CHAPTER I

INTRODUCTION

Cancer is the term used for the diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through blood and lymph systems. There are more than 100 different types of cancer, in which oral cancer is the cancerous tissue growth located in oral cavity. It may arise as a primary lesion originating in any of the oral tissues, by metastasis from a distant site of origin, or by extension from a neighboring anatomic structure, such as nasal cavity or the maxillary sinus.

Cancer is responsible for high morbidity and mortality rates worldwide. Among various types of cancer, oral cancer is the eighth most common cancer worldwide and is the leading cancer among males in India. Oral cancer ranks in top 3 of all cancers in India that accounts for over 30% of all cancers reported in country and oral cancer control is quickly becoming a global health priority (Coelho, 2012). Unlike in the West, where 65 per cent of oral cancers are tongue cancer, in India, oral cancer predominantly (60 per cent) is of the lining of mouth, lower gum and other mucosal regions of oral cavity, known as the Oral Squamous Cell Carcinoma of the gingivo-buccal region (OSCC-GB). Tobacco chewing is a major cause of OSCC-GB, which accounts for over half of oral cancers in India. More than forty thousand cases of oral cancer were undertaken, being diagnosed in the United States in 2012 (Coelho, 2012). This form of cancer accounts for about 3% of cancers in men (American Cancer Society, 2012) and 1.5% of cancers in women (American Cancer Society, 2012). The estimated annual worldwide number of incident oral cancers is about 275,000, with an
approximately 20-fold variation geographically. South and Southeast Asia, France, and Brazil have particularly high rates. In most countries, men have higher rates of oral cancer than women (Warnakulasuriya, 2009).

In India, due to cultural, ethnic, geographic factors and the popularity of addictive habits, the frequency of oral cancer is high. It ranks first in terms of incidence among men and third among women. Several factors like tobacco and tobacco related products, alcohol, genetic predisposition and hormonal factors are suspected as possible causative factors, although many oral cancer sufferers have no obvious risk factors. Infection with human papillomavirus (HPV-16) has been known to associate with a high risk of developing squamous cell carcinoma of the oropharynx (Mork et al., 2001).

Oral cancer is managed through various approaches like surgery and radiation, which can be used alone or in combination, often with chemotherapy. Chemotherapeutic drugs have side effects of indiscriminately killing normal cells along with the intended cancer cells. Newer therapeutic approaches use targeted drugs to specifically kill cancer cells by exploiting subtle differences between cell types at molecular level. Such targeted drugs approved for oral cancer treatment, work by targeting important events associated with oral carcinogenesis. A blocker agent, Cetuximab (NCI, 2009) blocks the entry of growth factor through epidermal growth factor receptor (EGFR), preventing tumorous cell growth. Another set of agents like Bevacizumab acts by targeting vascular endothelial growth factor (VEGF), a critical biomolecule necessary for angiogenesis (Bisen et al., 2012). The current approaches against cancer treatment are associated with adverse side effects and drug interactions. To avoid these
adverse effects, investigations have been extended to study novel molecules as new leads, which can offer better protection and lower incidences of adverse side effects.

A new approach in cancer treatment is to target the altered cell survival pathways, which are over-expressed in cancer cells (Downward, 2003). This approach is based on the assumption that targeting over-expressed pathways will be more selective in destroying the cancer cells and have fewer effects on normal lymphocytes. Protein kinases, being important constituents of cellular signal transduction pathways, are frequently altered or overexpressed in cancer cells (Cohen, 2002; McGovern and Shoichet, 2003). This observation has led to increased focus on kinases for targeted anti-cancer drug discovery efforts (Noble et al., 2004; Liao, 2007).

Protein kinases are regulators of cell signaling pathways controlling cellular growth, proliferation and apoptosis. The fact that the abnormal activity of some kinases can cause major diseases, and are readily inhibited by catalytic site-directed molecules, has resulted in their emergence as the drugable kinome that can be targeted for therapy of most human diseases. Kinase targeting is a central theme in drug discovery and molecular cancer therapy (Huse and Kuriyan, 2002; Bain et al., 2003; Druker, 2004; Vieth et al., 2004; Knight and Shokat, 2005), but a structural basis for rational design appears to be inconclusive (Huse and Kuriyan, 2002; Bain et al., 2003; Vieth et al., 2004; Bowman and Leong, 2006). In practice, most ligands or drug leads are actually discovered through screening techniques. While the paradigm of target specificity (Huse and Kuriyan, 2002; Bain et al., 2003) may be shifting to controlled multi-target impact (Bowman and Leong, 2006), the structural factors determining these possibilities are not yet fully understood, in spite of notable progress. For instance, the
accessible nonpolar surface is frequently invoked to assess protein associations (Chothia et al., 1985; Whittle and Blundell, 1994; Feng et al., 2005; Bowman and Leong, 2006). As a general rule in cancer therapy, an effective target for cancer treatment must have a biological function that is indispensible in the cell, its abnormal activity and deregulation must be related to the malfunction of the cell and be specific to cancer cells compared to the normal one (Ahmad et al., 2005). Based on growing evidence in cancer research, one can argue that protein kinase CK2, previously known as Casein Kinase II demonstrates these traits. Therefore, CK2 is put forth as a suitable target for cancer therapy in this study. Besides, it has been documented through a study wherein total serum glutathione-S-transferase levels were measured in patients (n = 27) with various stages of biopsy proven oral cancer (squamous cell carcinoma) and age and sex matched healthy human volunteers (n=10). In all patients with oral cancer, total serum glutathione-S-transferase (GST) was measured before the onset of treatment. There was a significant increase in serum total GST levels in patients with stage IV oral cancer as compared to stage II (P = 0.001) and stage III (P = 0.002) oral cancer. This shows that alterations in serum total Glutathione-s-transferase levels may have a role in cancer progression (Prabhu and Bhat, 2007). Recently, a study was undertaken to estimate and compare erythrocyte superoxide dismutase (E-SOD) and glutathione peroxidase (GPx) levels in oral submucous fibrosis, oral leukoplakia, oral cancer patients, and healthy subjects (Gurudath, et al., 2012). E-SOD and GPx levels were estimated in oral submucous fibrosis (OSF), oral leukoplakia and oral cancer patients with 25 subjects in each group. The results obtained were compared with the corresponding age-/sex- matched control groups. Statistically significant (P < 0.001) decrease in E-SOD and GPx levels were observed in OSF
oral leukoplakia and oral cancer groups as compared to the control group. Oral leukoplakia group showed lower levels in comparison with OSF (P > 0.05). Oral cancer group had the lowest levels amongst the study groups. Conclusively, an imbalance in antioxidant enzyme status may be considered as one of the factors responsible for the pathogenesis of cancer and may serve as a potential biomarker and therapeutic target to reduce the malignant transformation in oral premalignant lesions/conditions (Gurudath, et al., 2012).

