INTRODUCTION

1.1. Cancer

Cancer is a multistep process in which normal cells are transformed into cancer cells. It is characterized by a progression of changes on cellular and genetic level that ultimately reprogram a cell to undergo uncontrolled cell division, and thus forming a malignant tumor (Khan and Bisen, 2013). Globally, cancer is the second most leading cause of morbidity and mortality behind cardiovascular (CV) disease. Approximately six million people die due to cancer every year. It is estimated that by 2020 there will be 15 million new cases every year. Which mainly due to lack of hygiene, smoking, chewing tobacco leaves, regular alcohol consumption and other factors (Belpomme et al., 2007).

1.2. Oral cancer

Oral cancer, the sixth most common cancer globally, imposes a significant burden on public health in many region of the world. Oral cancer, a subtype of head and neck cancer (HNC), is cancerous tissue growth located in the oral cavity. It refers to tongue, lips, buccal mucosa, retro molar trigone, hard palate, alveolar ridges and floor of the mouth (Scully and Bagan, 2009). There are identified several class of oral cancers, such as squamous cell carcinomas (SCC), basal cell carcinomas (BCC), malignant melanoma (MM) nasopharyngeal carcinomas (NC), mucoepidermoid carcinoma (MEC), veracious carcinomas (VC) and ameloblastoma. Thus, 90% are SCC originating in the tissues that line the oral cavity. It accounts for 9 out of 10 oral malignancies and is the most common histological type of oral cancer. Oral SCC is preceded by oral preneoplastic lesions such as leukoplakia and erythroplakia (Neville and Day, 2002).

The World Health Organization (WHO) pointed out the oral cancer causes highest mortality of amongst all malignancies. Oral cancer can be life threatening if not diagnosed and treated early and promptly (Becker et al., 2000). Despite recent and sophisticated technologies are available for the treatment of oral cancer, the 5 year survival rate is not drastically changed
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during the past 3 to 4 decades. The main reason for the poor survival rate is due to late diagnosis and poor understanding about cancer development.

1.3. Epidemiology

Oral cancer epidemiological estimated annual incidence of over 300,000 cases, of which 62% arise in developing countries. There is a significant difference in the incidence of oral cancer in different regions of the world. The age-adjusted rates of oral cancer vary from over 20 per 100,000 populations in India, to 10 per 100,000 in the United States, and less than 2 per 100,000 in the Middle East (Krishnamurthy and Ramshankar, 2013). In comparison with the U.S. population, where oral cavity cancer represents only about 3% of malignancies, it accounts for over 30% of all cancers in India (Coelho, 2012; Sambasivaiah et al., 2003). While oral carcinoma of the tongue and floor of mouth is common in the western countries, cancers of the buccal mucosa are common in Asian countries such as India, Sri Lanka, Pakistan and Bangladesh (Kimple et al., 2014).

1.4. Oral cancer risk factors

Research has shown that people with certain risk factors are more likely than others to develop oral cancer. Tobacco (smoking, chewing and betel quid etc.,) and alcohol consumption is well established influence risk factors in oral carcinogenesis. Other some possible risk factors include virus, age, poor diet and lifestyle. Three out of four people with oral cancer have been used tobacco, alcohol and/or both (Madani et al., 2012; Haedicke and Iftner, 2013).

1.4.1. Tobacco

Tobacco is a major etiological factor for oral cancer. This carcinogenicity are rich evidences suggesting that tobacco in various forms, including smoking, chewing and in betel quid etc., the commonest form of tobacco used as smoking in many countries including India. The most important are tobacco-specific carcinogenic nitrosamines, such as N-nitrosonomicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and polycyclic aromatic
hydrocarbons (PAH) such as aromatic amines and benzo[a]pyrene found in tobacco. These carcinogens have been closely linked to oral cavity cancer (Petti, 2009). The action of carcinogens is generally exerted through generate free radical and DNA adducts (Hecht, 2003).

1.4.2. Alcohol

Alcohol is a toxin substance and constant exposure to this substance increases the risk of several human diseases including oral cancer. Numerous studies have suggested that excess of alcohol consumption to be a major risk factor for oral cancer (Van Zyl and Marnewick, 2012). Ogden and Wight, (1998), reported that alcohol may act as solvent and helps to the passage of carcinogens through cellular membranes. Secondly, alcohol augmented liver metabolising activity, thus, may activate carcinogenic substances. The alteration of intracellular metabolism of the epithelial cells at the target site by ethanol, which may also be aggravated by nutrition deficiencies (Rossing et al., 1989).

1.4.3. Lifestyle

The lifestyle behaviours associated to oral cancer with convincing evidence are tobacco use, betel quid chewing, alcohol drinking, low fruit and vegetable consumption, which act separately and synergistically (Kwan et al., 2010). Worldwide, 25% of oral cancers are attributable to tobacco usage (smoking and/or chewing), 7-19% to alcohol drinking, 10-15% to micronutrient deficiency, more than 50% to betel quid chewing in areas of high chewing prevalence (Petti et al., 2009).

1.4.4. Virus

Viral infection is most emerging area in research. Viruses are capable of hijacking host cellular apparatus and modifying DNA and the chromosomal structures and inducing proliferative changes in the cells (Zur Hausen, 2002). For example, human papilloma virus (HPV), herpes simplex virus (HSV) and Epstein-Barr virus (EBV) have been implicated in oral carcinogenesis.
1.4.5. UV radiation

Exposure of UV rays is considered as an important risk factor for lip cancer in outdoor workers. Lip cancer account for 60% of all cancers in countries that are closer to the equator region. UV rays mediated carcinogenesis by altering cell division, inactivating enzymes and by inducting mutations (Armstrong and Kricker, 2001).

