CHAPTER 1

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled cell proliferation and spreading of abnormal cells to other parts of the body. Worldwide, cancer is the most dreadful disease and is one of the major causes of morbidity and mortality. It was estimated that 14.1 million new cancer cases and 8.2 million cancer deaths occurred in 2012 worldwide (Ferlay et al. 2015) (Fig I). The long-term management of cancer requires efforts to minimize the risk of cancer while pursuing the intensive hunt for more effective treatments which are also cost effective and less toxic. The probability of a person being diagnosed with cancer is more than 40% during life time (Amin et al. 2009). However, cancer is known to be a preventable disease (Glade 1999a). Avoiding cancer-causing biological, chemical and physical agents and consuming diet that offers protection against cancer are the two major ways to lower cancer risk. Thirty to forty percent of cancer incidence can be prevented by maintaining ideal body weight, by doing physical activities and by increased consumption of vegetables and fruits (Jemal et al. 2008).

Fig: I. Worldwide incidence and mortality rates of cancer in 2012 (Adapted from Ferlay et.al)
The development of cancer is a multi-step process that requires the building up of several genetic changes over time. It can be divided into stages such as initiation, promotion and progression (Herrero-Jimenez et al. 2000). Initiation involves the exposures of normal cells to mutagens which causes some genetic alterations that accumulate and lead to promotion of cancer and finally to metastasis in an adequate microenvironment (Haldar and Basu 2011). The genetic alterations seen are conversion of proto-onco-genes to oncogenes, mutation of tumour suppressor genes and DNA repair genes. Oncogenes are genes that are involved in autonomous cell growth and are active in cancer cells with constitutive expression ie, independent of growth factors or other signals from outside. Proto-onco genes are their normal counterpart in normal cells. Onco proteins are similar to the proto-onco-gene products but are devoid of regulatory mechanisms and promote cancer cell growth. Conversion of Proto-oncogenes to oncogenes may be due to point mutation or may be the result of gene amplification leading to over expression of growth factors, production of constitutive signals of replication, elevated levels of transcription factors etc which all lead to uncontrolled growth of cells. The function of tumour suppressor gene in normal cells is to suppress the development of tumor by acting as receptors for the molecules that inhibit cell proliferation, inducing cell cycle check point, checking DNA damage and enhancing repair, promoting apoptosis and acting as negative regulators of cell signaling pathways. One of the major reasons for tumourgenesis, is by mutation or loss of function of some tumour suppressor genes which either leads to the production of defective truncated proteins or gene silencing. The retinoblastoma (Rb) is a tumour suppressor gene that regulates the cell cycle entry from G1 to S Phase. Rb protein interacts with transcription factor E2F and it stops production of cyclin E and halts cell cycle progression. When Rb protein is bound to the transcription factor E2F, transcription/replication is halted. Mutation of the Rb gene leads to release of E2F from Rb and the check point control is lost leading to uncontrolled cell
proliferation. There are yet another type of genes, involved in DNA damage repair that is known to provide stability to the genome (Levitt and Hickson 2002). The mutations in these genes confer certain hallmark characteristics to cancer cells like uncontrolled continuous proliferation, immortality and helps the cells to evade tumor suppressors activity, resistance to apoptotic cell death, angiogenesis, and promoting invasion and metastasis (Hanahan and Weinberg 2000). As normal cells are concerned, cell division and progression through the cell cycle is stringently governed by set of proteins that work together in a specific cascade of events. Checkpoints ensure that each phase of the cell cycle is completed accurately and that only completely replicated DNA is passed onto daughter cells. Main proteins involved are cyclin-dependent kinases (CDKs) and cyclins. CDKs are protein kinases that phosphorylates and activates downstream kinases or phosphatases leading to the progression of cell cycle. CDK activity is dependent on subunits known as cyclins that are synthesised and degraded in a cell cycle-dependent manner. CDK inhibitors tightly regulate the Cyclin-CDK complexes. Whether the cells should enter the cell cycle or not is determined at the restriction point or R point. This decision depends on the extracellular mitogenic signals received by the transcription factors (e.g. E2F) that are in the nucleus transmitted to them via signaling pathways. These regulatory checkpoint proteins activate the S-phase CDKs, which lead to DNA synthesis. Immortalisation is a vital step in the transformation of normal cells to malignant ones and can be attributed partly to the presence of telomerase. They are the enzymes which help in maintaining telomeres at the ends of chromosomes and function by extending telomeric DNA. Almost 90% of cancer cells are found to have extensive telomerase activity that counter the telomere shortening, preventing cell death and making cancer cells immortal leading to cell death unlike normal cells that have no detectable levels of telomerase activity. Metastasis is the hallmark of malignant tumors. It is the spread of cancer cells to various sites in the body via the blood stream or lymphatics and is the
most deadly stage of cancer. Metastatic cells lack adhesiveness as compared to normal cells and can invade, by moving through the basement membrane and infiltration (intrusion) into the interstitial tissues lying beneath (Kramer et al. 2013a). The invasion - metastasis step involves (a) acquisition of local invasiveness that is attained through overexpression of various matrix metalloproteinases (MMPs) that degrades the ECM (b) intravasation, transportation of cells through the blood/lymph vessels to distant organs or sites requires the epithelial-mesenchymal transition (EMT). It is a major event in tumor invasion and metastasis and is facilitated by reprogramming of epithelial cells (Wu et al. 2010a). It involves a change in the morphology of epithelial cells to a mesenchymal phenotype, that is characterized by marked decrease in expression of epithelial cell markers, like E-cadherin, α-catenin and γ-catenin, and a increase in mesenchymal markers, such as vimentin, N-cadherin and fibronectin (Yu et al. 2014). Cell motility is also controlled by G proteins that are activated by signalling pathways in cytosol (c) extravasation involves the escape of cancer cells from circulation and adapts to the local tissue environment and form colonies there. Recognition of all these well-established hallmarks of cancer cells is believed to make a significant difference while designing and developing new means to treat cancer.

