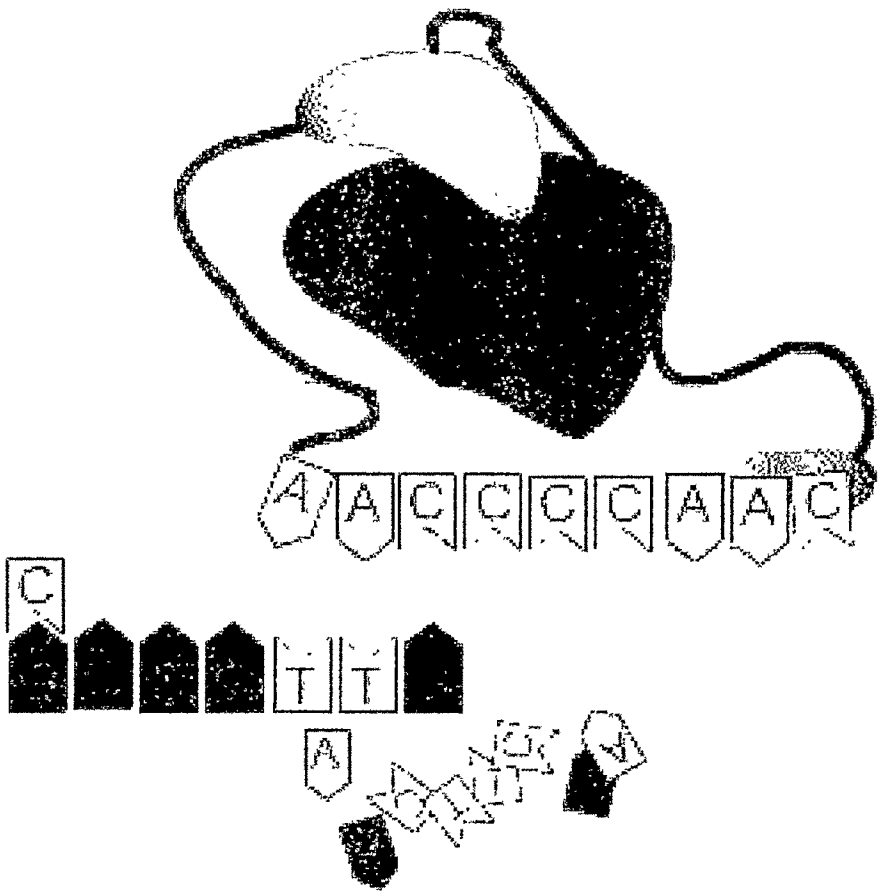


In its own microscopic way, becoming cancerous is about the most glamorous and successful thing a cell can do... ...

John Diamond

Prologue

Cancer has emerged as the most dreaded and life-threatening disease. It is still one of the most feared diseases at the rise of new millennium. Its incidence has been steadily rising in nearly all areas of the world. Globally, 10 million new cancer patients are diagnosed each year, which is projected to reach 20 millions by the year 2020. Thus, there will be a dramatic increase in the number of people developing cancer. The rising rate of morbidity and mortality due to cancer as well as the limitations of current therapeutic approaches to improve survival of the patients are the most strenuous problems for clinicians, researchers and health policy makers. Billions of rupees are spent annually on cancer research by drug industries, cancer charities and governments, but a cure for cancer appears illusive. However, understanding the disease at the molecular level seems to be a ray of hope in the dark for cancer patients.

