CHAPTER I

Introduction

The report of Public Health Statistic (1993), from the Ministry of Public Health, Thailand showed that cancer was the third most common leading cause of death in Thailand. The incidence of cancer seems to increase every year (Figure 1). An understanding of the basic knowledge of molecular biology in cancer should provide better diagnosis and treatment of the disease.

Nasopharyngeal carcinoma (NPC) is an epithelial tumour that occurs worldwide and shows great variation between different ethnic population. It is common among Southern Chinese, Hong Kong, Singapore, Taiwan, and Southeast Asia. The incidence rates are approximately 30/100,000 persons per year for male (Abderrahim and Esteban, 1995) and 13/100,000 persons per year for female(Muir et al., 1987). But it is rare among whites in Europe and North America (Muir et al.,1987; Choi et al.,1993; Zheng et al.,1994). NPC is found to be common in male with the male/female ratio of approximately 2.6 (Hung et al., 1991). In 1993, NPC was ranked the fourth among all cancers in male in an annual report of cancer at Chulalongkorn hospital and ranked the seventh in the annual report of National Cancer Institute (1993) of the Ministry of Public Health, Thailand.

An epidemiologic and etiologic studies demonstrate that NPC is closely associated with Epstein-Barr virus (EBV) infection. EBV is belived to be

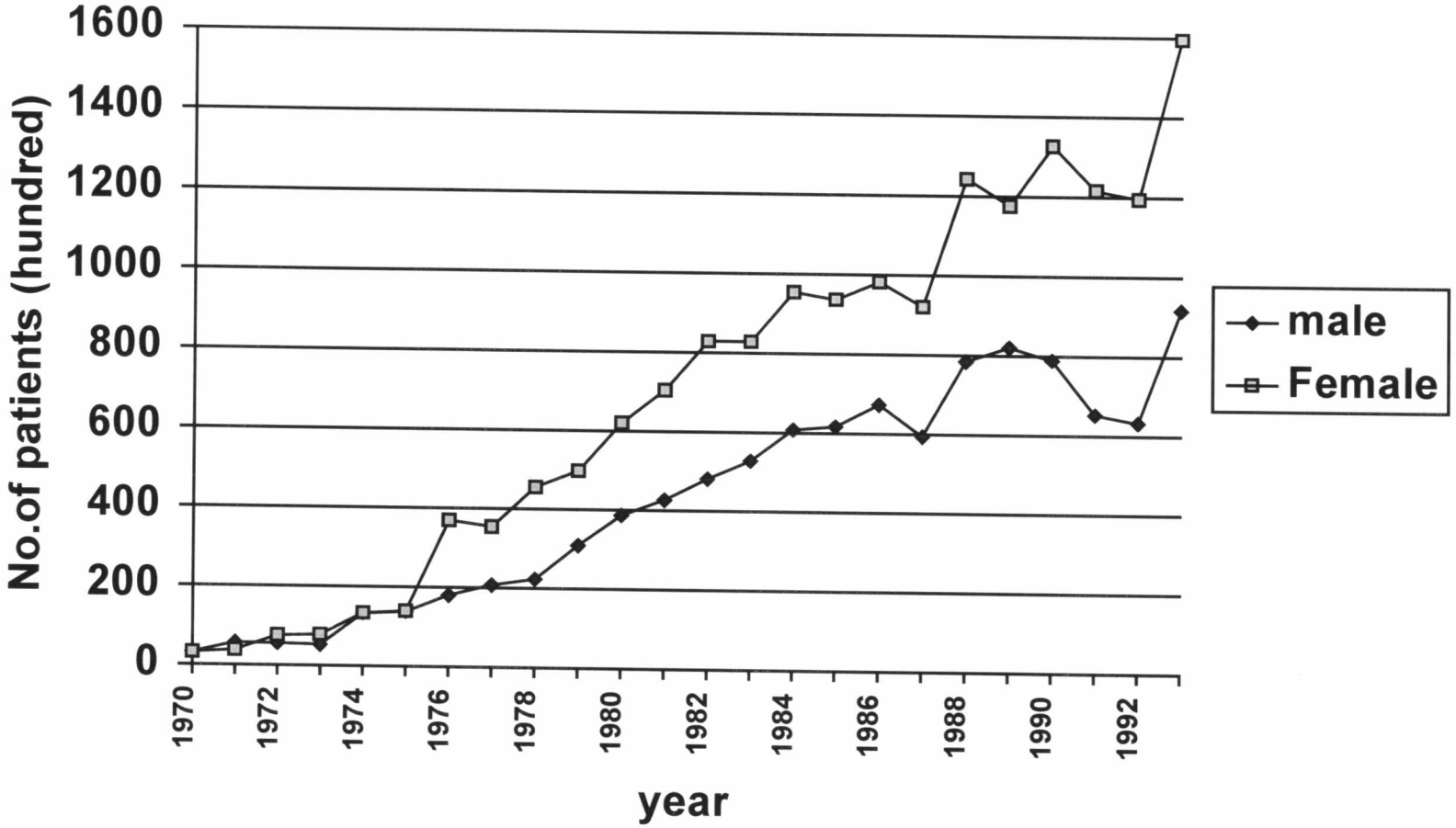

Figure 1 : Trends of cancer patients, 1970-1993 in Thailand.