1.4.6. Socioeconomic status

The association between socioeconomic status and oral cancer are controversial. A positive association between oral cancer and lower socioeconomic status and inverse association between oral cancer and higher socioeconomic status has been reported (Gold et al., 1985).

1.4.7. Gender

Men are more susceptible than women for the development of oral cancer. In the past, men had a six to one ratio of incidence of oral cancer than women. However; this ratio is narrowing and is now closer to a two to one ratio. In 2010, around 4,300 men and 2,200 women were diagnosed with oral cancer. More than a third of oral cancers in men and almost a fifth in women in the UK are linked to alcohol consumption (Muscat et al., 2010).

1.4.8. Age

Oral cancer incidence is strongly related to age are quite different for men and women. The age of those who have been diagnosed with oral cancer may indicate a time component in the aging processes of the cells contributes to the malignant transformation or decreased immune system competence (Warnakulasuriya, 2009). Most of the oral cancer patients are commonly occurs in middle aged and older individuals, although a disturbing number of these malignancies are also being documented in younger adults in recent years. However, recent data shows that the fastest growing segment of the oral cancer population is non smokers under the age of fifty, which could indicate a paradigm shift in the cause of the disease or the locations where it most frequently occurs. Individuals who are over the age of 60 have the highest incidence of oral cancer (Kademani, 2007).
1.4.9. Diet

Epidemiological studies worldwide have implicated dietary and nutritional factors in the development of oral cancer. A high intake of foods considered to be dietary staples in particular cultural groups, possibly indicating a generally impoverished diet, has been linked to excess risk (Winn, 1995). Indigenous dietary practices that in single studies were found to increase risk include a high intake of chili powder and wood stove cooking. Fruits and vegetables share some nutrients of suspected importance in cancer etiology, especially vitamin C, fiber, and β-carotene, a case-control study showed that less intake of vegetables daily has the risk of oral cancer (Moynihan, 2005).

1.5. Signs and symptoms

The most common signs and symptoms of oral cancer include

- A sore in the mouth that does not heal (most common symptom)
- A white or red patch on the gums, tongue, tonsil, or lining of the mouth
- A lump or thickening in the cheek
- A sore throat or a feeling that something is caught in the throat
- Numbness of the tongue or other area of the mouth
- Swelling of the jaw that causes dentures to fit poorly or become uncomfortable
- Looseing of the teeth or pain around the teeth or jaw
- Voice change
- A lump or mass in the neck
- Weight loss

The early and late signs and symptoms of oral cancer are given in the Table 1
Table 1.1. Frequent signs and symptoms of oral cancer

<table>
<thead>
<tr>
<th>S.No</th>
<th>Early</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Persistent red and/or white patch</td>
<td>Indurated area</td>
</tr>
<tr>
<td>2</td>
<td>Nonhealing ulcer</td>
<td>Paresthesia, dysesthesia of the tongue or lips</td>
</tr>
<tr>
<td>3</td>
<td>Progressive swelling</td>
<td>Airway obstruction</td>
</tr>
<tr>
<td>4</td>
<td>Unusual surface changes</td>
<td>Chronic earache (chronic serous otitis media)/otalgia</td>
</tr>
<tr>
<td>5</td>
<td>Sudden tooth mobility without apparent</td>
<td>Trismus</td>
</tr>
<tr>
<td>6</td>
<td>Unusual oral bleeding or epistaxis</td>
<td>Dysphasia</td>
</tr>
<tr>
<td>7</td>
<td>Prokanged hoarseness</td>
<td>Cervical lymphadenopathy, persistent pain (or) referred pain, altered vision</td>
</tr>
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</table>

1.6. Diagnosis

Diagnosing cancer at an early stage is crucial to improving survival rate and reducing morbidity. Currently, the most effective way to control oral cancer includes early diagnosis timely and appropriate treatment.

1.7. Prevention

The decreased prevalence of oral cancer, the following must be considered, avoiding tobacco, limiting alcohol intake, choosing a predominantly plant-base diet, reducing fat intake (Monosaturated type), red or processed meat, avoiding exposure of meat to open flames, usage of aluminum foil to wrap meat before roasting and using microwave ovens in order to reduce formation of heterocyclic amines. Intake of optimal levels micronutrients such as vitamin C, E, β-carotene, folate and antioxidant are recommended (Shenkin, 2006).
1.8. Treatment

A complete array of diagnosis could be more useful to plan the treatment strategy

1.8.1. Chemotherapy

Chemotherapy, is a most important cancer treatment, natural or synthetic anticancer drugs is used to slow, delay, or stop the growth of rapidly dividing cancer cells in the body (Donaldson, 2004). Chemotherapy may kill cancer cells that have moved elsewhere in the body.

1.8.2. Radiation therapy

Radiation therapy uses X-rays, radioactive substances to destroy cancer cells, shrink tumors, and/or alleviate certain cancer-related symptoms (Mazeron et al., 2009). Radiotherapy can be targeted to the specific area that needs treating to prevent damaging normal tissues nearby.