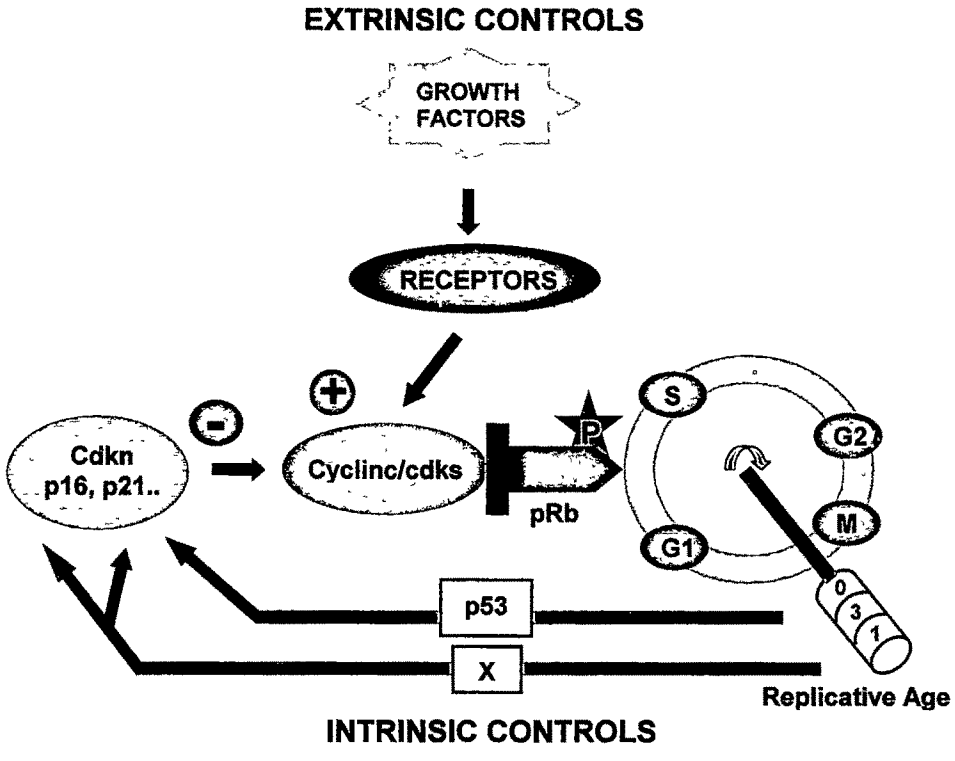
MOLECULAR ASPECTS OF CANCER

Carcinogenesis has been and will continue to be the subject of intense experimental, epidemiological and clinical research on its molecular, cellular and clinical aspects. Carcinogenesis at the molecular and cellular level is characterized by accelerated mutagenesis and proliferation. A fine balance between cell proliferation and cell death maintains the homeostasis of a fully developed organism (**Figure-1**).

Figure-1

Diagram illustrating the underlying mechanisms

of cell cycle control



Cdkn: Cyclin dependent kinsase inhibitors;
Cdks: Cyclin dependent kinases.

Extrinsic growth factors activate the stimulatory signal pathways, intrinsic inhibitory signals are activated by cell division counter through different pathways

The balance is unique, finely tuned and strictly regulated in each cell according to its need and role. The center stage of this balance is the complex interplay of cell cycle and cell death, both of which are controlled by the sequential assembly and activation/inactivation of crucial regulators. Growth factors stimulate the cell cycle machinery comprising of factors like cyclins and cyclin dependent kinases, which phosphorylate cell cycle regulators like pRb, which control G1→S phase wise progression. Any disturbance in this control along with other genetic changes like mutations in key oncogenes (e.g. ras, bcl2, etc.) and tumor suppressor genes (e.g. p53, etc.) may result into cancer. However, mammalian cells have evolved an intricate set of checks and balances against genetic damage and uncontrolled cellular proliferation. One of these is cellular suicide, or apoptosis, triggered by several genes in the presence of aberrant growth signals, recognizing the genomic damage and preventing Rb phosphorylation causing cell cycle arrest and cell death. The other factor is the progressive shortening of the ends of chromosomes, or telomeres, that accompanies normal cell division and contribute to cellular ageing. However, some cells escape these checks and finally transform into malignant phenotype. A key event during malignant transformation may be acquisition of the ability to escape the cell cycle control. Many hypotheses have been put forward as to how this occurs. Perhaps the most provocative model and one that has