closely associated with nasopharyngeal cancer (NPC), which the EBV is regulary found in the tumour cells and the specific anti-EBV antibody profiles presented in patients with NPC as compared to normal control population (Zheng et al., 1994; Rajadurai et al., 1994). Several studies, using several techniques exhibited the expression of specific EBV genome in NPC tumour specimens and suggested that EBV infection in NPC may be a prerequisite for the development of NPC (Levine et al., 1992; Hildesheim et al.,1992 ; Choi et al.,1993 ; Nieldobitex et al., 1993; Abderrahim and Esteban 1995; Rajadurai et al.,1995). Other etiologic factors that played an important role in the development of NPC are the genetic factors (Simon et al., 1974; Lu et al.,1990;Choi et al.,1993), environmental and dietary factors (Yu et al.,1990; Hieldesheim et al.,1992; Zheng et al.,1994), and chemicals such as nitrosamines (Huang,1983), arsenic compounds (Hawkins et al.,1990).

Cancer is a genetic disease at the cellular level that involves complex interaction between several factors, both exogenous (environmental) and endogenous (genetic, hormonal, immunologic etc.) , which stimulate changes of the state of cell. Tumourigenesis is a multistep process and the mutations of neoplasms may have a monoclonal composition, whereas aberrant differentiation processes give rise to a polycolonal composition (Fearon and Vogelstein, 1990).

The genetic damages may range from single base pair substitutions to gross chromosomal changes. This may lead to distortion of either the expression or the biochemical functions of genes (Bishop, 1987), particularly

those involved in the cellular proliferation and differentiation. Many of these genes have been classified as proto-oncogenes and tumour-suppressor genes, which products involved in the regulation of cellular proliferation and differentiation. Other genes that do not belong to one of these two categories may also play a role in carcinogenesis; for example those involved in cell recognition (histocompatibility complex, cell adhesion molecules) and metastasis (Haris,1992).

Like many other cancers, tumours of the NPC were belived to arise as a result of the accumulation of somatic mutations in tumour suppresor genes and proto-oncogenes. In 1971, Knudson was proposed the two-hit hypothesis model to explain the mutation processes of tumour suppressor gene that involved in the development of the inherited cancer syndrome, retinoblastoma. The first or predisposing event can be inherited through the germ line or arises in somatic cells. The second somatic event is required to inactivate the remaining normal allele by several mutational mechanisms such as base-substitution mutations, deletions, chromosomal nondisjunction and mitotic recombination. This results as a complete lost of the tumour suppressor gene functions and enhances the dysregulation of growth and differentiation leading to cancer. To investigate, the somatic event that affects the second (normal) allele is called "Allele loss or loss of heterozygosity (LOH)". The somatic event that affects the second (normal) allele and so exposed the recessive mutation can be detected by studying constitutional normal and tumour DNA polymorphism.

Several studies have used the restriction fragment length polymorphism (RFLPs) to detect the LOH in several cancer types (Vogelstein et al., 1989 ; Huang et al., 1991; Foulkes et al.,1993 ; Mitra et al., 1994). This type of assay is time consuming, required large amounts of DNA, limitted by the lower percent of heterozygosity. During the last 10 years, the discovery of short tandem repeat polymorphic markers (STRPs), the newly described DNA markers have been used as an alternative for LOH analysis. STRP is a short tandem repeat DNA sequence distributed throughout the human genome with highly polymorphism estimated more than 70% heterozygosity (Litt and Luty, 1989; Weber and May, 1989). The polymorphism at these loci is the results of the variation in length of the number of repeat blocks (Hearne et al.,1992; Weber,1990). The allele fragments can be generated by the polymerase chain reaction (PCR) assay using specific primer sequences closely flanking the repeat - containing units (Litt and Luty, 1989 ; Weber and May, 1989). In addition, this PCR based typing of STRP is more efficient, requires smaller amounts of genomic DNA than the standard RFLP analysis. STRP markers can be analysed simultaneously using mutiplex PCR methods(Huang et al.,1992). The PCR-generated DNA fragments are further resolved by polyacrylamide gel electrophoresis (Litt and Luty, 1989 ; Weber and May, 1989).

Because of their hypervariability and abundance, STRPs are ideal markers for LOH analysis of NPC. Several studies have used STRPs for detecting the LOH in several carcinoma and have shown that LOH at the specific chromosomal sites is frequently associated with development of

various cancers (Marshall, 1991; Goddard and Solomon, 1993). The nasopharyngeal carcinoma have been reported with a high frequency of LOH in chromosome arms 3p at D3S3 (3p14),RAF-1(3p25) (Huang et al.,1991;Choi et al.,1993) and 9p at 9p21-22 (Huang et al.,1994).

The aim of this study is to examine the LOH by using STRP markers to scan tumour genome on all the autosomal arms (allelotyping) which demonstrates the genomic region that contained candidate tumour suppressor genes responsible for nasopharyngeal carcinoma development.