Prologue

1. Scenario of oral cancer

Oral cancer ranks among the top three cancers in India, which accounts for over thirty percent of all cancers reported in the country. Age-adjusted rate of oral cancer in India is as high as 20 per 100,000 population (Coelho, 2012).

High incidence of oral cancer can be attributed to a number of etiological factors. The greatest, well established and well documented risk factor is tobacco. More importantly, tobacco consumption is the major cause of preventable deaths. It has been reported that the clinical, molecular and pathological profiles of tobacco-smoking and alcohol associated oral cancers are different from tobacco chewing associated oral cancers. Indians have distinct cultural practices such as betel-quid chewing as well as varying patterns of use of smokeless tobacco like gutkha, pan-masala, mava-masala and alcohol. These are important risk factors that predispose to cancer of oral cavity and significantly contribute to rising incidence rate of the dreaded disease. Since carcinogenesis is a multi-step process, several additional factors other than insults by tobacco-associated intra-oral carcinogens also play a vital role in synergetic manner in oral tumorigenesis. These factors include genetic susceptibility and external agents such as Human Papillomavirus (HPV). Recently, most of the epidemiological studies have suggested that High-Risk Human Papillomavirus (HR-HPV) play an important role in etiopathogenesis of oral cancer (Campisi *et al.*, 2007; Chocolatewala and Chaturvedi, 2009; Kumaraswamy and Vidhya, 2011). Further, molecular and clinico-pathological characteristics are different in HPV positive and HPV negative oral cancer patients. Also, HPV positive or negative oral cancers are considered as different entities in terms of outcome and response to treatment (Chen *et al.*, 2012). With advancing age, the risk of developing oral cancer increases. However, recently a trend of increased incidence of oral cancer in younger age groups has been observed. Among this young population, there is a sub-group of patients not exposed to the known major risk factors, in whom other factors such as HPV infection may be involved.

Interestingly, the variations in incidence and pattern of the disease have been observed across the country. It can be attributed mainly to regional differences in the prevalence of disease-specific risk factors. In Gujarat, Western India, this malignancy is highly prevalent with a serious trend of increased rate of oral cancer in the younger age groups (Patel *et al.*, 2009). Additionally, chewing mawa-masala and gutkha is the

predominant tobacco habit in population from Gujarat (Joshi *et al.*, 2010). The hospital based cancer registry (HBCR) of the Gujarat Cancer & Research Institute (GCRI), Ahmedabad, Gujarat have also shown that oral cancer is the leading malignancy among all cancers diagnosed and treated at the institute.

2. Oral Cancer: Challenges

Globally, the 5 year survival rate of oral cancer is low (Krishna Rao *et al.*, 2013) and has not changed significantly in recent years despite of the advances in surgery, radiotherapy and chemotherapy. Because of this high mortality rate the concern for oral cancer management has great importance. Therefore, oral cancer control has become the health priority in India. Even with the remarkable technological advancements and extraordinary efforts from researchers and clinicians, oral cancer is often diagnosed at a later stage. Oral cancer is also characterized by a high degree of local invasiveness and a high rate of metastasis to cervical lymph nodes (Singh *et al.*, 2010). Metastasis is a major problem in successful cancer treatment and it is reported that they originate very early in the growth of the primary tumor (Vicente *et al.*, 2005). Further, this malignancy is associated with high incidence of loco-regional recurrences, which account for the majority of treatment failures post-surgery and radiotherapy. Prognosis and outcome of oral cancer are mainly determined by TNM clinical classification (Brońić *et al.*, 2009). However, this does not provide any information on the biological characteristics and aggressive nature of the tumor. Hence, the drawbacks of conventional histopathology are becoming evident. Thus, high mortality rate is mainly attributed to late diagnosis, poor response of tumor to chemotherapy and radiotherapy as well as poor understanding of oral cancer biology for its application in early diagnosis and post-therapeutic monitoring.

There is abundant evidence that early diagnosis would reduce the morbidity and mortality of oral cancer. Molecular diagnostic aids/tools may help to achieve earlier diagnosis. Further, screening of the “at-risk” population, for early detection of invasion, metastasis and recurrences also help to improve the disease associated morbidity and mortality. This can be aided by studying and analyzing molecular signatures in a comprehensive manner.

3. Molecular pathogenesis of oral carcinogenesis in relevance to *p53*: the guardian of genome

Molecular pathology defines the molecular mechanisms that underlie the onset of oral cancer. Numerous genetic events that alter normal functions of oncogenes and tumor suppressor genes are involved in oral carcinogenesis. This may increase the production of growth factors, transcription factors or intracellular signal messengers. Together with the loss of tumor suppressor activity, these changes lead to a cell phenotype that can increase cell proliferation, with loss of cell cohesion, and infiltration of adjacent tissue thus causing distant metastasis (Campo-Trapero *et al.*, 2008). Thus, tumor suppressor mechanisms are very crucial for cells as it prevents the cells from development of malignant phenotype (Campisi, 2003). The tumor suppressor *p53* is the key player in stress responses that preserve genomic stability and it is suitably documented as "***Guardian of Human Genome***" (Partridge *et al.*, 2007). It is well-documented that p53 exercises its protective roles as the transcription factor. By binding to specific response elements in DNA, p53 modulates the transcription of genes that govern the major defenses against tumor growth, which include cell cycle arrest, apoptosis, maintenance of genetic integrity, inhibition of angiogenesis and cellular senescence (Partridge *et al.*, 2007). These molecular interactions might contribute to the inhibitory role of *p53* in tumorigenesis.

