CHAPTER II

BACKGROUND

2.1 Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC), also referred to hepatoma, is a malignant tumor composed of cells derived from hepatocytes. It occurs more frequently in males than females and usually in association with cirrhosis. The incidence varies from areas to areas perhaps because of differences in the frequency of hepatitis virus infection and in the degree of exposure of hepatocarcinogens. The prognosis is usually poor, depending on the extent of tumor invasion at diagnosis and the stage of diseases.

2.1.1 Epidemiology

The incidence of HCC varies considerably with the geographic region. Table 2 gives the age-adjusted incidence rates of primary liver cancer per 100,000 persons per year in various countries, areas, and ethnic groups. (Monoz and Bosch, 1987). The rates may be divided into the high incidence areas, more than 20 per 100,000/year, which include China, Taiwan, Chinese in Singapore, South Africa

and Japan. The intermediate incidence areas, 5 to 20 per 100,000/year are Malays in Singapore, Jamica, Cuba and the low incidence areas, less than 5 per 100,000/year, are Sweden, the United Kingdom, Ireland, Australia. HCC is relatively uncommon in western countries. In the United States, the annual report was usually only 4,000 to 5,000 cases per year (Silverberg and Lubera, 1990). However, it is one of the most prevalent cancers in Asia, Africa and the South Pacific Islands (Parkin, Stjernsward, and Muir, 1984; Okauda, 1986). HCC is so significantly high with an estimated annual incidence of 1,000,000 total cases (Di Bisceglie *et al.*, 1988; Lau and Lai., 1990). Thus, HCC is thought to be the most prevalent solid tumor and has a mortality ratio of 0.92 with accounting for 30 to 40 % of all cancer deaths in the countries with high incidence (Silverberg and Lubera, 1990).

2.1.2 Etiology

2.1.2.1 Hepatitis Virus

Epidemiological studies have suggested that there is a certain parallelism between the HBsAg carrier rate and the incidence rate of HCC. The carrier rate is high in the areas of high incidence of HCC and low in the areas of low HCC incidence as summarized in Table 3. (Hadziyannis *et al.*, 1980). HBsAg is also significantly

more often positive in patients with HCC than in those wihtout HCC and in controls. In Taiwan, Beasley (1982) reported that about 90% of patients with HCC were positive for HBsAg. There is close relation between hepatitis B virus (HBV) infection and HCC incidence. The discovery of integrated forms of HBV (Shafritz *et al.*, 1981; Brechot *et al.*, 1981) and the available of serological markers have been helpful in establishing this relationship. A similar association between HCC and non-A, non-B, hepatitis virus (NANBHV) was reported last 10 years (Gilliam *et al.*, 1984; Kiyosawa, *et al.*, 1984; Lefkowitch and Apfelbaum, 1987). The possible hepatocarcinogenic role of NANBHV had not been established until Choo *et al.*(1989) isolated a blood-borne NANBHV and designated as hepatitis C virus (HCV). This finding has led to the development of immunoassay for the detection of specific HCV antibody. It has been demonstrated that HCV is likely a causative agent of HCC (Husan *et al.*, 1990; Kew *et al.*, 1990; Saito *et al.*,1990). In Japan, Nishioka (1990) reported that the incidence of HCC per year in the HBsAg-positive population is only 0.25%, while it is 1.0% in the anti-HCV-positive population. This suggests a four times higher incidence of HCC among anti-HCV-positive persons than among HBsAg carriers in some parts of hepatitis C endemic areas such as Japan. There is no evidence that hepatitis A predisposes patients to develop HCC.

