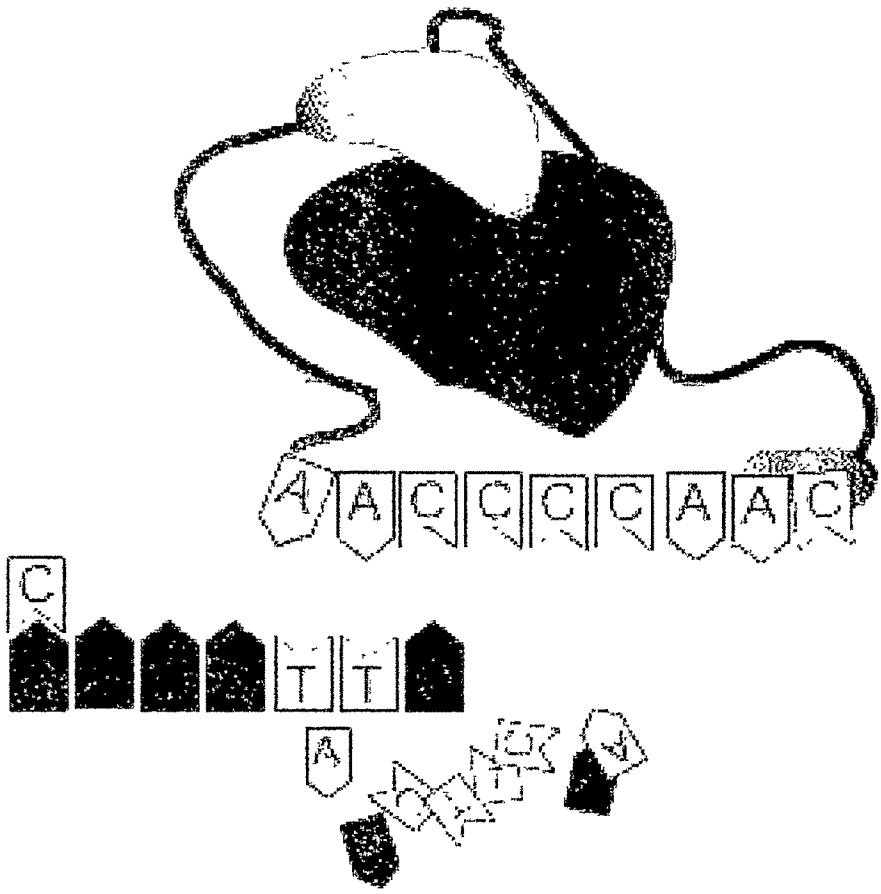


Those who donot study the past are condemned to repeat it... ...

Anonymous

Introduction

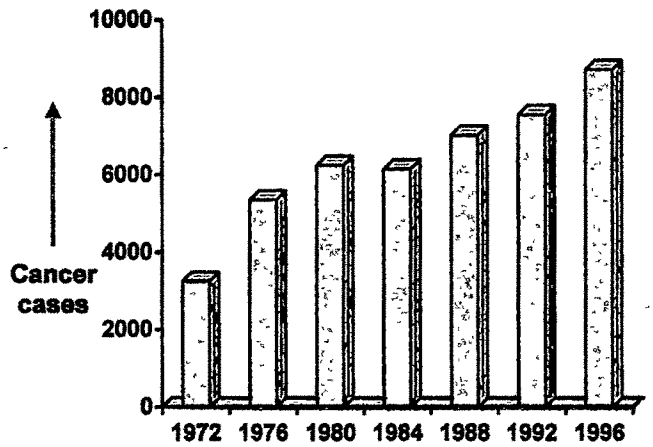
CANCER: THE DREADED DISEASE

The term "cancer" is derived from the Latin *cancer* and from the Greek *karkinos*, both meaning "crab". Its definition has changed over the centuries, as the number and types of lesions that are grouped under this term have expanded. Today's glossaries define cancer as malignant tumor, which spreads to rest of the body. For much of the 20th century, cancer was the most feared and, in many ways, the most mysterious of the major life-threatening diseases. It was the eighth leading cause of death. Even at the dawn of the 21st century, cancer is still a very real concern for public health both in perception and reality. In spite of the fact that advances in biomedical research, and the development of new strategies for disease prevention and therapies have controlled various diseases, the impact of cancer has continuously grown. Moreover, changes in lifestyle behaviours, especially tobacco use and dietary patterns, have led to significant increase in cancer incidence. Today, cancer has become the second leading cause of death (Seffrin, 2000). In west, the overall incidence rate is almost 310 per 100,000 per annum. There were estimated 8.1 million new cancer cases in 1990 all over the world (Parkin et al., 1999). In developing countries, cancer is still a major cause of death and because 70-80% of world's population lives in these areas, more than half the new cases of cancer recorded in the world occur in the so-called developing countries. **Figure-3** shows the

cases reported at The Gujarat Cancer & Research Institute during last several years. The Institute is the regional center for cancer treatment and research recognized by Government of India. As documented in the figure, cancer incidence has drastically increased during last several years.

Figure-3

Cancer cases reported at The Gujarat Cancer & Research Institute.



More dedication and immense efforts are necessary to understand etiology of the disease and to identify causative factors more precisely which may help in early diagnosis of the disease. The state of art treatment modalities including surgery, radiation, and neoadjuvant/adjuvant chemotherapy, have failed to completely cure the

disease or provide a longer disease free survival, possibly due to the lack of reliable prognostic markers. Recent developments in molecular analysis of tumors have given us some insights into these mechanisms. The growing understanding of the molecular mechanisms of carcinogenesis may help the clinicians in early diagnosis as well as in improving therapeutic strategies. Current literature on the subject emphasizes the importance of molecular markers for understanding the biology of cancer cells, and the need to further define molecular markers, which may provide better vision for carcinogenesis and tumor behavior (Cote, 2000).