1.8.3. Surgery for oral cancer

Oral cancer, which is detected at early stages, is treated with surgery. Oral cancer surgery can also be performed for patients with advanced-stage and recurrent cancers, often in combination with radiation therapy, chemotherapy or targeted therapy (Shah and Gil, 2009).

1.8.4. Biological therapy

Biological therapy uses monoclonal antibody, which blocks areas on the surface of cancer cells that can trigger growth (Scott et al., 2012).

1.9. Multistage carcinogenesis

Multiple studies suggest that human tumorigenesis is part of a multistep process. Each of these sequential steps reflects genetic changes that cause progressive alteration of normal human cells into malignant cells. The process in which normal cells convert into a cancerous state seems to require various rate-limiting steps that include genetic and epigenetic changes (Moolgavkar and Luebeck, 2003). Carcinomas accounts for more than 80% of human cancers
with oral, skin, lung, colon, breast, prostate and uterus as the most frequent sites. At least three important classes of genes play key roles in tumor initiation: proto-oncogenes, tumor suppressor genes, and genes involved in DNA repair mechanisms. Mutations, amplifications or deletions in these genes that may lead to a de-coupling of biological mechanisms involved in the regulation of normal cell growth and differentiation (Boyd and Berdchuck, 2005).

Cancer is a multistep process which consists of three phases: initiation, promotion and progression.

1.9.1. Initiation

Initiation is a rapid and irreversible process, whereby the carcinogen causes DNA adduct of target cells, including one or more simple mutation, transversions, translations and deletions. The three cellular function are important in initiation process namely, carcinogenic metabolism, DNA repair and cell proliferation.

1.9.2. Promotion

The promotion phase denotes the sustained clonal expansion of a mutated cell to form actively proliferating, multicellular premalignant lesion. The promotion phase is a protracted process that may be require several years or decades to establish and characterized by the clonal expansion of initiated cells by the induction of cell proliferation and or inhibition of programmed cell death (apoptosis).

1.9.3. Progression

Progression is the third and final stage of the carcinogenic process. This stage involves cellular and molecular changes that occur from the pre-neoplastic to the neoplastic state. This stage is irreversible and characterized by accumulation of additional genetic damage, leading to the transition of the cell from benign to malignant. This stage is characterized by genetic instability and disruption of chromosome integrity (Klaunig and Kamendulis, 2004). The multisteps of carcinogenesis are given in Fig. 1.1.
1.10. Biotransformation enzymes

Cytochrome-p450 (Cyt-P450) and Cytochrome b5 (Cyt-b5) are biotransformation enzymes, essential for initiating conversion of lipophilic xenobiotics into more hydrophilic, water-soluble metabolites. And together, induction of phase I enzymes are considered as potent risk factor for cancer due to the activation of carcinogens to form ultimate carcinogens (Rodriguez-Antona and Ingelman-Sundberg, 2006). The other components of this system viz. Cyt-b5, Cyt-P450 reductase function in a synergistic manner, facilitating the transfer of an electron to Cyt-P450 accepted from NADPH or NADH, thus enabling the proper functioning of the Cyt-P450 system (Karlgren et al., 2006).

Induction of phase II enzymes or detoxification enzymes is an important mechanism of chemoprevention. Glutathione-S-transferase (GST), reduced glutathione (GR) and DT-diaphorase are considered to be the major detoxification enzymes. The phase I carcinogenic enzymes are neutralize by phase II enzymes through conjugation process. GST is isoenzymes that catalyze the conjugation of glutathione (GSH) to a variety of electrophilic agent as the
first step in a detoxification pathway leading to the formation of mercapturic acid. Glutathione, consistently as the first line of defense against oxidative stress, is the most imperative cellular thiol enzyme that acts as a substrate for several transferases, peroxidases and other enzymes which prevent the adverse effects of oxygen free radicals (Surh, 2003).

1.11. Molecular markers

1.11.1. Apoptosis

Apoptosis is, capable the greatest defense against cancer development. Hence, it is cell-intrinsic machinery and helps to elimination of deleterious cells (Ding and Fisher, 2002). Intracellular or "intrinsic" cues, such as oxidative DNA damage, hypoxia, nutrient deprivation, or ROS, function via the mitochondrial pathway, which is tightly modulated by the Bcl-2 family proteins. Pro-apoptotic Bcl-2 proteins (Bax, Bim, Bid, Bak and Bad) are present in healthy cell and inactive form, while anti-apoptotic Bcl-2 proteins (Bcl-2 and Bcl-XL) are constitutively active and reside in the mitochondria outer membrane (Chan et al., 2004). Following the Bcl-2 family protein ratio (Bax/Bcl-2) which leads to form pores in the mitochondrial membrane, this facilitates the release of cytochrome c into the cytosol. Once cytochrome c accumulates in the cytosol, it complexes with procaspase-9 and Apaf-1 to form the "apoptosome," which in turn activates caspase-3. Thus, the default Bcl-2 signal is to preserve mitochondrial membrane integrity and prevent apoptosis and whereas apoptosis can only occur when the concentration of Bcl-2/Bax ratio at the mitochondrial membrane. It should be noted that the mitochondrial pathway can be indirectly triggered through less obvious extracellular signals. For example, following death receptor ligation, activated caspase-8 can cleave and activate Bid (Chan et al., 2004). Both intrinsic and extrinsic signaling cascades converge at the point of caspase-3 activation, which is often considered the "point of no return" in apoptosis (Fig. 1.2).
1.11.1. Tumor suppressor gene \((p53)\)

\(p53\), a tumor suppressor gene, is a transcription factor that protects the genome stability and normal cell growth. \(p53\) is the most common mutated tumor suppressor gene of several cancerous tissues. Under normal circumstances, \(p53\) does not allow the damaged cell into cell-cycle by acting as a molecular regulator. Enormous studies on experimental and human oral cancers painted out \(p53\) mutation as one of the common genetic in triggering neoplastic transformation in the oral tissues (Oren, 2003; Silvan and Manoharan, 2013).