Table 2 Age-Adjusted Incidence Rates of Liver Cancer in Various Countries, Cities, and Ethnic Groups

Country	Incidence Rate (per100,000 /y)	
	Males	Females
Mozambique, Lourenco Marques	112.9	30.8
Zimbabwe, Bulawavo	64.6	25.4
China, Shanghai	34.4	11.6
Gambia	33.1	12.6
Hong Kong	32.1	7.4
Singapore, Chinese	31.6	7.2
Pacific Polynesian Island	26.6	2.3
Cape, Bantu	26.3	8.4
Japan, Nakasaki	25.8	7.9
Philippines, Manila	19.9	6.2
United States, Sanfrancisco(Chinese)	19.1	3.6
Singapore, Malay	15.6	5.3
Nigeria, Ibadan	15.4	3.2
Singapore, Indian	14.1	2.8
Korea	13.8	3.2
New Zealand, Maori	11.2	4.2
Japan, Miyagi	11.2	4.0
Switzerland, Geneva	10.2	1.5
Argentina, Tandil	9.9	5.8
Italy, Parma	8.6	3.3
Hawaii, Chinese	7.8	2.4
Spain, Zaragoza	7.2	5.5
Canada, Eskimos	6.9	3.7
Jamaica	6.1	2.1
Czechoslovakia, Slovakia	5.1	2.8
Germany, Hamburg	4.5	1.7
India, Bombay	4.9	2.5
Peru, Lima	4.0	2.9
United States, Sanfrancisco(Black)	3.9	1.8
France, Doubs	3.7	1.0
Denmark	3.6	2.3
Brazil, Fortaleza	3.5	3.7
United States, Sanfrancisco(Japanese)	3.0	0.4
United States, Sanfrancisco(White)	3.9	1.8
New Zealand, Non Maori	2.4	1.1
Australia, South	2.0	0.7
Algeria	1.6	1.4
United Kingdom, England and Wales	1.6	0.8
Cape, Colored	1.5	0.7
Canada, Alberta	1.3	0.5
Cape, White	1.2	0.6
Pakistan	0.7	0.8
Ireland	0.1	0.3

(Bosch FC,. Munoz N. Hepatocellular carcinoma in the world :epidermiologic question. In : Tabor E, et al.eds. Etiology, pathology and treatment of hepatocellular carcinoma in North America. Woodlands Portfolio, 1991. p 35; Munoz N. Bosch X. Epidemiology of hepatocellular carcinoma. In: Okuda K,Ishak Kc, eds. Neoplasmas of liver. Tokyo, Springer, 1987, p. 3)

Table 3 *Correlation Between Prevalence of Primary Liver Cancer (Mortality) and HBsAg Carrier Rate*

Country	*Primary Liver Cancer Mortality (per 100,000/y)*	*HBsAg Carrier Rate Among Population*
High-Incidence Areas		
Mozambique	98.2	14.0
South African blacks	22.0	9.0
China	17	7.5–14.0
Intermediate-Incidence Areas		
India	—	2.5
Japan	15	2.6
Greece	12	5.0
Low-Incidence Areas		
United States	2.7–4.7	0.2
Scandinavia	2.1–3.5	0.1
Central Europe and United Kingdom	1–7	0.25

(Hadziyannis SJ. Hepatocellular carcinoma and type B hepatitis. Clin Gastroenterol 9:117, 1980)

2.1.2.2 Aflatoxins

Aflatoxins are mycotoxin, produced by Aspergillus flavus and Aspergillus parasiticus. They are divided into two groups. Aflatoxin B_1 is found more widly than aflatoxin G_1. Mycotoxin was proved as a cause of HCC since 1971 (Keen and Martin, 1971). Van Renburg *et al.* (1985) reconfirmed that there was a close correlation between HCC and aflatoxin B_1 level in food. The contamination of aflatoxins in several foodstuffs consumed in large quantities in those countries that has high incidence of HCC. In Thailand, aflatoxin is found contaminated in variety of market foods including peanuts, corn, wheat, millet, dried chili. A comparison of estimated HCC incidence and aflatoxin intake in two areas of Thailand showed that aflatoxin contamination was high in the area with the higher incidence of HCC (Srivatanukul *et al.*, 1991).

