CHAPTER 3

Overall research design and justification

Rationale of the study

Cancer of the oral cavity is the eleventh most common malignancy accounting for 12% of all cancers in men and 8% of all cancers among women. In parts of India and South East Asia, it is the most common cancer owing to the use of chewing quid's containing betel nut and tobacco, indicating a strong environmental and cultural influence in the prevalence of this disease. Even though these are important etiological factors, relatively few people exposed to them actually develop cancer, often despite years of exposure. There are also patients who develop oral cancer in the absence of such habits or other identifiable lifestyle or environmental etiological factors. Therefore host susceptibility may also play a role.

Nuclear DNA is under constant DNA damage stress induced by both endogenous (such as reactive oxygen species) and exogenous sources (such as radiation and environmental carcinogens). Proper recognition and repair of the DNA damage are essential for homoeostasis and normal functioning of multicellular organisms. DNA repair activities are maintained by the presence of different DNA damage sensor and repair mechanisms. Defects in the DNA repair pathways are often associated with excessive cell death or transformation of cells and variations in DNA repair genes are hypothesized to modify individual and population cancer risk. Mutations are early events in

carcinogenesis and defective DNA repair is a risk factor for many types of cancer.

There is substantial interindividual variation in DNA repair capacity (DRC) within a population. There is a large subgroup with reduced DRC who are likely to be at increased cancer risk, but are phenotypically normal. This risk subgroup needs to be identified and subjected to more intensive behavior modification changes and screening interventions. To date much success has been obtained in the identification of high penetrant cancer predisposing genes. However the challenge that remains is to identify those alleles conferring low to moderate cancer risk, like the DNA repair genes.

Genetic variations or polymorphisms in DNA repair genes leads to functional differences in the respective protein. The presence of these polymorphisms may be associated with increased extent of DNA damage and decreased repair. If genetic variations in DNA repair genes are a risk factor for malignancy, it should be reflected in the extent of actual DNA damage and repair, which can be assessed, by a number of *in vitro* assays like the Micronucleus assay and Host cell reactivation assay.

Radiotherapy is a major treatment modality for oral cancer treatment. However, radioresistance remains a major problem in the clinic. In spite of advances made in the management of cancer, the survival rate for patients

with oral squamous cell carcinoma remains unacceptably low. Even with multi modality treatment, the 5-year survival rates of oral cancer have remained at approximately 50% over the last few decades. In view of cancer heterogeneity and the availability of an increasing number of therapy options, identification of biomarkers that can predict tumor response to a given therapy is crucial in streamlining treatment and sparing patients from receiving often toxic and expensive therapies that are not likely to be effective.

Our approach to risk assessment has been multitiered, beginning with a detailed epidemiological assessment in case-control studies, followed by the application of phenotypic and genotypic markers of genetic susceptibility. The study emphasized on seven gene polymorphisms selected from the three important repair gene pathways. The polymorphisms include: XRCC1 *Arg*194*Trp* (C→T substitution at position 26304 in codon 194 of exon 6)*, Arg*280*His* (G→A substitution at position 27466 in codon 280 of exon 9)*, and Arg*399*Gln* (G→A substitution at position 28152 in codon 399 of exon 10) of the Base Excision Repair (BER) pathway; XPD *Lys*751*Gln* polymorphism, a A→C transversion in exon 23, ERCC4/XPF *Arg*415*Gln and* ERCC1 codon 118 polymorphism of the Nucleotide Excision Repair (NER) pathway and XRCC3 *Thr*241*Met* polymorphism of the Homologous Recombination Repair (HRR) pathway. Phenotypic assays like Cytokinesis blocked micronucleus assay and Host cell Reactivation assay was done to look into the actual extent of inherent DNA damage and DNA repair capacity of oral cancer

patients and the normal population. These were then correlated with genotyping results, thus establishing a genetic influence on phenotypic assays. In addition to SNPs as cancer susceptibility markers, we also looked into their possible role as biomarkers of radiation treatment (RT) response.

Objectives

Owing to the importance of maintaining genomic integrity in the prevention of carcinogenesis, genes coding for DNA repair molecules have been proposed as candidate cancer susceptibility genes. Tobacco users with diminished ability to excise or repair somatic mutations may be more susceptible to develop tobacco attributable cancers. Another issue of clinical significance was the role of DNA repair enzymes in response to anticancer treatments especially radiation therapy, which also paradoxically depends on the extent of DNA damage induced and its repair. Hence this study was planned to look into two major roles of DNA repair enzymes, viz its association with extent of DNA damage occurring during tumorigenesis in the oral cavity as well as its role in response to individual tumors to anti cancer treatment. The study was designed to look into the overall distribution of polymorphisms in DNA repair genes prevalent in our populations and see whether the presence of any polymorphic variant was associated with increased risk of developing oral cancer, associated with increased extent of DNA damage and repair and finally to see whether it influences treatment response for invasive oral cancer.

Specific objectives

1. To study the distribution of genetic polymorphisms of five DNA repair genes: XRCC1, XPD, ERCC4, ERCC1 and XRCC3 in normal subjects and those with squamous cell carcinoma of the oral cavity.
2. To correlate the presence of these genetic polymorphisms with the actual extent of *in vitro* DNA damage and DNA repair.
3. To correlate the presence of genetic polymorphisms of the five genes with the clinico-pathology of the malignant lesions.
4. To correlate the presence of the genetic polymorphisms, extent of invitro DNA damage with the treatment outcome of oral cancer.

Fig 3.1

OUTLINE OF THE STUDY