1.11.2. B cell lymphoma gene 2 (Bcl-2)

Bcl-2 family proteins are comprised of both pro-apoptotic (Bax and Bak) and anti-apoptotic (Bcl-2 and Bcl-XL) proteins. Bcl-2 is located to the outer mitochondrial nuclear envelop and the member of endoplasmic reticulum.
Imbalance of pro and anti-apoptotic proteins expression might cells to neoplastic transformation. Bcl-2 is an apoptosis rather than inducing cell proliferation. Over expression were shows in various tumor tissues including oral tumor tissues (Borner, 2003).

1.11.4. Bcl-2 associated X protein (Bax)

Bax, a pro-apoptotic protein, is present in viable cells and they are activated by pro-apoptotic stimuli. Bax induces apoptosis in response to genotoxic stress, and thus protects cells from neoplastic transformation. It binds to Bcl-2 and inactivates the anti-apoptotic role of Bcl-2. While excess of Bax causes cell death, excess Bcl-2 prolongs cell survival. Bcl-2/Bax ratio thus determines the apoptotic cell death. Down regulation of proteins was reported in oral carcinogenesis (Lindsten et al., 2000; Letchoumy et al., 2007).

1.11.5. Cystein-aspartic proteases (Caspase-9 and -3)

Caspase-3 is an important apoptotic marker, exists in the cytosolic fraction of cell as an inactive pro-caspase-3. The process programmed cell death (apoptosis) is executed by a class of cysteine proteases known as capases (Hu et al., 1998). Caspase-3 shares may of the typical characteristics common to all currently-known caspases. The inactive form is converted to active form by proteolytic signals received for apoptosis to remove the damage cells. Caspase-3 plays crucial role in apoptotic chromatin condensation and DNA fragmentation in all cell types examined. Caspase-9 is the functionally important initiator of the apoptotic cascade. Caspase-9 triggers a signalling cascade to induce apoptosis. Decreased levels of Caspase-3 and -9 were reported in oral carcinogenesis (Hakem et al., 1998; Vidjaya Letchoumy et al., 2006).

1.12. Inflammatory markers

In cancer, chronic inflammation is one of the early pathological alterations for neoplastic transformation (Gimenez-Conti and Slaga, 1993). Chronic inflammations are regulated by several inflammatory markers. Dysregulation of the inflammatory molecular events responses leads to the development of various pathological states including cancers (Ben-Neriah and Karin, 2011).
1.12.1. Nuclear factor-κB (NF-κB)

NF-κB is one of the major inflammatory transcription factor activated by several stimuli such as oncogenes, viral proteins, carcinogens, cytokine and growth factors. Thus activated NF-κB regulates several downstream target genes that mediate transformation, proliferation, invention, angiogenesis, apoptosis, and metastasis. Over-expression of NF-κB was observed in oral carcinogenesis (Oeckinghaus et al., 2011).

1.12.2. Tumor necrosis factor alpha (TNF-α)

TNF-α is an adipokine involved in pathological inflammation. It is produced chiefly by activated macrophages, although it can be produced by many other cell types such as CD4+ lymphocytes, NK cells and neurons. The activation of TNF-α can leads to degradation of IκB-α and activation of NF-κB resulting in inflammation and other biological process. Over expression of TNF-α were observed in several type of human cancer and experimental model (Kassouf and Thornhill, 2008).

1.12.3. Inducible Nitric Oxide synthase (iNOS)

The inflammatory mediator enzyme of iNOS is synthesizes NO from L-arginine using NADPH and oxygen molecules (Murakami and Ohigashi, 2007). As plays an important role in numerous pathophysiological including oral cancer. Over expression of iNOS was observed human and experimental animal model (Chen et al., 2000).

1.12.4. Cyclooxygenase-2 (COX-2)

Cyclooxygenase-2 (COX-2) the rate limiting enzymes in prostaglandin synthesis, is stimulated by several factors including mitogens and tumor promoters. Ample evidences pointed out that COX-2 inhibition may be a valid target for the prevention of precancerous and cancerous lesions in several organs. Over expression of COX-2 was observed in oral cancer (Turini and Dubois, 2002; Pannone et al., 2004).

1.12.5. Interleukin-1 (IL-1) and Interleukin-6 (IL-6)

IL-1 and IL-6 is a multifunctional cytokine that controls many cellular processes such as inflammation, differentiation of immune cells, and hepatic
regeneration in response to diverse stimuli (Heinrich et al., 2003). IL-6 plays vital role in angiogenesis since it has been demonstrated that in vivo expression of IL-6 accompanies vascularization during wound healing, psoriasis and tumor growth. Its biological activity is mediated through its binding to the membrane-bound glycoprotein IL-6 receptor chain (gp80), which is present on several target cells, including ECs. This leads to form dimerization of the ubiquitously expressed gp130 and to the activation of several intracellular signal transduction events, including Janusactivated kinase (JAK)/signal transducers and activators of transcription (STAT) signaling cascade (Lamy et al., 2012).

