CHAPTER 1: INTRODUCTION

Oral squamous cell carcinogenesis is a complex and dynamic process. Together with squamous cell carcinoma of the other regions in the head and neck, it is ranked as the sixth most common malignancy worldwide (Parkin et al., 1999). It is predominantly a disease of the old age and occurs generally in the fifth and sixth decades (Siar et al., 1990). Nonetheless, there have been reports of younger patients (arbitrarily defined as age less than 45 years**,** Llewellyn et al., 2003) contracting the disease with or without major risk factors (Chen et al., 1999; Chitapanarux et al., 2005). Well-recognized risk factors include tobacco (Warnakulasuriya et al., 2005) and alcohol usage (Petti & Scully, 2005), however, in parts of south East Asia, the habit of chewing betel quid has contributed to the prevalence of oral squamous cell carcinoma (OSCC) cases in certain ethnic groups (Zain, 2001; Scully & Bedi, 2000).

Treatments for oral squamous cell carcinoma include surgery (Petruzzelli et al., 2003), radiation (Yao et al., 2005) or combination of both (Kasperts et al., 2005). It is generally believed that prognosis is better if the disease is detected, diagnosed and treated early. However, prognosis of oral squamous cell carcinoma has not improved much over the past decades (Woolgar, 1997). Besides possible diagnostic delay (Allison et al., 1998), poor prognosis could be due to inherent weaknesses in current prognostication benchmarks such as the TNM system and histopathological grading. Recognizing the problems, suggestions have been put forth by several studies to improve the predictive power of the two systems. Jones et al. (1993), Hall et al. (1998) and O-charoenrat et al. (2003) all showed room for improvement in the TNM system. Bryne et al. (1992), on the other hand, had shown that grading the most invasive part of the tumour yielded better prognostication. This newer

histopathological system, the Invasive Front Grading, has been shown to be a better system when compared to the conventional Broders' classification in terms of prognosis and reproducibility (Bryne et al., 1995; Bryne et al., 1991; Bryne et al., 1989).

With the recent explosion in technological advances in genomics and proteomics methodologies, several breakthroughs have been achieved in cancer genetics. Immunohistochemical analysis of oestrogen receptor and ERBB2 (also known as HER2/NEU) expression can now be used to predict responses to designer drugs like Tamoxifen and Herceptin respectively (Berns et al., 1995; Nahta & Esteva., 2005). More recently, STI-71 (Gleevec), a tyrosine kinase inhibitor has been used to treat leukaemic patientswith BCR-ABL gene translocation (Druker et al., 2001). Furthermore, by using cDNA arrays, two subtypes lymphoma were recognized by differing patterns of gene expression within the morphologically homogenous large B-cell lymphoma suggesting that the clinical presentation of the disease might be similar but the genetic background might differ (Alizadeh et al., 2000). However, deciphering the molecular pathway of oral carcinogenesis has not been as smooth as the paradigm stepwise progression of colorectal carcinoma put forth by Fearon and Vogelstein (1990).

Carcinogenesis is a probabilistic phenomenon and three basic principles were outlined by Cohen & Ellwein in 1991. Firstly, cancer arises due to genetic alteration. Secondly, more than one genetic alteration is required and thirdly, DNA replication is not 100% precise. Cellular homeostasis is a delicately and tightly regulated event. Disruption in genes that control cell cycle undoubtedly causes turnover imbalances and ultimately tumour development (Partridge, 2000). There are two classes of genes in which damages cause

transformation: 1) Oncogenes, generated when a celluar proto-oncogene that is involved in normal cellular functions is inappropriately activated and represents a gain-of-function. 2) Tumour suppressors, when inactivated, represent loss-of-function in genes that usually impose some constraints on the cell cycle or cell growth; the release of the constraint is tumorigenic (Lewin, 2000).

Before the discovery of the tumour suppressor genes, the oncogenes had held centre stage in attempts to explain and understand the origins of cancer. The role of several oncogenes had been investigated in head and neck cancers. Irish and Berstein (1993) found that epidermal growth factor receptor (EGFR) overexpression was closely related to tumour staging as compared to other oncogenes like K-ras, raf and erb-B. There was also interest in assessing the proliferative rate in order to determine prognosis for the patients by the pathologist. Found expressed in cells in proliferation only, Ki-67 had recently emerged as a popular marker for proliferation due to the ease of the methodology and availability of the reagents (Hall and Levison, 1990).