2.1.2.3 Contraceptive Drug

Although the role of contraceptive in hepatocarcinogenesis in women is not clear. Several reports claimed oral contraceptives for the and development of HCC (Neuberger *et al.*, 1980; Goodman and Ishak, 1985; Pamlmer, Rosenberg, andKaufmann, 1989). However, there are fewer number of HCC caused by oral contraceptives than that caused by hepatitis virus and aflatoxins.

2.1.2.4 Chemical carcinogens

Synthetic carcinogens, including food additives such as nitrosamine is also another cause of HCC (Adamson, 1989; Lau and Lai, 1990). Azo dyes, aromatic amine, chlorinated hydrocarbon and organochlorine pesticides were found to be hepatocarcinogen (Okuda *et al.*, 1993).

2.1.2.5 Smoking

In 1966, Hammond reported a high mortality from cancer of the liver and bilitary tract among smokers than among non-smokers. Another reports suggested that tobacco smoking is a possible cause of the development of HCC (Trichopoulos *et al.*, 1980; Lam, 1982; Yu *et al.*, 1983; Mayans *et al.*, 1990). Yet tobacco smoking is the risk factor to HCC is remained unclear.

2.1.3 Pathology

HCC, grossly forms a grayish white to bright yellow in color with solidly tumor mass. According to growth pattern, HCC is divided into three forms. Most common pattern is those grow in small nodular form studded with tumor. The second pattern is the massive form which grow in large, single mass and may be associated with satellite lesions. The last pattern is the diffuse form,

which grow diffusely with fine scattered foci throughout the liver and rarest in number. The occurrence always associate with cirrhosis (Okuda *et al* , 1977)

Microscopically, HCC has eight different histological features as shown in Table 4. The hepatic type, also called trabecular type is the most common form. The fibrolamella type is noteworth, as it is typically solitary and has a better prognosis than oridinary HCC (Buamah *et al*., 1986; Ruffin, 1990). It occurs primarily in women aged between 15 to 30 years and is not associated with cirrhosis. The clear cell type may also be associted with a prognosis that is better than typical HCC.

Table 4 Histological types of hepatocellular carcinoma

Histological type
Hepatic or trabecular
Pleomorphic
Adenoid or acinar
Sclerosing
Giant cell
Clear cell
Fibrolamella
Mixed

Mostly, HCC showed direct invasion such infiltration to the diaphragm and intrahepatic portal veins but less often invades the hepatic vein or bile duct. Frequently, regional lymph nodes are involved with tumor. At autopsy, 40 to 50% of patients with HCC have distant metastasis. The lung is the most common site of metastasis. The presence of metastatic lesions is rarely altered the prognosis. The hepatic artery invasion or tumor embolism lead to short survival with rupture of varix.

2.1.4 Clinical Presentation

HCC usually does not produce any signs and symptoms until advanced stage. The most common presenting symptom is abdominal pain, abdominal distension, fatigue, anorexia, weight loss and low fevers. On physical examination, all patients have hepatomegaly. At almost terminal stage will notice the ascites, edema, and /or jaundice.

2.1.5 Diagnosis

The diagnostic workup of a patient suspected of having HCC is usually to test liver function, blood chemistry including alpha-fetoprotein test. The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase levels are increased but lower than that of hepatic cases even at terminal stage. Bilirubin level is increased over 50% of patients. The synthetic ability of the liver is mostly decreased. Albumin level is mostly not changed or only mild decreased because HCC produces itself even the patient apparently presents the cachexia. Prothrombin and partial thromboplastin times may be increased. A mild anemia and reactive leukocytosis frequently occur. Hypoglycemia occurs in two distinct groups of patients. The first is patients with good

performance statuses but have an acquired form of glycogen storage disease. The second group is terminal patients in whom hepatic gluconeogenesis is impaired (Wing *et al.*, 1991). Hypercholesterolemia is also common in patients with HCC.