1.13. Cell proliferative markers

1.13.1. Proliferating cell nuclear antigen (PCNA)

PCNA, a 36 kDa non-histone protein play an important role in several cellular process including DNA replication, chromatin remodelling, DNA repair, sister-chromatid cohesion and cell cycle control. PCNA is one of the helper proteins of DNA polymerase σ, which is a key protein in cell cycle regulation and a trigger of cell proliferation and differentiation. PCNA is considered to be a marker of cell proliferation in various cancers. PCNA is cell specific protein, maximally expressed in late G1 and S phase of cell cycle. The investigation of PCNA would help to assess invasion and metastatic features of cancer cells. Over expression of PCNA was demonstrated in oral carcinogenesis (Staibano et al., 1998; Poosarla et al., 2015).

1.13.2. Cyclin D1

Cyclin D1, a critical cell cycle regulatory protein, derived the cell cycle from the G1 to the S phase. Several studies suggested that Cyclin D1 expression can be used both as a prognostic marker and as a target in the treatment of the disease. A positive correlation between Cyclin D1 and the members of the AP-1 family, c-jun and c-fous was shown. Deregulation of Cyclin D1 involved in the regulation of cell cycle, is one of the common phenomenon in tumor development. Upregulation and amplification of cyclin D1 was shown in oral cancer (Fu et al., 2004).
1.13.3. Ki-67

The nuclear protein Ki-67 is strongly correlated with cell proliferation. It is expressed in all phases of the cell cycle, except G0, making it an ideal marker for neoplastic tissue. Immunohistochemical staining of Ki-67 is widely used for the analyze carcinogenesis prognosis in tumour tissue. Despite over expression of Ki-67 was reported in head and neck cancer metastases and decreased survival (Klimowicz et al., 2012).

1.14. Transcriptional regulatory genes

1.14.1. Activator protein-1 (AP-1)

AP-1 is a DNA site specific transcription factor and it composed of heterodimer proteins belonging to the c-Jun, c-Fos, ATF, and JDP families. These dimers can bind AP-1 DNA recognition elements (5’-TGAG/CTCA-3’). It is activated by several stimuli including cytokines, growth factors, oncoproteins, serum, stress, UV radiation, chemical carcinogens, bacterial and viral infections (Eferl and Wagner, 2003). AP-1 transcriptional factor has been implicated in regulation of several genes involved in apoptosis and proliferation and may promote cell proliferation by activating the Cyclin D1 gene, and suppress tumor-suppressor genes, such as p53, p21cip1/waf1 and p16. These oncogenic properties of AP-1 are primarily dictated by the dimer composition of the AP-1 family proteins and their posttranscriptional and translational modifications (Young et al., 2003).

1.14.2. Signal Transducers and Activators of Transcription-3 (STAT-3)

STAT-3 are family of cytoplasmic proteins. It act as signal messengers and transcription factors and participate in normal cellular responses to cytokines and GFs (Growth Factors). Activation of STAT-3 leads to regulation of cell proliferation, cell cycle progression, apoptosis, angiogenesis and immune evasion; and exerting effects in cancer stem cells (Mali, 2015). STAT-3 is a most often implicated in human cancer progression by controlling cell cycle
progression and apoptosis. STAT3 is generally overexpressed in Head and Neck Cancer (HNC). Inhibition of STAT3 in HNC leads to induce apoptosis, inhibit cell proliferation and tumor development (Pectasides et al., 2010).

1.14.3. The PI3K/AKT pathway

Phosphatidylinositol-3 kinase (PI3K), AKT, and mammalian target of rapamycin (mTOR) are major junction of the PI3K pathway and are typically activated by upstream signaling of tyrosine kinases and other receptor molecules such as cytokine and growth factors (Vivanco and Sawyers, 2002). Aberrant activation of the PI3K and its downstream molecules AKT, mTOR has been widely implicated in cell growth, cell proliferation, cell survival and angiogenesis in many types of cancer, including oral cancer.

1.14.4. Phosphatidylinositol 3 kinase (PI3K)

Phosphatidyl-inositol-3-kinases (PI3Ks) composed a lipid kinase family characterized by the capability to phosphorylate inositol ring 3\'-OH group in inositol phospholipids. PI3K, Akt, are major junctions in the PI3K signaling pathway and are typically activated by upstream signaling molecules of tyrosine kinases. Aberrant activation of the PI3K and its sub active proteins has been widely implicated in the transforming activity of viral oncogenes and several types of cancer, including breast, lung, colon, and oral cancer (Vivanco and Sawyers, 2002).

1.14.5. AKT

AKT, a serine/threonine protein kinase, it act as downstream mediator of phosphatidylinositol-3-kinase (PI3K) to regulate many biological processes, such as proliferation, apoptosis and growth, but other signalling pathways are also known to be regulated by PI3K activity and might be involved in PI3K-mediated tumorigenesis. This signalling activated by growth factor stimulation and subsequent activation of receptor tyrosine kinases at the cell membrane (Engelman, 2009).
1.15. Angiogenesis markers

1.15.1. Vascular endothelial growth factor (VEGF)

VEGF is a 34 to 42-kDa, dimeric, disulfide-bound glycoprotein. The growth of solid tumors is dependent on the process of angiogenesis. Therefore, solid tumors must produce angiogenic factors at an early point in tumor development. Vascular endothelial growth factor (VEGF) is an important pro-angiogenic molecule, which plays a crucial role in tumor angiogenesis, by increasing blood vessel permeability, endothelial cell growth, proliferation, migration, and differentiation (Ferrara and Kerbel, 2005). High tumor expression of VEGF protein has been linked to poor clinical outcome in several human tumor sites including oral, stomach, ovary, esophagus, breast, colorectum, lung, and bone. In OSCC, VEGF levels are elevated and may be related to poor prognosis (Salven et al., 1997).