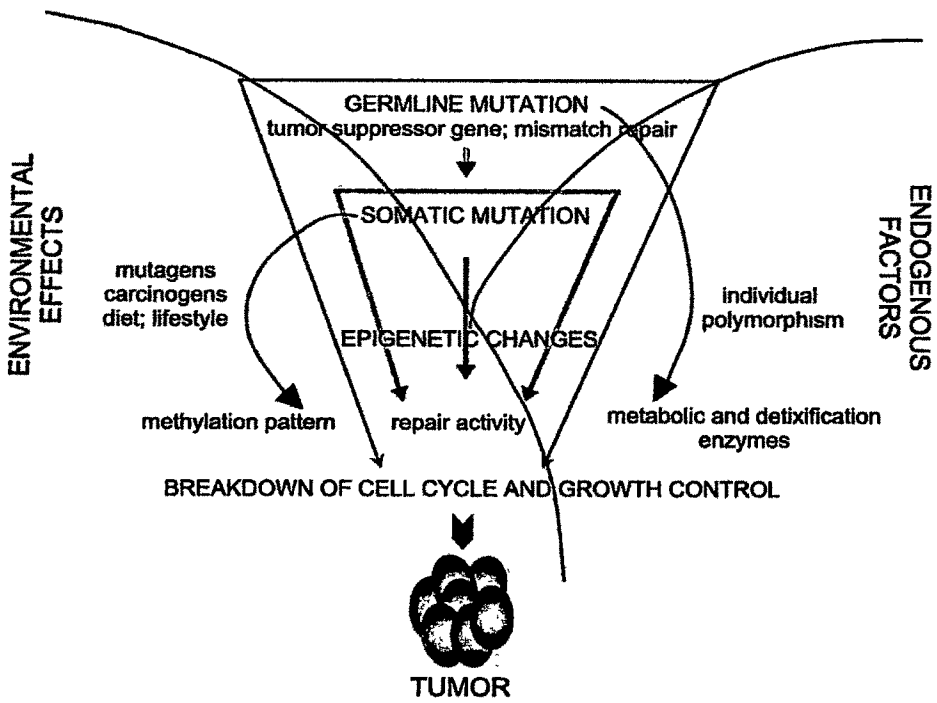
CANCER CAUSATIVE FACTORS

Cancer is a heterogeneous disease arising from accumulated mutations leading to clonal promotion of a transformed cell. Most cancers result from the interactions of genetic mutations, environmental factors and their cellular targets (**Figure-4**). Germ line mutations play a key role in the relative risk of cancer predisposition, but for the most part, they are limited to those that meet the criteria of familial cases (Minamoto et al., 1999). Genetic factors by themselves are thought to explain only about 5% of all cancers (Venitt, 1994). The remainder could be attributed to external, environmental factors that act in conjunction with both genetic and acquired susceptibility. A large body of evidence either

confirms or implicates various environmental factors in the development of a wide range of malignancies (Minamoto et al., 1999).

Figure-4

Environmental and endogenous factors leading to cancer



The ability of environmental factor to cause cellular transformation hinges on an individual's genetic make-up, as well as on the activity of cellular defense proteins (Bartsch and Hietanen, 1996). Among molecular targets of environmental influences on carcinogenesis are somatic mutations (genetic changes) and aberrant DNA methylation (epigenetic change) at the genomic level and post-translational

modifications at the protein level. At both levels, changes elicited affect the stability of the activity of key regulatory proteins, including oncoproteins and tumor suppressor proteins. Together, via multiple genetic and epigenetic lesions, environmental factors modulate important changes in the pathways of cellular carcinogenesis. Among the key environmental factors are chemical carcinogens (e.g. tobacco and dietary contaminants) and physical carcinogens (e.g. UV irradiation, asbestos and radon). Infectious agents which include pathogenic bacteria and viruses, such as *Helicobacter pylori,* human papilloma virus (HPV), and human hepatitis B and C virus (HBV/HBC) are also known to have important role in the etiology of cancer. Lifestyle factors such as tobacco consumption, dietary inadequacies, etc. are the integral environmental factors that contribute to cancer development (Minamoto et al., 1999). Tobacco is the largest avoidable public health problem on the planet. It is the largest cause of dreaded diseases and increasing morbidity worldwide. Most recent estimates at the world level amount to an annual death toll of 4 million, projected to increase to 10 million by the year 2030 due to tobacco consumption (The World Health Report, 1999). There are an estimated 1,164,700,000 adult cigarette smokers worldwide, which accounts for 20% of the world population (The World Bank, 1999). Furthermore, in India, apart from cigarette smoking, tobacco is consumed in a variety of different ways

viz. tobacco chewing, bidi smoking, gutka, betel quid chewing, etc., which are more harmful (Notani, 2000). The harmful effects of betel quid, the famous Indian "pan", and oral cancer has been observed since a long time in India (Thomas and Kearsley, 1993). Fells in 1908 observed that chewing betel quid was a popular habit in India and that *"favourite spot for cancer to originate is just the spot where the quid lies"*. Today the severity of this observation has increased all the more because of availability of tobacco pouches called pan masala, chewing of tobacco with lime and also because of heavy advertising aimed at younger generation with highly commercial approach.

Tobacco contains several carcinogens including tobacco-specific nitrosamines (TSNA), polycyclic aromatic hydrocarbons, volatile aldehydes, lactones, like benzo(a)pyrene, nickel, cadmium, radioactive polonium-210, uranium, and hydroquinones. Nicotine, the primary alkaloid in tabacco, yields primarily N'nitrosonornicotine (NNN), and 4(methylnitrosamino)-1-(3- pyridyl)-1 butanone (NNK). The TSNAs are further activated via the microsomal cytochromes to yield reactive electrophiles, which bind to the cellular macro-molecules and cause aberrations in specific genes, increasing risk of cancer development. The TSNAs are the most abundant and most potent carcinogens in tobacco (Saranath, 2000).