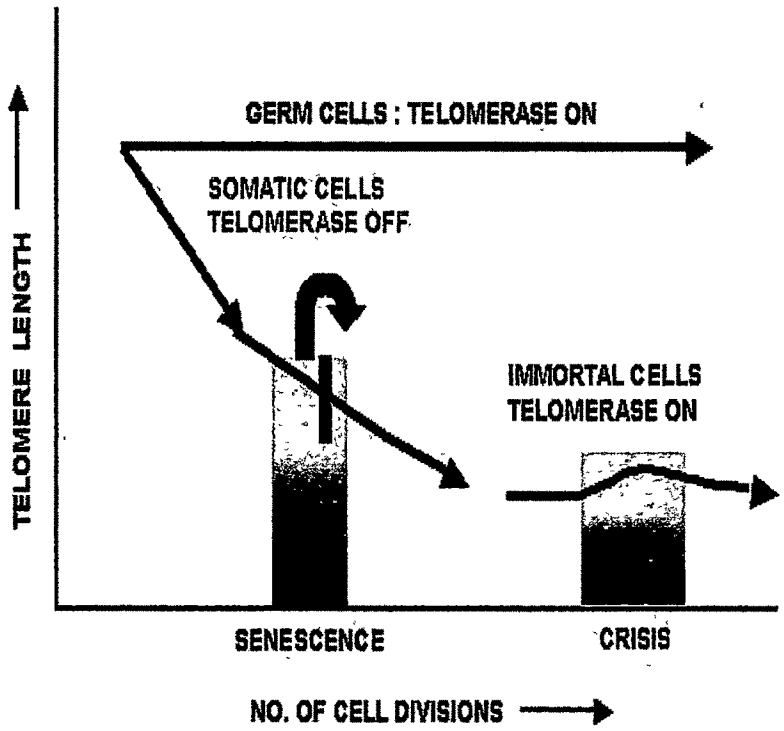
received the most attention recently is the telomere-telomerase hypothesis, which states that shortening of telomeres triggers senescence and telomerase activation leads to indefinite cell proliferation.

THE TELOMERE-TELOMERASE HYPOTHESIS

Telomeres form the structural ends of chromosomes. The presence of telomeres protects chromosome ends from enzymatic end-degradation and illegitimate recombination. Telomeres also provide a reservoir against the loss of few base pairs taking place at every cell replication cycle due to the end replication problem. This shortening of telomeres at every cell cycle represents a genetic mitotic clock, which counts the number of cell divisions rather than chronological or metabolic age. When telomeres shorten critically, the cells are signaled to pull out of the cell cycle pool and enter into the senescence phase. Telomere shortening thus appears to constitute nature's timing mechanism that leads to cell senescence and death. Perturbations in this clock may contribute to the pathologies associated with certain diseases including cancer. Activation of telomerase, an enzyme that synthesizes and maintains telomeric DNA, is one of the major mechanisms adapted by most of the cancer cells to overcome the phase of senescence (**Figure-2**).

Figure-2

The Telomere-Telomerase Hypothesis



An emerging hypothesis suggests that the up-regulation or re-expression of telomerase is a critical event responsible for continuous tumor growth. Telomerase at present is at the center stage of cancer research and is looked upon as the magic bullet for cancer therapy. The

interest of cancer researchers was aroused when it was proposed that telomerase is not expressed in human somatic tissues and that telomerase is required for cancer cell growth. Telomerase activity was first reported to be present in almost all malignant tumors and some of their benign counterparts but not in somatic cells by Kim et al. (1994). Jerry Shay did the pioneering work of studying telomerase activity in human cancer, in collaboration with Geron Corporation, USA. Since then, telomerase activity has been extensively studied all over the world in variety of malignancies. Telomerase is reported to be undetectable in normal somatic cells with some exceptions, but activated in embryonic cells, adult male germ line cells and almost all cancers. The telomere-telomerase hypothesis of cancer has become a new model for carcinogenesis studies. It suggests that telomere length is maintained by a balance between processes that lengthen telomeres (e.g. telomerase) and processes that shorten telomeres (e.g. end replication problem). It is generally observed that telomeres are stabilized at a shorter length in cancer cells. Telomere length is reported to decline in most of the cancerous tissues as compared to their normal counterparts.