4. Hypothesis of the present study

The normal functioning of tumor suppressor gene - *p53* is the potent barrier to cancer. Hence, the understanding of ***why p53, the guardian of genome, is not able to guard against the tumor formation*** might provide an answer to the certain key questions in oral carcinogenesis. The normal function of *p53* is affected by mechanisms like, somatic mutations and germ line polymorphisms in the gene itself or its regulatory genes. Mutations in the *p53* causing its malfunctioning are the hallmarks of most human cancers (Rivlin *et al.*, 2011) and oral cancers are not an exception. However, in the tumors harboring wild-type *p53*, there might be alterations in the regulation of *p53*. Thus, understanding why *p53*, is unable to perform its role as a tumor suppressor in these wild-type tumors is very crucial and critical. Germ-line polymorphisms in *p53* are also anticipated to cause measurable disturbances in p53 function (Whibley *et al.*, 2009). Polymorphic variants of other genes in the p53 pathway like Mouse Double Minute 2 (*MDM2*), a key negative regulator of *p53*, also have biological consequences either individually or in combination with *p53* variants (Whibley *et al.*,

2009). Moreover, molecular epidemiological studies have recently suggested that degradation of p53 by E6 protein of high risk HPV is one of the alternative mechanisms which attenuate p53 responses (Campisi *et al.*, 2007; Chocolatewala and Chaturvedi, 2009; Kumaraswamy and Vidhya, 2011) (Figure 1.1).

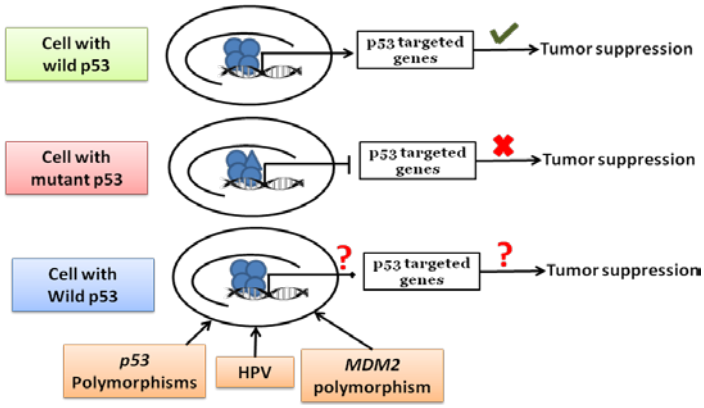

Figure 1.1: Alterations in *p53* determine its fate as a tumor suppresser gene (Tumor suppressive function of a guardian; *p53* is also influenced by polymorphisms in *p53* and *MDM2* genes as well as HPV infection in cells with wild type *p53*)

These alterations in p53 responses provide limitless replicative potential to cell in oral carcinogenesis. The subsequent progression of oral cancer includes immortalization, angiogenesis, tissue invasion and metastasis. Further, reports have also suggested that mutant p53 also play a key role in malfunction of almost all hallmarks of cancer (Freed-Pastor and Prives, 2012) (Figure 1.2). Thus, it is biologically plausible that alterations in p53 responses other than *p53* mutations also influence the genes involved in major hallmarks of carcinogenesis.

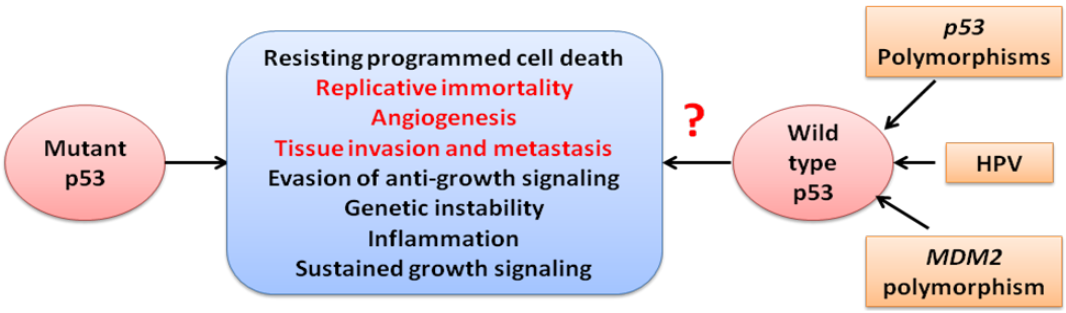

Figure 1.2: Alterations in p53 responses play an important role in tumorigenesis (p53 mutants have been found to actively contribute and play a central role in tumorigenesis, impacting nearly all of the hallmarks of cancer. Thus, it is biologically plausible that alterations in p53 responses due to presence of polymorphisms in *p53* and *MDM2* gene or HPV infection also influence the genes involved in major hallmarks of cancer.)