Laboratory test can be helpful in the diagnosis but mostly are nonspecific. The most useful laboratory test in diagnosing HCC is the α-fetoprotein (AFP). AFP is a glycoprotein synthesized by fetal normal liver, fetal intestine, and yolk sac cells. AFP is high at pregnant and the fetus prior to birth and fall to adult levels of less than 20 ng/ml. AFP is increased in about 75% of patients with HCC (Waldman and Mc Intire, 1974). The levels of AFP in HCC patients is significantly increased than other malignant disorder, makes it useful as a tumor marker. However, AFP can not be used for follow up of the clinical course. Ferritin levels are increased in patients with uncomplicated cirrhosis (Okada, Ohtisuki, and Obata, 1985) and do not correlate with therapeutic response as α-fetoprotein.

For documenting the presence of a hepatic mass and/or following the clinical course after diagnosis is confirmed and treatment was given, computerized axial tomography and magnetic resonance imaging are the standard

imaging techniques for visualizing liver tumors. They provide a superior image which help in ruling out the other or metastasis and allow examination of the entire abdomen. However, by themselves they do not provide a definitive diagnosis. Intraoperative ultrasound is the most sensitive radiographic technique for detecting hepatic lesions.

Twenty-five percent of HCC have AFP negative and small questionable masses refer to the necessity of pathological diagnosis for an absolute confirmation. However, there is disadvantage in the biopsy of hemangioma. Tissue biopsy will be performed in only for special confirmation. Thus large masses with high level of AFP need not to be confirmed by biopsy.

2.1.6 Prognosis

HCC is an aggressive tumor that usually leads to grave prognosis. The median survival in untreated patients from the time of diagnosis varies from 3 to 6 months under general treatment. Nowadays, there are two attempts that provided a relative good prolong life. One is tumor resections and the second is ethanol-direct local freezing. The laboratory findings fit for criteria of baseline is that the liver functions has to be within normal level especially bilirubin levels, prothrombin and

partial thromboplastin times. Patient has to have no ascites or signs of portal hypertension. Patients met with category mostly had good prognosis but after hepatic resections most cases had tumor recurrence within two years. Pateints with fibrolamella type probably survive longer than classic HCC growth. Metastatic diseases grow very fast and are associated with a shorter survival rate.

Other important prognostic factors include the patient's age, sex, country of origin, and the presence or absence of cirrhosis. Patients under 45 years of age have a better prognosis than over 45. Female patients survive longer than males counterparts. For unclear reasons, South african blacks with HCC have a shorter survival than North American blacks. Patients with no cirrhosis survive longer and respond better to therapeutic agent than patients with cirrhosis.

2.1.7 Treatment

Several modalities have been used in the treatment of HCC. These modalities include surgical resection, radiotherapy, chemotherapy, immunotherapy, and other modalities. Practically these modalities are performed either alone or in combination.

As follow-up of high risk group makes a possibility in diagnosis. Small tumors (less than 3 cm in diameter) which are confined to one lobe of liver, is technically easily removable. Surgical resection is the most interesting procedure that promises one to two years prolong survival rate. Therefore, more endeavour for the complete cure is needed. Liver transplantation is also technically provided another chance but the donor and unresectable cases are the other obstructive problems (Iwatsukietal, 1985; Ringe, 1991). Among resectable cases, about 75% of survival cases develop recurrent tumor within 1 to 3 years either post-transplantation or posthepatectomy (Jenkins, Pinson, and Stone, 1989).

The benefit of external beam radiation is limited because of the inherent intolerance of normal liver tissue to radiation. Volberding, Friedman, and Phillips (1980) suggested that radiotherpy is more effective when combined with chemotherapy, as response rates increased from 35 to 46 percent. Polyclonal antibodies labelled with either iodine-131, indium-111 and yttrium-90 have been tried (Klein *et al.*, 1989; Order, 1990; Order, 1991). Iodine conjugated showed satisfactory response rate at 50 out of 105 patients (Stizmann, 1989). Again, this "magic bullet" mostly use animals' immunoglobulins which cause human antimouse immunoglobulin (HAMA) before the dose could

reach the maximum requirement.