TOBACCO CONSUMPTION HABITS IN INDIA

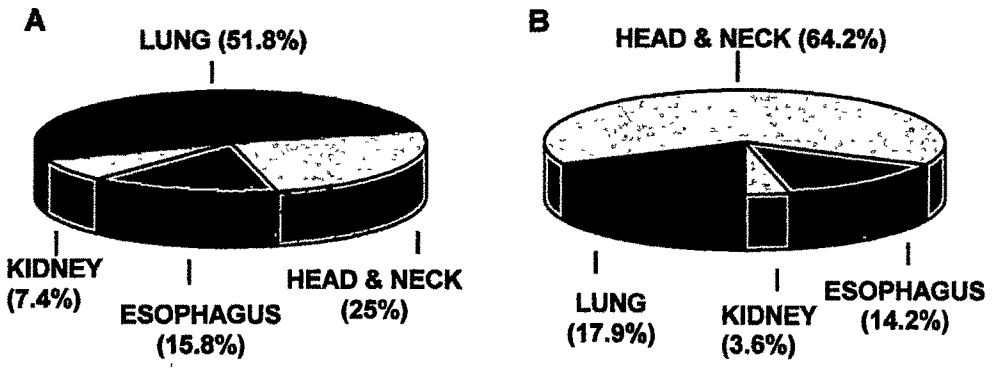
Tobacco has been consumed in a variety of ways for centuries in many parts of the world. Cigarettes have become by far the most common form in which tobacco is consumed throughout the world. In many parts of the world tobacco is chewed or snuffed which are referred as the smokeless forms. These practices have been common for centuries in Asia, Africa and few other areas of the world. In Asian countries, particularly in India, tobacco is consumed in the smokeless form more commonly. The habit of chewing betel quid (betel leaf, areca-nut and lime) is at least two thousand years old. Carcinogenic potential of tobacco increases many folds by mixing it with areca-nut and lime. Lime cuts the mucosal layer and thus facilitates penetration of tobacco. Placing tobacco or tobacco/lime mixtures (nass, nasswar) into the lower or upper groove, brushing the teeth and gums with tobacco paste, blowing tobacco dust into the nostrils (nasal snuff), are commonly observed habits in Indian population. Khaini i.e. sun dried tobacco which frequently contains several spices of molds is commonly used as plugs and by betel quid chewers. Fermented tobacco pieces and tobacco pastes containing a great variety of perfumes and spices are frequently used in the preparation of a betel quid. Bidi is also a very popular form of tobacco consumption in India, smoked by over 30 million persons. The use of attractively packed pouches containing tobacco along with other

unknown ingredients has tremendously increased among the youngsters in recent years (IARC Scientific Publication, 1990; Daftary et al., 1991). Thus, in India, modes in which tobacco is consumed are different and more harmful as compared to Western countries.

Tobacco is considered to be the major etiological factor along with alcohol and diet for development of cancer (Brenner et al., 1995a). It has been shown that individuals who smoke heavily have a risk of developing cancer, which is six times that of individuals who have never smoked. In India, where tobacco chewing is prevalent, the risk of cancer increases to 25-30 times (Daftary et al., 1991). According to a recent report, there are about 1.3 million cancers worldwide attributable to tobacco, which amounts to 16% of total cancer cases. Lung, head and neck, esophagus and kidney are the major sites affected by tobacco. Lung cancer is the most common of all tobacco related cancers worldwide (Parkin et al., 1999). However, as clear from **Figure-5**, the scenario is completely different in India, where head and neck is the leading site amongst the tobacco related cancers (Notani, 2000). According to The Hospital based Cancer Registry data, head and neck cancer forms 51.8% of the tobacco related cancers at The Gujarat Cancer & Research Institute (G.C.& R.I.), Ahmedabad. Thus, head and neck is the major site affected by tobacco in Indian population.

Figure-5

Incidence of tobacco related cancers



Worldwide G.C.& R.I., Ahmedabad

HEAD AND NECK CANCER: INDIAN SCENARIO

Most of the head and neck cancers are squamous cell carcinomas and include cancer of oral cavity, i.e., of the lip, tongue, and other intra-oral sites, pharynx and larynx. Cancers of these sites are clubbed together as head and neck cancer because of the common etiological/risk factors associated with these sites. Globally head and neck cancer is one of the 10 most common malignancies. It represents approximately 5% of all human cancers with an incidence of 500,000 cases per year worldwide (Parkin et al., 1999). More than 50% (256,700 new cases in 1990) of the total head and neck cancers occur in Asian countries (Parkin et al., 1999). In India, head and neck cancer is highly prevalent, comprising