The acute toxicity related with conventional treatments like chemotherapy and radiation therapy and some impairment of functional structures as in case of surgery affects the overall survival rate of cancer patients. It is, thus, inevitable to explore additional alternative strategies for the control and management of cancer. As discussed earlier, cancer is a result of multistage and multi-mechanism process that involves mutagenic, cell death and epigenetic mechanisms, involving three distinct stages like initiation, promotion, and progression. Absolute reversion of the initiation phase is difficult so the most successful intervention would be at the promotion stage (Trosko 2005). Chemoprevention is the use of both natural and/or synthetic agents to
reverse, suppress, and/or prevent initiation, promotion and progression of tumorigenesis (Wu, Patterson and Hawk 2011). According to the application of this strategy at different stages of carcinogenesis, it is divided into primary secondary or tertiary chemoprevention (Fig II). Primary chemoprevention is targeted towards high-risk individuals and implies to agents that prevent incidence of precursor lesions; secondary chemoprevention is directed towards the regression of prevalent precursor lesions; and tertiary chemoprevention is aimed at preventing the recurrence of secondary primary tumors (SPTs) and/or the spreading of primary tumors to other organs (Davis and Wu 2012).

**Fig II: Schematic diagram illustrating primary, secondary and tertiary chemoprevention at various stages of carcinogenesis**

The area of chemoprevention has gained attention in the recent years due to the success in clinical trials. Tamoxifen approved by FDA to use in breast cancer, dutasteride and finasteride that have shown chemopreventive potential in prostate cancer patients are just two examples (Davis and Wu 2012). Currently, there are numerous potent anticancer agents in clinical trials for various cancers (Meyskens and Szabo 2005).
The process of carcinogenesis involves multiple steps and arresting any one or more of these steps may halt or delay the development of cancer. This has been well explained in studies involving precancerous and cancerous lesions of the head and neck, which focus on oral premalignant lesions (leukoplakia and erythroplakia) and their associated increased risk of progression to cancer. There are several chemoprevention trials conducted in HNSCC (Lippman et al. 1993, Hong et al. 1986b, Shin et al. 2001, Tsao, Kim and Hong 2004). But toxicity is always a primary concern in studies involving human subjects. The main characteristics of an ideal chemopreventive agent is that it should be less toxic, effective at lower doses, cheap and easily available. Several epidemiological researches have reported that consumption of fruits, vegetables and grains as well as phytochemicals from non-dietary sources confers protection against wide variety of malignancies (Singh and Agarwal 2003). Although limited progress has been made in head and neck cancer chemoprevention, the field still remains viable though with challenges of identifying safe and effective agents that can be used in high risk individuals as well as cancer patients.