1.16. Glycoconjugates and cancer

Glycoproteins are a family of complex proteins have oligosaccharide chain covalently linked to their polypeptide backbones. The predominant sugar moieties in glycoprotein are glucose, galactose, fucose, mannose and derivatives of sialic acid as well as acetylated derivatives of hexosamine. Glycoproteins play an essential role in cell differentiation, cell proliferation, cell-cell interaction (Rabinovich et al., 2007). It also been implicated in the transport of metabolites across cell membranes and performs basic biological functions including in protein sorting, immune defense, receptor function, cell recognition, inflammation, pathogenicity, fertilization, and degradation of blood clots, metastasis and other cellular process (Baxi, 1991).
1.17. 7,12-dimethylbenz[a]anthracene

7,12-dimethylbenz[a]anthracene (DMBA) is a member of polycyclic aromatic hydrocarbons that are present in the environment as products of incomplete combustion of complex hydrocarbons. It is one of the reference compounds most often used as a site specific carcinogen in experimental carcinogenic studies (Lim et al., 2009). These toxic metabolites of DMBA are capable of binding to adenine residues of DNA, causing chromosomal damage. DMBA and its metabolites are documented to mediate their mutagenic and carcinogenic effect via ROS generation that acts complementary to the mutation induced by diepoxides. DMBA cause to DNA adducts which may induce G→A transition or A→T transversion. Such mutations have been observed frequently at exons 5–8 of the p53 gene, and at codon 61 of the Ki-ras gene in DMBA-induced hamster oral carcinomas (Li et al., 2002; Johnstone et al., 2002).

1.18. DMBA induced hamster buccal pouch carcinogenesis

DMBA is commonly employed to develop oral carcinoma in the buccal mucosa of golden Syrian hamsters (Suresh et al., 2010). DMBA-induce buccal pouch carcinogenesis in golden Syrian hamsters is a well-accepted experimental carcinogenesis model, which is similar to human oral carcinogenesis (Gimenez-Conti and Slaga, 1993). Repeated and regular interval of topical application of DMBA resulted in tumor formation in the buccal pouch of hamsters. DMBA mediates carcinogenesis in the buccal mucosa through induction of chronic inflammation and the formation of DNA adducts as well as by causing reactive oxygen species mediated oxidative DNA damage (Li et al., 2002). DMBA and its metabolites are documented to mediate their mutagenic and carcinogenic effect via ROS generation that acts complementary to the mutation induced by diepoxides (Chang et al., 1996). DMBA induced hamster buccal pouch carcinogenesis is thus considered as an ideal experimental model to study the anticancer potential of natural products and synthetic entities.
1.19. Chemoprevention

In general, chemoprevention is an important strategy to inhibit the cancer burden. It has been use of natural, synthetic, or chemical agents to reverse, inhibit, or prevent transformation of premalignant cells to a malignant geno/phenotype. Hence, chemopreventive agents are classified into two categories:

- Chemopreventive agents to prevent or suppress carcinogens from reaching and reacting with critical cell target sites, i.e., they inhibit metabolic activation of carcinogens and their induction of detoxification.
- Suppressing agents may impair the malignant transformation in target cells, at both early and late stages of carcinogenesis (Surh, 2003).

The natural and nutraceuticals product used in modern medicine for the treatment or prevention of carcinogenesis is an important aspect. Plant derived natural products have been utilized for carcinogens therapeutic. Herbal medicine is still the mainstay of about 75-80% of the world population, particularly in the developing south Asia countries such as India, China, and Srilanka for major health care due to better cultural tolerability, compatibility and efficacy with the human body and lesser side effects. Providentially, many plant derived phytochemicals and antioxidant nutrients (vegetables, fruits, and spice) own a great impact from both the scientific community and the general public due to their various health promoting effects including cancer therapeutic (Aggarwal and Shishodia, 2006).

Cancer chemoprevention, the most promising areas and appealing strategy in experimental oncology, deals with the use of natural, synthetic or biologic substances to prevent, reverse or suppress the development of cancer in normal or precancerous tissues. Chemoprevention has earned serious consideration as a potential means of controlling cancer incidence (Kelloff et al., 2006). Primary chemoprevention deals with the use of chemopreventive agents in patients with premalignant lesions whereas secondary chemoprevention is directed at patients with cancer.
The most preferred chemopreventive approach lines in the intervention at the early stage of carcinogenesis to eliminate premalignant cells before they become malignant or protect normal cells from undergoing neoplastic transformation. Extensive studies reported the chemopreventive efficacy of natural or synthetic entities against chemical induced oral carcinogenesis (Anusuya and Manoharan, 2011; Manoharan et al., 2012). The mechanistic pathway so far reported for the chemopreventive potential of natural products or synthetic entities include antioxidants, anti-lipid peroxidative, anti-inflammatory, anti-cell proliferative, anti-angiogenic and apoptotic potential during carcinogenesis. The list of chemopreventive agents and their possible mechanisms were reported against oral carcinogenesis shows in Table 2.
Table 1.2. List of phytochemicals on cellular and molecular changes in different experimental models