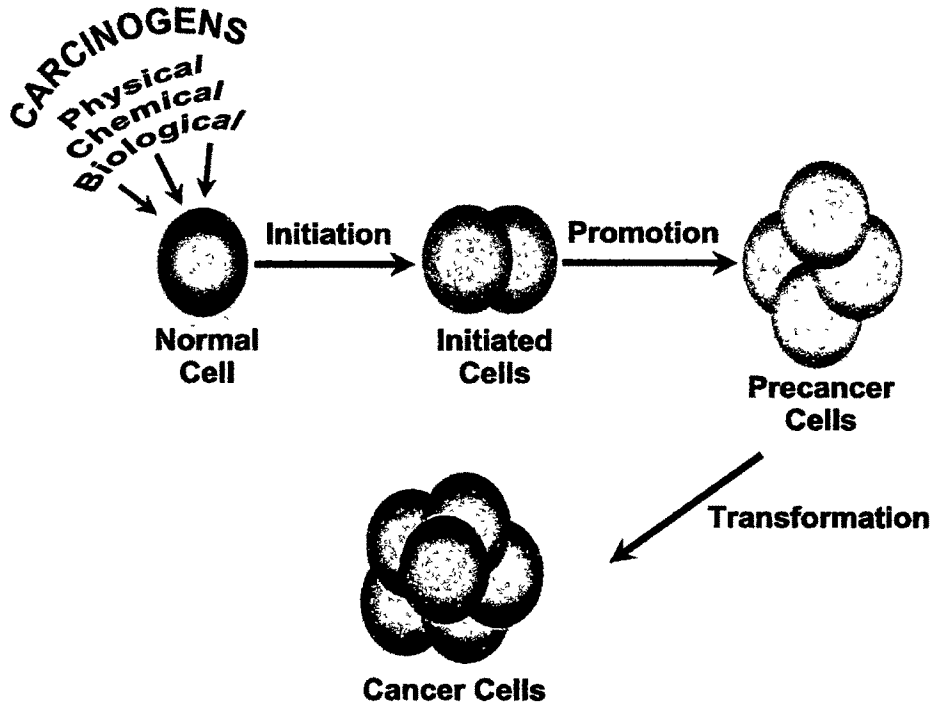
about 40% of all malignancies (Jayant and Notani, 1991 Notani, 2000). This can be attributed to the fact that tobacco consumption, particularly tobacco chewing in the form of betel quids, tobacco mixtures, pan-masala etc., is commonly observed in the population. Local customs and habits play an important role for higher incidence of head and neck cancer in India. The exceptionally high incidence of this cancer in the Indian population far exceeds that of Western countries. Also the picture of head and neck cancer is distinct in Indian population from that observed in Western population in several aspects. The incidence rate for oral cancer in females is equivalent to that in males, in India. Whereas, in other countries, the disease is found to be at lower rates in females, than that in males for oral cancer. Further, cancer of mouth constitutes the major bulk of head and neck cancers in India (Saranath et al, 1993). Clinical, epidemiological and laboratory studies confirm an etiological relationship between prolonged tobacco chewing and oral cancer in India (Gupta et al., 1987; Jussawala and Deshpande, 1971). At The Gujarat Cancer & Research Institute, oral cavity cancer account for almost half (49.3%) of the head and neck cancers, while pharyngeal cancer and laryngeal cancer represent 39% and 11.7%, respectively. These facts reflect the lifestyle habits in the population of Gujarat and its neighboring states.

HEAD AND NECK CANCER: A MULTISTEP PROCESS

Head and neck carcinogenesis is a multi-year process of clonal selection and evolution of genetically damaged cells, leading to the abnormal precancer phenotype that may eventually lead to invasive cancer (**Figure-6**). The genetic model that is continually being refined show that the sequence of genetic damage is of multiple choice, multiple path and by nature stochastic. During this process, some initiated and preneoplastic cells never progress to cancer cells but may remain for years or even for a lifetime in a dormant stage. The initiated cells originate by somatic mutations/selection process, and these early genetic changes are irreversible (Cohen and Ellwein, 1991; Knudson, 1991; Nowell, 1976; Weinstein, 1988). However, multiple and sequential mutations are required for tumor progression. These mutations can occur randomly at an early stage due to errors in DNA repair, segregation, and replication, or may occur later following exposure to various carcinogens or mutagens, which induce genomic instability and chromosome abnormalities and genetic damage (Christian et al., 1995). Preneoplastic cells are an intermediate stage between normal cells and cancer cells (Christian et al., 1995; Gray, 1993; Lupulescu, 1983). These precancerous lesions or conditions occur for a varying length of time before malignant transformation in some cases.

Figure-6

Model for multistep carcinogenesis.



Interestingly, these precancerous lesions share the same etiologic factors with head and neck cancer, particularly the use of tobacco, and exhibit the same site and habit relationships. Many of them show a high potential for neoplastic changes. Even though only a small proportion of "precancers" actually progress to cancer, this development forms a source of over 70% of oral cancers in India. Individuals with precancerous diseases run a risk that is 69 times higher for them to develop oral cancer compared to tobacco users who do not have

precancerous diseases (Mehta and Hamner, 1993). Some of the precancerous conditions or lessons are described below:

Leukoplakia

Leukoplakia is defined as *"a raised white patch of the oral mucosa measuring 5 mm or more, which cannot be scraped off and cannot be attributed to any other diagnosable diseases"*. Leukoplakia can regress spontaneously in about 40% of the cases while 2.8-5.2% per year recur. It is classified into following types based on the clinical appearance and the natural history i.e. the long-term behavior of each type.

Erythroplakia

Erythroplakia is a rare but severe precancerous lesion. It describes a bright-red, velvety plaque, which cannot be characterized clinically or pathologically as any other lesion. Microscopically 91% of them show squamous cell carcinoma or moderate to severe epithelial dysplasia.

Oral Submucous Fibrosis

Oral submucous fibrosis is a chronic mucosal condition, characterized by mucosal rigidity of varying intensity due to fibroelastic transformation of the juxtaepithetial connective tissue layer. It is a high-risk precancerous condition. Oral submucous fibrosis is not known to regress either spontaneously or with the cessation of the areca-nut chewing habit.

ETIOLOGY AND MOLECULAR CHANGES IN HEAD AND NECK CANCER

As described earlier, the form of tobacco consumption in Indian population (bidi smoking, tobacco chewing, snuff, etc.) is very much different compared to the Western countries where cigarette smoking is the major mode of tobacco consumption. Therefore, etiology of tobacco related cancer in India is different from that in Western countries. The differences in the etiological factors are shown to reflect in molecular changes in tumor characteristics. The p53 mutations are common in tumors from the West (47%) but are infrequent in the East (7%). Tumors from India are characterized by the involvement of ras oncogenes, including mutation, loss of heterozygosity (H-ras) and amplification (K- and N-ras), events that are uncommon in the west (Paterson et al., 1996; Saranath et al., 1993). Thus, it is important to study molecular markers for head and neck carcinogenesis in Indian population.