Currently, telomerase is extensively studied all over the world. It is reported to be a ribonucleoprotein reverse transcriptase. It has a RNA component (hTR), which acts as a template for synthesis of telomeric

repeats onto the chromosome ends. It also consists of a catalytic unit (hTERT) with reverse transcriptase activity, which adds the telomeric repeats on the chromosome ends. It is also reported to possess a telomerase-associated protein (hTP1), which is essential for telomerase activity. All these components of telomerase enzyme are also extensively studied in human cancers and are believed to be essential for enzymatic activity.

The current understanding of normal function of telomerase indeed suggests that this enzyme may represent an ideal target for anticancer therapy. Anti-telomerase agents could be developed to treat tumors possessing telomerase activity. Lot of research is being done in this field too. Oligonucleotides binding hTR have been reported which can block telomerase activity. Compounds binding the catalytic subunit of telomerase hTERT have also been documented. Many anti-telomerase agents have been reported till date, but it is still a long way before any of these compounds could be clinically used to treat cancer. It would also be very beneficial to isolate telomerase negative tumors, as these tumors would contain cells with limited proliferative capacity. These tumors could be particularly targeted for anticancer therapy. Thus, telomerase has certainly become a very exciting area of research

currently, offering great prospects for diagnosis and management of cancer.

HEAD AND NECK CANCER IN INDIA

Head and neck cancer is one of the 10 most common malignancies in the world. Its incidence in Asian countries is increasing day by day. The scenario is more alarming in India. Head and neck cancer is the most commonly occurring malignancy in males and second to gender associated cancers in females in India. Tobacco consumption is considered to be the major etiological factor along with alcohol and diet, for development of cancer. The World Health Organization has listed tobacco control at the top of the cancer priority ladder. Moreover, Indian population consumes tobacco in a variety of forms, which are more harmful. In western countries, the most prevalent form of tobacco consumption is cigarette smoking, often with filters attached. On the other hand, tobacco chewing is very common in Indian population. Because of tobacco chewing, the epithelial cells are directly exposed to tobacco, which contains ample number of carcinogens. Eating tobacco in the form of gutka (a mixture of tobacco, areca nut, lime, synthetic flavoring agents, etc.) is considered to be very fashionable, particularly in youngsters who are easily peer-influenced. Nowadays, increasing incidence of oral precancers in youngsters due to consumption of gutka is very alarming. Many people have the habit of keeping tobacco in their

mouth for long hours. Bidi, which contains raw tobacco, is also very common in the socioeconomically poor group of the population. Other common forms of tobacco consumption are pan, reverse smoking, nass, snuff, etc. Thus, the etiology of head and neck cancer in Indian population is very much different as compared to the western countries. These differences in the etiological factors are reported to reflect in molecular changes in the tumor characteristics. Many reports from India show different status of a variety of molecular markers as compared to the western studies. Therefore, it becomes imperative to study telomerase and telomeres, which is the center of attraction for cancer researchers globally, in Indian population too.

During the last few years, we have witnessed the burgeoning of telomere-telomerase hypothesis as a major field of cancer research. A large body of literature validates clinical usefulness of telomerase and telomere length in cancer. However, there are very few studies on telomerase activity reported from India. Moreover, there are no reports available on telomere length in cancer in Indian population. It would be interesting to study telomerase activity and telomere length in Indian cancer patients. Any possible correlation between telomere length, telomerase and tumor characteristics need to be elucidated.

Therefore, the present study was undertaken to evaluate the clinical significance of telomerase activation and telomere length in head and neck cancer in Indian population as well as to elucidate any possible correlation of these parameters with clinicopathological characteristics of the tumors and survival of the patients.