Chemotherapy has the most longest history in trying to overcome the cancer but most of endeavours were close to failure. Doxorubicin is the most interesting drug for HCC. In dose ranging from 20 to 75 mg/m^2, only showed the response rates from 9 to 32 percent with the median survival 4 to 15 months (Volgel, Bayley, and Brockes, 1977; Johnson *et al.*, 1978; Falkson *et al.*, 1978; Olwey *et al.*, 1980; Melia, Johnson, and Williams, 1983; Ciarrino *et al.*, 1985). Mitoxantrone and epidoxorubicin have compatible activities with doxorubicin (Lai *et al.*, 1989; Shin *et al.*, 1988). Response rate of both oral and intravenous of 5-fluorouracil alone was very low under 10 percent (Davis, Ramirez, and Anfield, 1974; Link *et al.*, 1977; Falkson *et al.*,1978). Various combination regimens were studied, it showed no specific improvment compared to the single-agent chemotherapy (Patt *et al.*, 1983). The attempt has been progressed in administrating a chemotherapeutic agent through the hepatic artery, intra-arterial infusion, and tumor site for enhancing the therapeutic efficacy (Chenand and Gross, 1980). Even the response rates is higher than intravenous chemotherapy. This modality still can not reach the success because there is the difficulty in administration optimum concentration of drugs and its rapid total body clearance together with side effects on the

hematopoietic system and cachexia. For example, with 5-fluorouracil, the response was 54 percent (15 out of 28 patients)(Wellwood, Candy, and Oberfield, 1979). Doxorubicin showed response at 40 to 50 percent of all patients (4 out of 10; 6 out of 13; 8 out of 19) (Bern, McDermott and Candy, 1978; Olweny *et al.*, 1980; Uris and Balch, 1983). Intra-arterial combination of mitomycin-C, 5-fluorouracil, vinblastin, vincristin and doxorubicin showed the response rate 53 percent of 8 out of 15 patients (Douglas, 1980).

In early, 1975 after Kohler and Miller have estabished cell hybridization that provided hybridoma and the secretion, named monoclonal antibodies (MAbs). Since that a new system in sending drugs and toxins more specific and accuracy come to the reality. The development of biotechnology and genetic engineering made possible for producing highly pure immunomodulators and immunostimulators. Interferon and interleukin are another promising agents that time this magic bullet was highly expected and tremendous efforts were made. After clinical trails around 1986 have come to the conclusion that MAbs is good immunolocalization but for immunotherapy, it needs more endeavour. Progressive in cancer immunology is very wide and remarkable in every field of them. As for clinical trails, lymphokine-activated killer cells (LAK), and tumor infiltrating lymphocytes (TILs) are used to activate some

specific tumor killer cells (Ohnishi *et al.*, 1989; Lai *et al.*, 1989). Fortunately, HCC has TIL cell, the effort on this system seem to be another choice in overcoming the HCC. Even immunological modality which once was out of list in expecting the possibility to damage or to kill the tumor cell, is now come to the new, front line of hope. In this thesis, the IFN-α was selected for evaluating its effect against human hepatoma cell lines. As Laohathai (1985) found inclusion body in cytoplasm in her pilot study (unpublished data). The study is emphasized on finding the time that the inclusion body and segmented nucleus occur whether these are ralated.

Other modalities such as hyperthermia has been evaluated. In the study of Nagata and his colleague (1990), the clinical hyperthermia for malignant liver tumor was evaluated for 41 patients with HCC. The result showed partial remission occurring in only 28 percent. Ethanol injection responded significantly with small HCC. This technique is now widely adopted (Shen *et al.*, 1987; Livraghi et al., 1990). Ebara et al. (1990) have suggested that ethanol injection significantly prolonged life.