The recent advances in the understanding of the genetic basis of carcinogenesis in head and neck cancer have provided the multistep tumorigenic progression model based on genetic alterations as mentioned earlier. These alterations are hallmarks that signal stages of carcinogenesis between initiation, development and progression of a

malignant tumor. A flourish of intermediate markers are being characterized in head and neck cancer and include categories such as cytogenetic changes including gain or loss of the Y chromosome and abnormalities at multiple other loci, loss of heterozygosity (LOH) at chromosomal loci which can be detected by microsatellite markers, mutations of specific genes e.g. p53, abnormal gene expression e.g. over expression of ras and bcl2, as well as some epigenetic alterations (Oh and Mao, 1997; Mahale and Saranath, 2000; Saranath et al., 1999, Scully et al., 2000). These markers are helpful in detection of premalignant lesions, tracking of the carcinogenic process *in vivo* and obtaining prognostic information about the disease. In the recent years the development of sophisticated technical approaches have substantially contributed in understanding the disease at the molecular level. Several molecular markers have emerged recently, out of which telomerase, also known as the immortality enzyme, is the most outstanding and highly promising for fulfilling the demand of the day in cancer research (Krupp et al., 2000; Meyerson, 2000; Shay et al., 2001; Shay and Gazdar, 1997; Shay and Wright, 2001).

CELLULAR AGEING

Before the 1960s, human cells that replicated in the body were thought to be capable of dividing endlessly. Hayflick (1965) demonstrated unequivocally that this notion was incorrect. They first described the limited replicative capacity of normal human fibroblasts. The finite replicative life span *in vitro*, or the Hayflick limit, has since been demonstrated for many other somatic cell types such as keratinocytes, endothelial cells and lymphocytes and generally ranges between 50 and 100 population doublings (Cristofalo and Pignolo, 1993). It is now known that somatic cells derived from human new borns will usually divide 80 to 90 times in culture, whereas those from a 70 year old are likely to divide only 20 to 30 times (Greider and Blackburn, 1996). When human cells that are normally capable of dividing stop reproducing, they enter the phase of senescence. Senescence is characterized by the withdrawal from cell cycle, chromosomal instability and various morphological and biochemical changes. Furthermore, altered pattern of gene expression has been reported between young and senescent cells (Cristofalo and Pignolo, 1993; Dimri et al., 1995; Linskens et al., 1995a,b; West et al., 1989; Winstrom and Villeponteau, 1992). These post-mitotic senescent cells remain viable for extended period of time provided that appropriate growth conditions are maintained (Linskens et al., 1995). Accumulating evidences suggest

that *in vitro* replicative senescence has biological significance in *in vivo* aging. The cellular replicative life span decreases with increasing age of the donors, presumably reflecting an increased number of cell divisions occurring with age (Stanulis-Praeger, 1987). These results suggest the presence of a genetic mitotic clock, which counts the number of cell divisions rather than chronological or metabolic age (Harley, 1995). Perturbations in this clock may contribute to the pathologies associated with certain diseases. In 1970s, this programmed cessation of cell division was linked to the gradual loss of chromosome ends due to a special dilemma termed end-replication problem by Watson (1972) and A.M.Olovnikov (1973) independently.

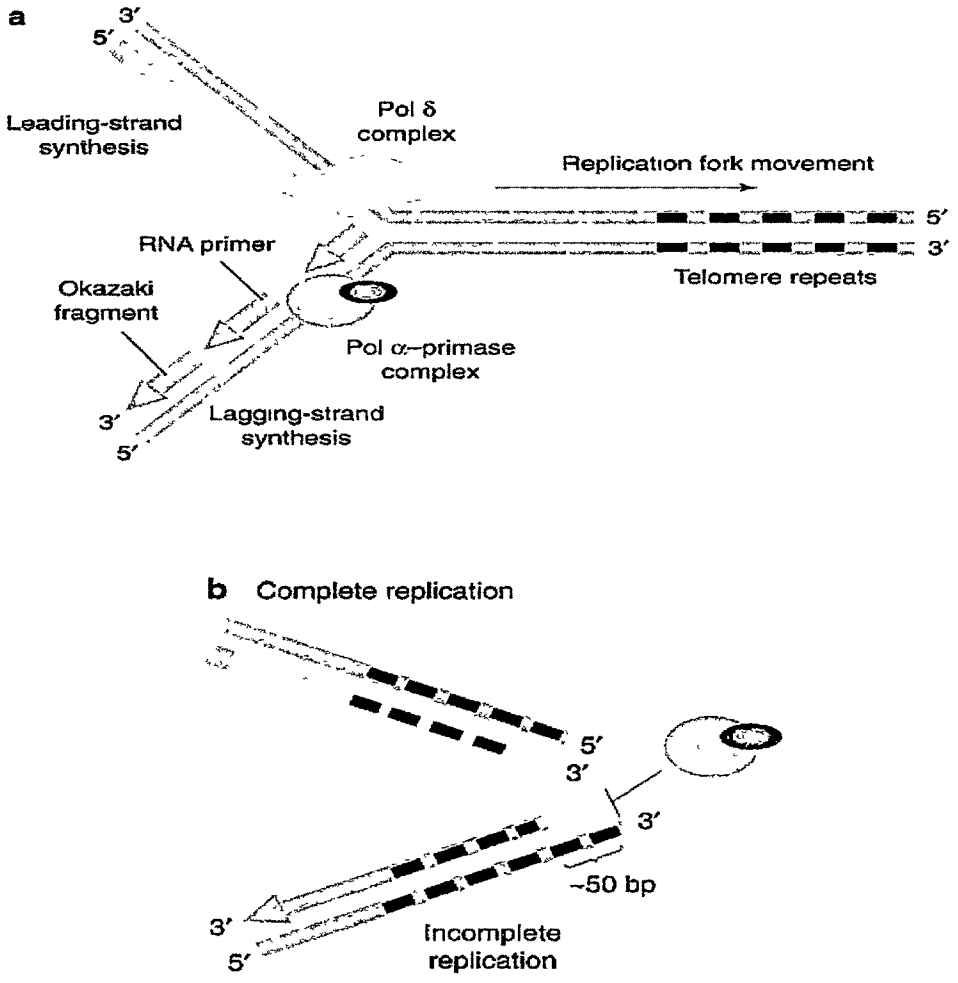
THE END REPLICATION PROBLEM

When cells grow and divide, each newly formed cell retains the entire set of chromosomes which are faithfully replicated prior to division, but eukaryotic chromosomes, being linear in nature, encounter a special problem called the end replication problem which is depicted in **Figure-7** (Olovnikov, 1973, McKenzie et al., 1999, Watson, 1972). All human DNA polymerases can synthesize DNA in one direction only (in the 5'-3' direction). The two antiparellel DNA strands of a replication fork are therefore replicated differently. No known human DNA polymerase activity can initiate a deoxyribonucleotide chain. All the