One of the most potent tumour suppressor genes is the TP53 gene located on 17p13.1. This gene encodes a 53kD phosphoprotein, the p53 protein, which carries many functions in cell cycle control and maintaining genomic integrity (Hall, 1996). Over 50% of human solid cancers haboured mutations in this gene (Greenblatt et al,, 1994). The p53 protein is involved in cell cycle arrest, DNA repair and apoptosis when the damage to the DNA is beyond repair (Wiesmuller, 2001). The p53 protein from a mutated gene has a longer half life and can be detected by immunohistochemical staining. This overexpression of the protein has been assessed in relation to the prognosis of many cancers (Ostrowski et al., 1991; Hamelin et al., 1994) including oral squamous cell carcinoma (Kerdpon et al., 2001).

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However, its usefulness in predicting the survival of diseased patients has not been established (Raybaud-Diogene et al., 1996).

Assessing overexpression of p53 protein alone in predicting prognosis in diseased patients may not be sufficient as cancer is a result of stepwise progression of multiple genetic alterations (Nylander et al., 2000; Schneider et al., 2000). Moreover, p53 protein stabilization and hence its detection can be due to mechanisms other than mutations (Patridge et al., 1999). Two other molecules that are closely related to the p53 are the MDM-2 and Bcl-2.

MDM-2, murine double minute 2, is a 90kD phosphoprotein encoded by chromosomal 12q13-14. This gene is a downstream target of the p53 protein (Juven et al., 1993). It forms a protein complex with the p53 protein and blocks transactivation of p53's gene targets (Momand et al., 1992). Amplification and overexpression of this gene had been implicated in the development of tumors like gastric carcinoma (Gunther et al., 2000) and possibly in oral squamous cell carcinogenesis (Huang et al., 2001). Bcl-2 protein, on the other hand, is a prototype for a big family of proteins involving in the apoptotic pathway.

The Bcl-2 gene was first described in the 14; 18 translocation associated with follicular lymphoma in 1979 (Fukuhara et al., 1979). It is now believed that the Bcl-2 protein is found and acts on the cytoplasmic face of mitochondria and/or endoplasmic reticulum (Riparbelli, et al., 1995). This protein was also found mainly expressed topographically at the proliferating cell zones i.e. the basal cell layer of the epidermis, the basal cells of prostate epithelium and hence Bcl-2 expression seemed to be restricted in tissues where apoptosis shapes the developing structure or accounts for cell turnover (Hockenbery et al., 1991). Subsequently, expression of Bcl-2 had been assessed in a variety of cancers

including oral squamous cell carcinoma, either alone or with expression of other proteins and its bearing on the prognosis of the cancers (Ohbu et al., 1996; Pezzella et al., 1993; Kannan et al., 1998; Jackel et al., 2000).

To date, several studies on the immunohistochemical expression of the proteins mentioned above and other proteins had been carried out in Malaysia and special attention had been paid to their expression at the tumour invasive front. George (2002), recomfirmed the high percentage of overexpression of the p53 protein in OSCC and found that this overexpression at the invasive front might have some prognostic value in OSCC at the buccal mucosa. Meanwhile Abdul Jalil (2003) compared the expression of two proliferative markers, the Ki-67 and PCNA, at the tumor invasive front as well, and even though there was no significant correlation with any of the clinicopathological parameters, it was found that most expression was accumulated at the invasive front. Lee (2004) found that none of the proliferative and apoptotic markers, i.e. Ki-67, MDM-2 and Bcl-2, were associated with any clinicopathological parameters. It was suggested that a further study to be carried out that includes the assessment of the p53 protein in addition of the markers in that study since p53 is linked to the regulation of the investigated markers. Therefore, the current study aims at assessing the expression of p53 protein and its closely related proteins, the MDM-2 and Bcl-2, and also the proliferating index Ki-67 Labeling Index (Ki-67 LI) at the invasive front of OSCC and their relationship to clinincopathological parameters.

The current study hence proposed the hypothesis that there is no correlation between the expression or coexpression of Ki-67, p53, MDM-2 and Bcl-2 with demographic or clinicopathological parameters.

The specific objectives of the study are to:

1. To describe the demographic and clinicopathological characteristics of the samples.

2. To describe the expression of Ki-67, p53, MDM-2 and Bcl-2 in OSCC

3. To correlate the expression of Ki-67, p53, MDM-2 and Bcl-2 to:

    a) demographic factors (age, sex and gender)

    b) clinicohistopathological grading (TNM staging, Broders and pattern of invasion)

4. To correlate the co-expression of the markers to above mentioned parameters.