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemicals</th>
<th>Mechanisms</th>
<th>References</th>
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<tbody>
<tr>
<td>1</td>
<td>Capsaicin</td>
<td>Inhibit NF-κB/Rel and AP-1 transcription factors</td>
<td>Han et al., 2001</td>
</tr>
<tr>
<td>2</td>
<td>Tea and curcumin</td>
<td>Inhibition of cell proliferation in DMBA induced oral carcinogenesis</td>
<td>Li et al., 2002</td>
</tr>
<tr>
<td>3</td>
<td>Garlic</td>
<td>Induces apoptosis during DMBA induced HBP carcinogenesis</td>
<td>Balasenthil et al., 2002</td>
</tr>
<tr>
<td>4</td>
<td>Resveratrol</td>
<td>Prevention of tumor growth by inhibiting PCNA and cyclin-D1</td>
<td>Reagan-Shaw et al., 2004</td>
</tr>
<tr>
<td>5</td>
<td>Gingerol</td>
<td>Inhibition of COX-2 mRNA and protein, as well as NF-κB translocation.</td>
<td>Kim et al., 2005.</td>
</tr>
<tr>
<td>6</td>
<td>Turmeric</td>
<td>Dietary turmeric modulates DMBA-induced p21ras, MAP kinases and AP-1/NF-κB pathway to alter cellular responses during HBP carcinogenesis</td>
<td>Garg et al., 2008</td>
</tr>
<tr>
<td>7</td>
<td>Green tea</td>
<td>Modulate phase I and phase II detoxification enzymes</td>
<td>Srinivasan et al., 2008</td>
</tr>
<tr>
<td>8</td>
<td>Resveratrol</td>
<td>Suppression of tumor progression and tumr development</td>
<td>Berta et al., 2010</td>
</tr>
<tr>
<td>9</td>
<td>Diosgenin</td>
<td>Inhibited tumor development in DMBA induced experimental carcinogenesis</td>
<td>Rajalingam et al., 2011</td>
</tr>
<tr>
<td>10</td>
<td>Rosmarinic acid</td>
<td>Induce apoptosis in DMBA induced HBP carcinogenesis</td>
<td>Anusuya and Manoharan, 2011</td>
</tr>
<tr>
<td>12</td>
<td>Myricetin</td>
<td>Modulation of central kinases such as MEK, JAK1, Akt, and MKK4 kinase by direct binding.</td>
<td>Kang et al., 2011.</td>
</tr>
<tr>
<td>S. No</td>
<td>Phytochemicals</td>
<td>Mechanisms</td>
<td>References</td>
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<tr>
<td>13</td>
<td>Chlorophyllin and Ellagic Acid</td>
<td>Reverse inflammation and angiogenesis gene expression in experimental carcinogenesis</td>
<td>Priyadarsini et al., 2012</td>
</tr>
<tr>
<td>14</td>
<td>Glycyrrhizic acid</td>
<td>Prevention of photoaging via Modulation of NF-κB and MMP</td>
<td>Afnan et al., 2012</td>
</tr>
<tr>
<td>15</td>
<td>Ellagic acid</td>
<td>Inhibit Wnt/β-catenin and NF-κB signaling pathways to induce intrinsic apoptosis in an animal model of oral oncogenesis</td>
<td>Anitha et al., 2013</td>
</tr>
<tr>
<td>16</td>
<td>Shogaol</td>
<td>suppresses cancer cell invasion and inflammation through modulation of NF-κB and Nrf2-Keap1 signaling pathways</td>
<td>Gan et al., 2013</td>
</tr>
<tr>
<td>17</td>
<td>Naringenin</td>
<td>Inhibit cell proliferation and induce apoptosis in carcinogenesis model</td>
<td>Sulfikkarali et al., 2013</td>
</tr>
<tr>
<td>18</td>
<td>Black tea</td>
<td>Black tea polyphenols suppress cell proliferation, angiogenesis and induce apoptosis on DMBA induced HBP carcinogenesis</td>
<td>Letchoumy, et al., 2007</td>
</tr>
<tr>
<td>19</td>
<td>Astaxanthin</td>
<td>inhibits NF-κB and Wnt/β-catenin signaling pathways via inactivation of Erk/MAPK and PI3K/Akt to induce intrinsic apoptosis in a hamster model of oral cancer</td>
<td>Kavitha et al, 2013</td>
</tr>
<tr>
<td>20</td>
<td>Rutin</td>
<td>inhibiting JNK and p38 MAPK signalling in rotenone induced human dopaminergic cells</td>
<td>Park et al 2014</td>
</tr>
<tr>
<td>21</td>
<td>Arctiiin</td>
<td>Modulation of Wnt and MAP kinase signaling pathways via microRNA expression.</td>
<td>Lee et al, 2014</td>
</tr>
</tbody>
</table>
1.20. Free radicals and their origin