enzymes extend a previously started chain from a free 3'-OH end. Therefore, an RNA primer is required to initiate a chain, which is synthesized by RNA polymerase. The leading strand is then replicated continuously by the polymerase δ (Pol δ)-PCNA (proliferative cell nuclear antigen) complex. The lagging strand is replicated discontinuously and backwards relative to the movement of the replication fork. This requires multiple RNA-primed reinitiations of DNA synthesis by the polymerase α (Pol α)-primase complex and the subsequent elongation and splicing of the resulting (Okazaki) fragments. Each replication fork is a part of a replication bubble, with a mirror image replicating fork moving in the opposite direction. At the ends of both lagging strands at the tips of chromosomes, the Pol α-primase complex runs out of space, leaving a variable length of the lagging strands unreplicated. This leads to the generation of a 3' overhang and loss of about 50bp per cell-doubling in human cells. This is called the end replication problem. Eukaryotic chromosomes solve this problem by having telomeres, which serve as a buffer against, or a means to compensate for incomplete replication of chromosome ends at every cell division (Blackburn, 1991; Blackburn and Gall, 1978).

Figure-7

End Replication Problem



(a) Lagging strand is replicated in form of okazaki fragments;

(b) Incomplete replication in the lagging strand because the very end cannot be replicated.

TELOMERES: THE MITOTIC CLOCK

The term "telomere" was coined by Muller (1962) from the Greek for "end" (telos) and "part" (meros). Telomeres, the end parts of chromosomes, are composed of a DNA component and multiple protein components. The telomeric DNA consists of noncoding tandemly repeated sequences, with the exact repeat sequence varying from one species to the other. In humans and other vertebrates, the repeat unit is the hexanucleotide 5'TTAGGG3' (Blackburn, 1991). Characterization of vertebrate telomeres has lead to recognition of rapidly increasing list of proteins reported to bind telomeres and/or affect telomere length. TRF1 was the first telomere-binding protein identified (Zhong et al., 1992), followed by TRF2 (Broccoli et al., 1997), a distantly related homolog of TRF1. Both proteins have a single myb repeat at the carboxyl terminus and an internally located dimerization domain. They form homodimers and bind very specifically to double-stranded telomeric sequence. TRF1 is a negative regulator of telomere length: over expression of the full-length protein results in telomere shortening, interfering allele leads to telomere lengthening. TRF2 serves two functions at the telomeres. First, it is needed to prevent telomere fusions. Second, it somehow interacts with the cell-cycle machinery to prevent senescence (Griffith et al., 1998; Smith and deLange, 1997). Tankyrase was the first telomere protein to be identified that has enzyme activity (Smith et al., 1998).

This 142 kd protein co-localizes with TRF1 at telomeres. The central TRF-1 interacting region of the protein contains 24 ankyrin repeats whereas the carboxyl terminus has a PARP (poly ADP ribosyl polymerase)-related catalytic domain. Recently one more telomere protein has been identified, hnRNPA1, hnRNA-binding protein. Deletion of Hnrpa1 gene causes telomere shortening whereas restoring expression of either A1 or a fragment of A1 (UP1) increases telomere length (LaBranche et al., 1998). The length of telomeres varies among different species. Humans have telomeres 8-14 kb long. Telomeres protect chromosome ends from enzymatic end degradation and illegitimate recombination. Telomeres also appear to play a role in the correct pairing and movement of chromosomes at meioses. They serve as the attachment points to the nuclear matrix (Hastie and Allshire, 1989; Zakian, 1989). Thus, one important function of telomeres is to ensure that chromosomes are retained and properly inherited with each cell division. Chromosomes with truncated tips fuse with other chromosome (forming dicentric or ring chromosomes) or are lost during cell division causing mitotic instability and even cell death (Greider, 1998, Harley, 1991). Apart from providing stabilization and protection to the chromosomes, telomeres carry out another important function in replicating cells: their structure allows the end of coding DNA to be replicated completely. Therefore, although telomeres shorten by

50-200 bp at every cell cycle, the coding region of the chromosome is completely replicated. In 1990, Harley et al. proposed that shrinking telomere may be the cell's measure of the mitotic age of the organism. The loss of telomeres represents a molecular equivalent of ageing, with the end-point being an exit from the cell cycle and senescence. Thus, telomeres are known as the mitotic clock driving the programmed arrest at senescence (Counter et al., 1992; Harley et al., 1990). Published measurements of telomere lengths have been variable due to true biological variations in telomere repeat number between chromosomes, plus the fact that southern blot measurements rely upon measuring a terminal restriction fragment (TRF) which also include a subtelomeric region which itself varies between chromosomes (Autexier and Greider, 1996).