Hence, we hypothesized that alterations in p53 responses in comprehensive way influence cell cycle regulation as well as apoptosis and provide limitless replicative potentials to cell. Further, these alterations in p53 responses together also influence

major genes involved in immortalization (hTERT), angiogenesis (VEGF), invasion and metastasis (MMPs). These ultimately aid in subsequent progression of oral cancer (Figure 1.3).

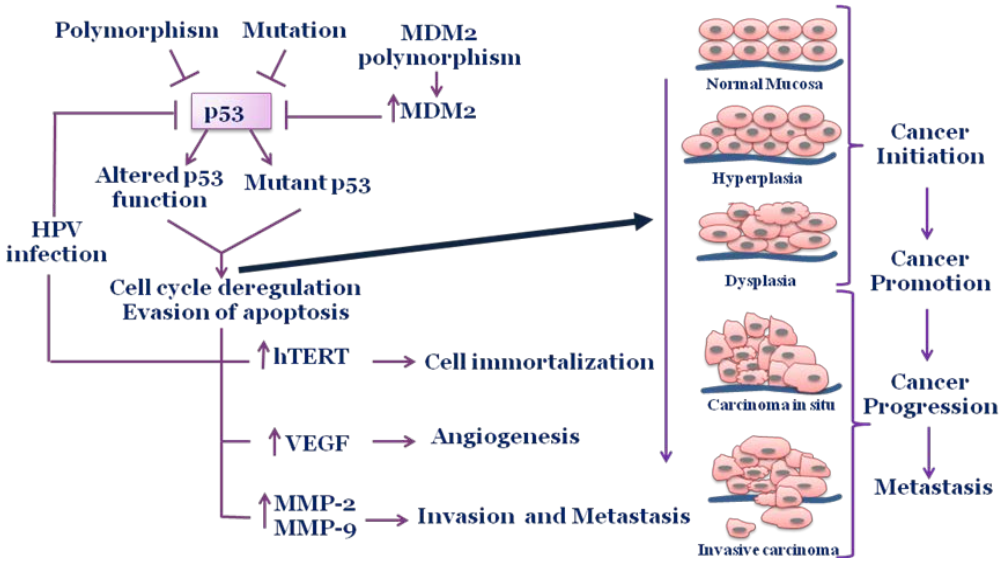

Figure 1.3: Hypothesis of the present study

5. Scope of the study

The cellular and molecular heterogeneity of oral cancer implicates that a large number of genes encompassing various hallmarks of cancer are potentially involved in oral carcinogenesis. Therefore, evaluation of multiple molecular pathways simultaneously would aid in improved understanding instead of single molecular event. Analysis of various molecular alterations in different relevant combinations might be helpful to identify nature of the disease at an early stage. Many p53 family transcriptional targets have been identified as having the capacity to modulate various cellular processes (Bai and Zhu, 2006; Vousden and Prives, 2009; Freed-Pastor and Prives, 2012). In fact, it has become evident that this key tumor suppressor is a molecular node at the crossroads of an extensive and complex network of stress response pathways (Bai and Zhu, 2006). Deregulation of p53 has enormous influence on carcinogenesis.

Evaluation of polymorphisms in *p53* and *MDM2* gene simultaneously might be helpful to identify at risk individuals, which might have great impact on prevention, early detection, follow-up strategies and genetic counseling. Exploring mechanisms by which p53 responses is affected (polymorphisms, mutations, *MDM2* polymorphism and HPV infection) together with evaluation of molecular markers that

represent major hallmarks in association with *p53* status might offer more detailed insights into molecular mechanisms underlying the oral carcinogenesis. This comprehension ultimately might aid to predict aggressive potentials of tumor and hence, prognosis of oral cancer patients for further better management.

Therefore, the present investigation was aimed to study comprehensively numerous molecular mechanisms related to susceptibility, immortalization, angiogenesis, invasion and metastasis and their potential role in the etiopathogenesis of oral cancer keeping *p53* as a vital and key molecule. The use of these molecular events as an adjunct to routine histopathological examination may eventually help in prognostication and effective management of oral cancers.

6. Major objectives

- To estimate the risk of oral cancer associated with *p53* and *MDM2* gene polymorphisms in the population of Gujarat, West India (single gene, gene-gene and gene-environment interaction analysis).
- To evaluate *p53* mutations and the HPV status of the oral cancer patients from Gujarat, West India and its association with *p53* and *MDM2* gene polymorphisms.
- To investigate expression levels of genes involved in major hallmarks of cancer i.e. immortalization (*hTERT)*, angiogenesis (*VEGFA*, *VEGFC* and *VEGFD*), invasion and metastasis (*MMP2* and *MMP9)* in oral cancer patients and their association with *p53* status.