2.2 Interferons

2.2.1 Introduction

The exist of interferon (IFN), virus inhibitory factor, was discovered since 1940. The reconfirmations of interferon were reported during 1956-1958. Its component was identified as protein by Isaacs and Lindermann (Isaacs and Lindermann, 1957). From the observation on the viral infected cells in culture, revealed that a protein was released, which is identified as IFN. These viral infected cells will also resisted to other viral infection. During the first twenty years, reserach on the interferons were hampered by difficulties in mass production and its purification. This difficulties were solved since 1970s by use of recombinant DNA technology and monoclonal antibodies. IFN genes of vertebrates showed that they composed sugar family and was counted as one of cytokines. The IFN has multifunctions such as a positive growth factor for immunocytes, which is useful for hosts to defense against viral, parasitic infections and some tumors. Its certain function is involved with the proliferation and differentiation. During the past 10 years, clinical trial of IFN on infectious and malignant disease have were attempted.

2.2.2 IFN Genes

IFNs were initially classified by their sources of secreting cells: the leukocytes, fibroblasts and immunocytes. Type I IFN, is a group of IFNs that were secreted by leukocyte and fibroblast. Type II IFN, an immune IFN, is secreted by immunocytes. Recent nomenclature is based on sequencing, the leukocyte secreting IFN is named as IFN-α, the fibrobast IFN as IFN-β and the immune IFN as IFN-ɣ. In human, there are at least 18 IFN-α genes but only one IFN-β genes. The IFN-α and IFN-β genes form a cluster on the short arm of chromosome 9 (Balkwill, 1989) and the single IFN-ɣ gene is located on chromosome 12 (Naylor *et al.*, 1983). Both IFN-α and IFN-β contain no intron while IFN-ɣ has three intron. The mature IFN-α and IFN-β have 165-187 amino acids with molecular weight of 17-25 kD (Balkwill, 1989). After losing its secretory signal peptide, the secreted IFN-ɣ has 166 amino acids (Weissmann and Weber, 1986).

2.2.3 IFN Biosynthesis.

Low levels of IFNs can be detected in human tissues, even in the absence of a specific inducer. Biological stimuli include viruses, bacterias, mycoplasma and protozoa, and exposure to certain cytokines and growth

factor such as colony stimulating factor-1 (CSF-1), interleukin (IL)-1 , IL-2 and tumor necrosis factor enhancing IFN-α/β biosynthesis. Double-stranded (ds) RNA is a potent inducer and is the IFN-inducing side product or intermediate product during viral replication. Probably most differentiated cells are capable of producing IFN-α/β except undifferentiated mouse embryonal carcinoma cells (Harada *et al.*, 1991). In contrast, IFN-ɣ is produced by related lymphocytes which is induced by phytohemagglutinin (PHA)(Katayama et al., 1993). T cells and natural killer (NK) cells, stimulated by IL-2, can produce IFN-ɣ. Non-specific T cell activator such as concanavalin A is inducers for IFN-ɣ systhesis.

2.2.4 IFN Receptors

IFN must first interact with specific cell membrane receptors. This binding is generally species-specific and has repertory function. Human IFNs bind to human cells with a much higher affinity. Two distinct IFN receptors are widely distributed in the body (Auget and Mogensen, 1983). The IFN-α and IFN-β bind to the same receptor with its molecular weight 110-130 kD, whereas IFN-ɣ binds to a different receptor (Andrew *et al.*, 1980). The receptor genes for the IFN-α/β and IFN-ɣ are located on chromosome 21 and 6, respectively (Balkwill, 1989). The

similar structural features of human and murine IFN-ɣ receptors were shown in figure 1. Analysis of the human IFN-ɣ receptor cDNA and protein sequences revealed that the receptor consisted of 499 amino acids with a MW of 52 kD. This molecule is oriented around a single 23 amino acid transmembrane domain. The extracellular domain consists of 228 amino acids, including 10 cystein residues and 5 potential N-linked glycosylatation sites. The receptor has a large cytoplasmic domain of 221 amino acids. Interestingly, this portion of the molecule is particularly rich in serine and threonine and at least some of these residues become phosphorylated following interaction of the receptor with ligand in fuctionally responsive cells (Schveiber and Farrar, 1993).