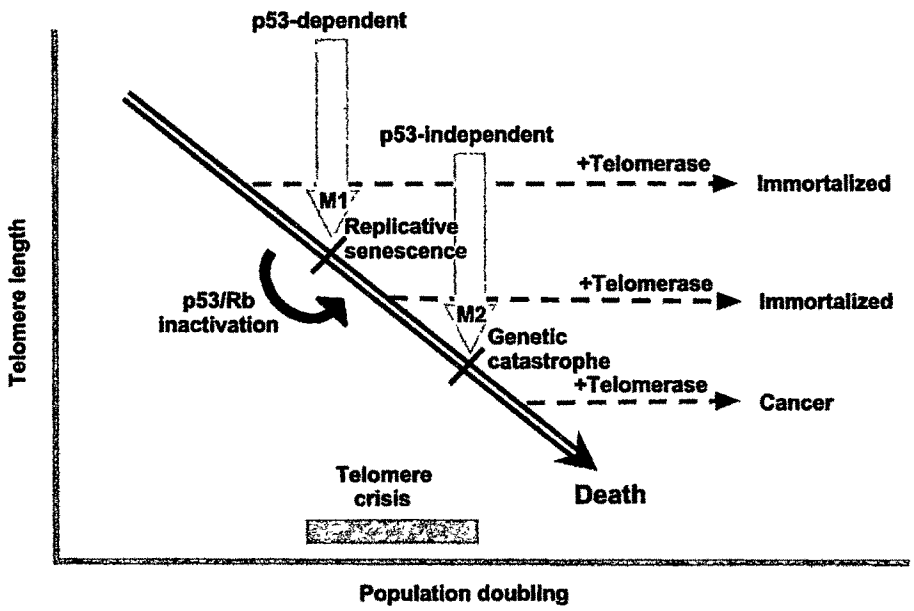
THE TELOMERE-TELOMERASE HYPOTHESIS

The telomere-telomerase hypothesis is depicted in **Figure-8** (Artandi and DePinho, 2000; Greider and Blackburn, 1996; Rhyu, 1995; Stewart and Weinberg, 2000; Wright and Shay, 1995). Telomeres shorten as a function of proliferative age. Human diploid fibroblasts have a mean TRF length of 7-9kb in the adults, which falls to around 5-7kb by the time the cells reach the senescence limit described by Hayflick (Allsopp et al., 1995; Allsopp and Harley, 1995; Harley et al., 1990). When the

telomere length is reduced to a critical point, a signal is given to stop further cell division, which is the hallmark of cellular senescence (Hayflick, 1976). Cells in culture are thought to stop dividing because of activation of an antiproliferative mechanism termed "mortality stage 1" (M1). The stimulus for the induction of M1 may be DNA-damage signals from the altered expression of subtelomeric regulatory genes or from a critically shortened telomere.

Figure-8

Diagramatic representation of telomere-telomerase hypothesis



p21, p16, p53 and pRb are believed to be involved in the execution of M1 (Bryan and Reddel, 1994; Hara et al., 1996; Noda et al., 1994). If these cell cycle regulators are activated or mutated/blocked, the cells continue to divide and thus the telomeres continue to shorten. The telomere function appears to be critically diminished when mean telomere length falls to around 1-2 kb, although this possibly reflects the near total loss of telomeres from a subset of chromosomes, due to the second independent block in proliferation called the "mortality stage 2" (M2) or "crisis". This stage is punctuated by another surge of cell death. While most cells do not survive M2, a subpopulation may escape from this crisis point at a low frequency by activating an enzyme telomerase, which synthesizes telomeres. These cells manage to stabilize their chromosomes, giving rise to immortal cells, which have unlimited proliferative potential (Meyerson, 2000).

An intricately balanced control of cellular proliferation and death maintains normal tissue homeostasis and is accomplished by a network of genes. An important hallmark of cancer is aberrant growth control. Genetic changes that confer a growth advantage to the tumor cell are observed on numerous levels. However, in addition to aberrant growth control, many cancer cells possess another important feature, which distinguishes them from normal somatic cells: unlimited replicative

capacity. This characteristic is said to be conferred by activation of telomerase, the enzyme synthesizing telomeres. Kim et al. first reported association of human telomerase activity with immortal cells and cancer in 1994. Subsequent studies in recent years have added significantly useful data in this most exciting area.

TELOMERES, TELOMERASE AND CANCER

Telomerase activity is found to be repressed or inactivated in the majority of human tissues except some tissues like fetal and adult testes, ovarian follicles, etc. In contrast, telomerase activity is detectable in most tumors (Kim et al., 1994). Numerous published reports have documented the involvement of telomerase function in acquisition of immortality in cancer cells. Telomerase activity has been detected in most of the specimens of all types of cancer including gastric, breast, liver, prostate, head and neck, lung, neuroblastoma and leukoplakia, and also in some specimens of the benign and premalignant tissues like gastric adenoma, breast fibroadenoma, cirrhosis, colon adenoma, leukoplakia, etc. (Kim, 1997; Kim et al., 1994; McKenzie et al., 1999; Shay and Bacchetti, 1997; Shay and Wright, 1996a,b; Tsao et al., 1998). It has been suggested that the patients showing telomerase activation in the benign or premalignant tissues are at a higher risk for malignant transformation. There are a number of studies on telomerase activation

in head and neck cancer showing similar trends of telomerase activation in majority of malignant tumor tissues (Califano et al., 1996; Mao et al., 1996; Mutirangura et al., 1996). Telomerase activity has also been analyzed in the normal tissues adjacent to the tumor. Very few adjacent normal tissues have revealed positivity for telomerase. The pathway by which telomerase is activated is not elucidated completely. However, recently it has been shown that the human papilloma virus (HPV) E6 oncogene and MYC directly up-regulate telomerase. Expression of MYC activates telomerase by up-regulating the mRNA encoding the catalytic subunit of telomerase by E6 might also be a MYC-mediated event. Expression of E6 leads to the activation of telomerase in epithelial cells (Greider, 1999; Veldman, 2001). Recent developments report that human T lymphocytes regulate telomerase function through novel events independent of hTERT protein levels, and hTERT phosphorylation and nuclear translocation may play a role in regulation of telomerase function in lymphocytes (Liu, 2001). It is also shown that direct or indirect phosphorylation of telomerase proteins regulate telomerase and that PKC-zeta isotype that functions *in vivo* in nasopharyngeal cancer cells (Yu et al., 2001). Rb, E2F-1 and autocrine transforming growth factor beta are also known to have a role in telomerase regulation (Crowe and Nguyen, 2001; Yang et al., 2001).