Protein kinase CK2 is involved in many fundamental aspects of normal cell life, including cell cycle regulation, development, proliferation, signal transmission and apoptosis. CK2 is a potent suppressor of apoptosis. It strongly promotes cell survival and strengthens the multi-drug resistant phenotype. Further, the pro-survival/anti-apoptotic signaling pathway induced by P13 kinase (P13K) targets protein kinase B (PKB) or Akt that induces survival through direct inhibition of caspase 9 and pro-apoptotic protein BAD. This pathway is regulated by tumor suppressor PTEN (phosphatase and tensin) homolog deleted on chromosome 10. Removal of PTEN renders P13K-mediated Akt induction constitutively active (Duncan and Litchfield, 2008). CK2 intervenes at two sites in this transduction pathway. First CK2 targets PTEN for degradation by the proteasome (Torres and Pulido, 2001). Second, CK2 directly phosphorylates Akt and promotes its activation to further induce cell survival (Di Maira et al., 2005, Di Maira et al., 2009). Overall, CK2 over-expression and dysregulated activity perturbs significant pro-survival and anti-apoptotic signaling pathways leading to cell transformation and tumorogenesis.

CK2 has been found to be upregulated and over-expressed in almost all malignancies. Dysregulation of CK2 kinase activity was found to enhance the oncogenic ability of some
genes and lead to malignant transformation of tissue. Since CK2 is involved in several essential cellular pathways and implicated in tumorogenesis and transformation of tissues CK2 can be considered a promising target for cancer therapy (Battistutta, 2009). Fortunately, protein kinases can be shut down using small molecules targeting their ATP binding sites. There is a growing list of small ATP-site directed molecules that can be used to inhibit CK2 kinase activity.

Nature is the source of several anti-cancer compounds, which act as antioxidants and anti-proliferative agents. Some of these compounds have a general acceptance as dietary elements and also have a well-established safety profile. According to one of the estimates by the World Health Organization (WHO), approximately 80% of the world’s population relies on traditional medicine for their primary health care (Farnsworth et al., 1985). Drugs derived from these natural sources have become potential source of alternative medicine that can be used alone or in combination with chemotherapy to manage cancer (Bisen et al., 2012). This has led to a growing interest in therapy using naturally occurring compounds.

Ellagic acid (EA) (Fig.1) is a potent plant derived antioxidant (Atta-Ur-Rahman, et al., 2001; Festa, et al., 2001) and anti-proliferative (Narayanan, et al., 1999; Seeram, et al., 2005) compound found in numerous fruits, nuts and vegetables including pomegranates, pecans, raspberries, strawberries, walnuts showing high selectivity and specificity towards CK2, and is thus documented to be an efficient molecule for CK2 inhibition (Festa, et al., 2001). Since the CK2α- and α'-subunits exhibit substrate specificity that is different from the CK2 holoenzyme, one might speculate that the asymmetric distribution of the CK2 holoenzyme and the CK2 catalytic subunits may have regulatory functions (Anderson et al., 2011). EA is
derived from ellagittannins (ETs) present as dietary polyphenols found in fruits and nuts (Doyle and Griffiths, 1980; Boukharta et al., 1992; Leonardo et al., 2011).

Figure 1: Ellagic acid

This study involves the use of computational drug design approaches to assist in the drug design process against cancer using molecular docking analysis of proteins relevant to cancer with selected ligands of natural and synthetic origin. The computational drug design approach helps to increase the efficiency of the drug discovery process as well as reduce the experimental work done. Three target molecules, viz., Glutathione-S-transferase (chapter-III), Glutathione Peroxidase (chapter-IV) and CK2 (chapter-V) were selected as target molecules against cancer. The availability of X-ray crystal structure of these enzymes allowed the exploration of both the structure-based and ligand-based drug design strategies. EA was selected as one of the natural compounds used for molecular docking studies with two major enzymes (GST and CK2) selected for this study against cancer.
OBJECTIVES

- Molecular docking and critical analysis of proteins and their ligands relevant to oral cancer
- Identification, characterization and interactional studies of the selected ligands of natural and synthetic origin against selected proteins with respect to oral cancer