Group of atoms with unpaired electrons are called free radicals, which can involve in chain reactions and highly reactive molecules. Free radical generated from mitochondria and its electron transport chain reaction. Reactive oxygen species (ROS), reactive nitrogen species (NOS), carbon-centered radicals, and sulfur-centered radicals are the highly dangerous free radicals (Hermes-Lima, 2004). In living systems, NOS present in the form of nitric oxide (NO) and nitrogen dioxide. The unpaired electron or free radical of NO can produce hydroxyl radicals and nitrogen dioxide radicals. Most of free radicals are generated in living biological systems. The generation of ROS is derived from the contradictory roles that oxygen plays in metabolism. In other hand oxygen is essential for living system and aerobic respiration has significant advantages, however the process of oxygen reduction to water can generate ROS as intermediates with the potential to cause cellular damage (Davies, 1995). The free radicals such as hydrogen peroxide (H$_2$O$_2$), superoxide radical (O$_2$•$^-$), and hydroxyl radical (OH•) are primary sources of ROS generation in human. The superoxide radical is generated in the processes involving oxidases such as NADPH oxidase, xanthine oxidase, and the auto-oxidation of hemoglobin. Superoxide is not particularly reactive by itself, but can be catalytically converted by superoxidase dismutases (SOD) to hydrogen peroxide, which decomposes to yield the highly reactive OH• in the presence of iron (Kowaltowski et al., 2001). The equation of the free radicals generations are given below:
1.21. Antioxidants

Antioxidants are natural or synthetic substances that may prevent or delay some types of cell damage and oxidation of biological molecules. In a biological system they may protect cells from damage caused by unstable molecules known as free radicals. Antioxidants terminate these chain reactions by removing free radical’s intermediates and inhibit other oxidation reaction (Lobo et al., 2010). They are believed to play a role in preventing the development of such chronic diseases as cancer, heart diseases, stroke, Alzheimers disease, rheumatoid arthritis and cataracts (Aruoma et al., 1989). Excess generated free radicals neutralized by antioxidants such as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). Non-enzymatic antioxidants are represented by ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants (Bouayed and Bohn, 2010).

1.21.1. Antioxidants and free radicals

Many human degenerative diseases are caused by oxidative stress that results from an imbalance between formation and neutralization of antioxidant and pro-oxidants (Pham-Huy et al., 2008). High concentrations of ROS are toxic to cells and it has many biochemical changes (Apel and Hirt, 2004). Free radicals are reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage. The recent evidence suggesting the involvement of oxidative stress in the pathogenesis of various diseases has attracted much attention of the scientists and general public to the role of antioxidants in the maintenance of human health (Andre et al., 2010).

1.22. Ginger

Ginger (Zingiber officinale), is one of the most widely used species of the ginger family (Zingiberaceae) and is a common condiment, spice for various foods beverages and exhibits characteristic odors and flavors with a pungent taste (Jolad et al., 2005). It has a long history of cooking and therapeutic use dating back 2,500 years in China and India. Ginger contains a number of
pungent constituents and active ingredients such as gingerols and their dehydration products of [6]-shogaol, zingerone and paradol are major class of bioactive compounds found in small amounts in fresh ginger and in larger amounts in dried gingers (Bode and Zong, 2011).

1.22.1. Pharmacological activities

The dietary supplement of ginger has been considered as an important ingredient in Ayurvedic, Unani and Chinese herbal medicines for the treatment of various digestive ailments such as asthma, dyspepsia, colic, gingivitis, catarrh, toothache, stroke, constipation, diabetes, and rheumatism (Ali et al., 2008). Numerous investigation documented that ginger, have serval pharmacological properties such as antimicrobial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer activities (Zick et al., 2008; Dugasani et al., 2010).

1.23. [6]-shogaol

In our study, we focused on phenolic agent of [6]-shogaol (Fig. 1.3), the dehydrated product of [6]-gingerol, is a major bioactive compound in the rhizomes of ginger. It is structurally similar with other ginger pungent compounds. As dehydrated products of [6]-shogaol less amount exist in fresh ginger, nevertheless maximum amounts present in dried or thermal treated ginger, as conversion of some gingerol to [6]-shogaol (Chrubasik et al., 2005). [6]-shogaol exhibit strong antioxidant properties which can be attributed to the presence of α, β- unsaturated ketone moiety and long side chain length (Dugasani et al., 2010). [6]-shogaol has been shown to inhibit ovarian, lung, breast, colon, and skin cancer cell growth (Rhode et al., 2007; Tan et al., 2013). As evidence that [6]-shogaol induce apoptosis via the production of ROS and activation of caspase in human colorectal carcinoma cells (Pan et al., 2008b).

In another study, [6]-shogaol reduce gastric cancer cell viability by impairing tubulin polymerization (Ishiguro et al., 2007). Moreover, dietary agent of [6]-shogaol has been shown to be effective at inhibiting the expression of inflammatory mediators, inducible iNOS and COX-2 induced by either
lipopolysaccharide or phorbol 12-myristate 13-acetate (PMA) (Pan et al., 2008a). Furthermore, Park et al., (2009) reported that it can act as suppressor of the TRIF-dependent signalling pathway of Toll-like receptors by targeting TBK1. In addition, [6]-shogaol inhibit metastasis in cancer cells through suppression of NF-κB activity (Ling et al., 2010).

![Ginger](image)

[6]-shogaol
- Chemical name: 1-(4-Hydroxy-3-methoxy-phenyl) dec-4-en-3-one
- Molecular formula: C\(_{17}\)H\(_{24}\)O\(_3\)
- Molecular mass: 276.38 g·mol\(^{-1}\)

Fig. 1.3. Molecular structure of [6]-shogaol