# CHAPTER I

## INTRODUCTION

The health problems of Thailand have changed dramatically during the past two decades. From Public Health Statistic (1992), Ministry of Public Health, Thailand, the death due to infectious disease, malnutrition, maternal and child health problems have declined, while the non-communicable diseases have risen significantly to become the leading cause of death. The first leading cause of death was heart disease, the second was accident and the third was cancer. At present, the cancer incidence rate has climbed up to reach 200 new cases per 100,000 populations (Vantanasapt *et al.*, 1993).

Hepatocellular carcinoma (HCC) is the most frequent malignant neoplasm of males in Thailand, and the second importance in females as shown in Table 1 (National Cancer Institute, 1990). Previous report showed the incidence as high as 16.3% of new cases HCC among males and 5.5% for females (Srivantanukul *et al.*, 1988) and median survival was 4.3 months from diagnosis (Cheirsilpa *et al.*, 1989). Unless early detected, the prognosis is often poor. Most cases are inoperable at presentation. Radiotherapy has

been shown to be ineffective because of heterogeneity of cell sensitivity. Conventional chemotherapeutic agents have produced objective remission in small proportion. Although the responses have been in short duration, it has little impact on survival (Chlebowski *et al.*, 1982). Complete removal of HCC by surgery offers the only hope of cure. However, patients considered to be good surgical candidates are few (Kanematsu *et al.*, 1989). The Liver Cancer Study Group of Japan (1987) reported that the hepatectomy could be performed only in 699 cases out of 1760 patients with HCC and the rate of resectability was 35.2%.

One of the intensive efforts has been made to improve the management of HCC by using biological response modifiers (BRMs). These agents are single substance which can influence the immune response effectively by modifying a variety of immunocytes. Many BRMs such as interferon, interleukin, colony stimulating factor are in various stage of clinical trails. But the most widely used immunomodulator so far is the interferon (Chatamara, 1991). The recent development of biotechnology and genetic engineering, allowing for the synthesis of highly purified BMRs, made biotherapy possible.

Interferons (IFNs) are a group of inducible protein which can be divided into 3 major classes based on the type

of secreting cells and the inducers together with their biological and physiological properties (Steward, 1980).

Type I interferons consist of interferon alpha (IFN-α), produced by leukocytes, and interferon beta (IFN-β), produced by fibroblasts after exposure to virus or double-stranded RNA (Steward, 1980). They share a common surface receptor (Andrew, Branca, and Corrado., 1981).

Type II interferon or interferon gamma (IFN-γ) is produced by T-lymphocytes in response to mitogenic or antigenic stimuli (Steward, 1980). This interferon binds to its receptors on target cell surface distinct from that of IFN-α and IFN-β (Andrew, Branca, and Corrado., 1981). It has been known that IFNs have antiviral activity (Isaacs and Lindermann, 1957; Baglioni, 1979) and exhibit antitumor activity either directly or indirectly via immune response (Djeu *et al.*, 1979; Heron *et al.*, 1979). Several of these activities, perhaps in combination, give IFNs more potent antitumor activity and make IFNs received wide attention in therapeutic value for treatment of certain type of cancer (Pherson and Tan, 1980; Sherwin, Knost, and Seymour, 1982; Sikora and Smedley, 1983).

IFN-α and IFN-γ have strong antitumor activities because they directly inhibit cell proliferation and

differentiation (Flishmann *et al.*, 1984). In additon, they have been shown to indirectly enhance the activity of natural killer cells (NK cells) and macrophages (Kuwahara *et al.*, 1987) including enhancing the expression of surface antigens such as HLA (Balkwill *et al.*, 1987) and tumor-associated antigen (Greiner *et al.*, 1984 ). Reports on clinical studies of IFNs confirmed that IFN-α is one of the most effective therapy in hematological malignancies such as hairy cell leukemia (Doane, Ratain, and Golomb, 1990), chronic myelogenous leukemia (Alimena *et al.*, 1990), and lymphoma (Vegna *et al.*, 1990). IFN-ɣ, on the other hand, is an effective therapy for solid tumor such as renal cell carcinoma (Thomas *et al.*, 1987), breast cancer (Muss *et al.*, 1986), and cervical carcinoma (Iwasaka *et al.*, 1990). For HCC, extensive *in vitro* studies using different types of IFNs and different human hepatoma cell lines were reported (Desmyter *et al.*, 1981; Hinoue, 1985; Motoo, Hill and Osther, 1985; Duke *et al.*, 1986; Ilan *et al.*, 1988). Duke *et al.* (1986) reported that IFN-α has powerful antiproliferative effect on human hepatoma cell line PLC/PRF/5 in dose-dependent manner, both *in vitro* and *in vivo* after implantation in nude mice. Ilan *et al.*(1988) also reported the successful inhibition of PLC/PRF/5 and SK-Hep1 hepatoma cells growth by IFN-α. Additionally, Ren *et al.*(1988) has shown that 100 IU/ml of IFN-ɣ induced high level of MHC class I when exposed to different types of human hepatoma

cell lines. These results suggested possible therapeutic approach for suppressing tumor cell growth and enhancing tumor cell recognition by the host immune system. However, Nair *et al.*(1985) and Sach *et al.*(1985) reported that in patients with advanced HCC, IFN-α induced no tumor shrinkage instead undesirable side-effects were observed.

The anticellular activity of IFNs on morphological change in several cancer cells provided the basis for therapeutic use of IFNs in the treatment of human cancer. Iwasaka *et al.*(1986) demonstrated that the characteristic cellular changes in a cervical cancer cell line after treatment with IFN-ɣ caused cytoplasmic vacuolization and various nuclear changes, including enlargement and deformity as well as pyknosis and karyolysis.

Results of our preliminary study on exposure of a Thai hepatoma cell lines to IFN-α showed lethal effects on these cells as shown by inclusion body in cytoplasm and segmented nuclei. From the described rational, IFNs were found to exert different properties useful for HCC treatment. In order to evaluate the actual efficacy of IFN-α on hepatoma, this thesis was carried out to confirm the process of cytoplasmic changes relating to the occurrence of segmented nuclei together with the pattern of surface antigen of hepatoma cell lines (Laohathai and

Bhamarnpravat, 1985) with anti-hepatoma monoclonal antibody (anti-hep Mab) by using electron microscopy and immuno-electron microscopic techniques. This study will also evaluate the reversibility of treated cells and resisted cells. The results from this research study will provide more informations on therapeutic efficacy of IFN-α in the treatment of HCC.

Table 1 The common malignancy in Thai people

| Site | Male(%) | Site | Female(%) |
|---|---|---|---|
| Liver | 40.5 | Cervix | 23.4 |
| Lung | 25.0 | Liver | 16.3 |
| Stomach | 5.8 | Breast | 13.5 |
| Colon | 5.5 | Lung | 12.1 |
| Oral cavity | 4.9 | Ovary | 4.5 |
| Urinary bladder | 4.9 | Oral cavity | 4.5 |
| Leukemia | 3.8 | Thyroid | 3.6 |
| Skin | 4.6 | Colon | 3.7 |
| Lymphoma | 3.5 | Leukemia | 3.2 |
| Esophagus | 4.1 | Skin | 3.7 |
| Nasopharynx | 3.3 | | |
| Larynx | 4.0 | | |

From: National Cancer Institute, Department of Medical services, Ministry of Public Health. 1990.