The central fact that has emerged from numerous studies is that the lack of telomerase expression appears to curb the growth of rapidly proliferating cancer cells, while an increase in telomerase permits indefinite proliferation. The finding that the majority of human tumors contain elevated telomerase levels coupled with the fact that the ability to diagnose cancers in early stages of development is often translated into increased survival rates, has hastened the use of telomerase as an indicator of cellular transformation in clinical settings. The telomere hypothesis of cellular senescence and cancer has a stunning implication: inhibition of telomerase might limit the growth of tumors without significantly affecting normal, non-proliferative tissues. This has led to the hypothesis that telomerase is a novel target for chemotherapy (Aszolas and Eckhardt, 1997; Davis and Siu, 2000; Pitts and Corey, 1999; Rousseau and Soria, 2000; Rowley and Tabler, 2000; White et al., 2001).

Telomerase is a specialized reverse transcriptase that synthesizes telomeric DNA (Blackburn, 1992; Morin, 1997; Weilbaecher and Lundblad, 1999). Human telomerase is a large complex up to 1000 kd consisting of two core subunits – an RNA domain that acts as a template for replication (hTR) and a protein domain that catalyzes

nucleotide polymerization (hTERT). Telomerase associated protein (hTLP) is also required for its activity.

The catalytic subunit of telomerase, hTERT, is found to be the limiting component for telomerase (Meyerson et al., 1997; Nakamura et al., 1997). Its expression at the mRNA level is strongly associated with enzyme activity and concomitant immortalization. The introduction of hTERT into normal human epithelial cells and fibroblasts is shown to be sufficient to reconstitute telomerase activity, arrest telomerase shortening, and extend the life span *in vitro* (Bodnar et al., 1998; Nakayama et al., 1998; Wang et al., 1998; Weinrich et al., 1997). Moreover, hTERT expression has been found to correlate strongly with telomerase activity in various cancers (Ito et al., 1998; Kanaya et al., 1998; Takakura et al., 1998). The telomerase associated protein (TP1) is another essential component of telomerase. This protein has the WD-40 repeats that are related to protein-protein interactions. However, the expression of this protein is not correlated with the level of telomerase activity (Harrington et al., 1997). The RNA component of telomerase, hTR, has been well studied. It is approximately 445 nucleotides long, although deletion experiments have shown that the minimal function region comprise nucleotides 44-203. Within this RNA, nucleotides 46-56 (5'CUAACCCUAAC) serve as a binding site for telomere ends and acts

as a template for the addition of telomeric repeat. This template region of hTR is complementary to the human telomere sequence (TTAGGG)n (Feng et al., 1995). Germ line tissues and tumor cell lines are shown to express more hTR than normal somatic cells and tissues, which have no detectable telomerase activity. Human cell lines that expressed hTR mutated in the template region generated the predicted mutant telomerase activity. HeLa cells transfected with an antisense hTR are reported to die after 23 to 26 doublings (Feng et al., 1995). hTR is also known to be up-regulated in different types of malignancies.

Telomere dynamics is another aspect, which is being studied in cancer cells in relation to the mechanism of cancer development. Cancer cells are shown to have short telomeres in general. However, the telomere length is not shown to be shortened in all cases examined, especially in solid tumors. Telomere length is not determined simply by balance between the total number of cell divisions and telomerase activity. Telomere length is also regulated by some telomere binding proteins. Some of these proteins negatively regulate telomere length, probably by inhibiting telomerase activity (Colgin and Reddel, 1999). Also, there is a great deal of subclonal cell to cell variations in the telomere length. High degree of heterogeneity is found in telomere length with some telomeres being very long and some short. Thus, there has been lot of

variations in the reports on telomere length in different types of cancers (Autexier and Greider, 1996).

Among the available reports on telomeres, telomerase and cancer, most of the studies are from the Western countries and some Asian countries like Japan. There are very few reports from India on telomerase and cancer. In fact there are only two reports from India. The first report is from Kerala on telomerase activity in oral cancer by Kannan et al. (1997), and the second from New Delhi on telomerase activity in aerodigestive tract cancer (Sharma et al., 1999). However, there has been no detailed analysis of telomere length and telomerase activation in head and neck cancer in India as yet.

OBJECTIVES OF THE STUDY

If all the points mentioned before are taken together, it appears that head and neck cancer is one of the common human cancers. Despite improvements in diagnosis and treatment of head and neck cancer in the past two decades, overall survival of these patients remains poor and mortality rate has not declined. The scene is similar in Western and Eastern countries, with the five year survival rates reported from thirty European countries only marginally better than those seen in developing country like India. For patients with localized disease,

unsuccessful eradication of the primary tumor or locoregional metastasis remains the principal cause of treatment failure. The data emphasizes the necessity of early detection of the disease as well as identifying patients with residual/occult malignant disease who may be in need of adjuvant therapies. Early detection of the disease or the identification of a high-risk population can allow the clinicians to initiate treatment earlier and eventually to improve the survival rate. There has been a lack of cellular or biochemical markers that would reveal and quantitate cell or tissue damage. The etiology of head and neck cancer in India is completely different, its biology might be different and therefore it is most essential to further study the disease at molecular level. After intense research on telomerase activation, researchers are optimistic for the successful application of the advances in telomere-telomerase research in the clinic. Considering all these facts, the present investigation was undertaken to:

- evaluate telomerase activation in tumor tissue specimens of head and neck cancer patients.
- study telomerase activation in precancerous tissue specimens of patients with related precancerous lesions/conditions.
- assess telomerase activation in normal tissue specimens adjacent to the tumor in head and neck cancer patients.

- evaluate telomere lenth in tumor tissue specimens of head and neck cancer patients.
- study telomere length in normal tissue specimens adjacent to the tumor in head and neck cancer patients.
- establish any possible correlation between telomerase activation and clinical and pathological characteristics in patients with head and neck cancer.
- find out any possible correlation between telomere length and clinical and pathological characteristics in patients with head and neck cancer.
- document whether status of telomerase activation can be helpful in predicting disease course after anticancer treatment, and thus in discerning the patients in need of aggressive anticancer treatment.
- establish whether status of telomere length can be helpful in predicting disease course after anticancer treatment, and thus in discerning the patients in need of aggressive anticancer treatment.