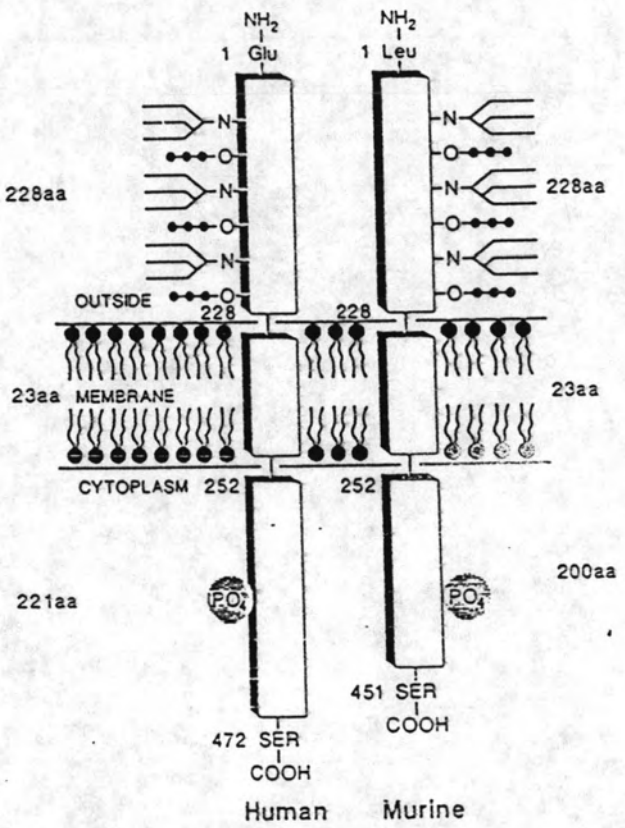

Figure 1. Schematic representation of human and murine IFN-ɣ receptor.

2.2.5 Proteins Induced by IFNs.

The mRNAs encoded by the IFN-activatable genes are translated into the IFN-inducible proteins. Some of these proteins may initiate a second cascade of activation of a second set of genes. The number of IFN-inducible proteins exceeds 30 types. Some are induced by all types of IFNs, and some are induced preferentially or even exclusively, by IFN- α/β or IFN-γ as summarized in Table 5. The IFN-induced proteins include several enzymes for example 2',5'-oligoadenylate synthetase (2',5'-oligo-A synthetase), protein kinase, indoleamine 2,3-dioxygenase and tryptophanyl-tRNA synthetase. Both 2',5'-oligo-A synthetase and protein kinase exert antiviral activity. Indoleamine 2,3-dioxygenase has been implicated as the mediator of inhibition of the replication of the intracellular protozoa Toxoplasma gondii and Chlamydia psittaci by IFN-γ (Pfefferkorn, 1984). The inhibition is thought to be caused by tryptophan starvation. It is intriguing that IFNs especially IFN-γ, also induce the synthesis of a further enzyme affecting tryptophan such as tryptophanyl-tRNA synthetase (Rubin *et al.*, 1991). Although the mechanism of some IFN-induced proteins are not completely understood, studied on these proteins have led to a new concept of host defence and to important therapeutic efficacy.

Table 5 Partial list of IFN-inducible proteins whose cDNA have been isolated.

Designation	Characteristic	Inducer
2',5'-$(A)_n$ synthetase	2',5'-oligo-A synthetase	α, β>γ
p68 kinase	Protein phosphorylation	α, β>γ
Indoleamine2,3-dioxygenaes	Tryptophan degradation	γ>α, β
P56	Tryp-tRNA synthetase	γ>α, β
DBP/γ67	Guanylate binding	γ>α, β
Mx families	Anti-influenza virus	α, β>γ
IRF1/IRF2	Transcription factor	α, β, γ
MHC class I	Immune system	α, β, γ
MHC calss II	Immune system	γ
β_2-microglobulin	Immune system	α, β, γ
IP 10	Platelet factor4 related	γ>α, β

2.2.6 Mechanism of IFN Action

2.2.6.1 Antiviral Action

The IFN can impact various steps of viral replication including penetration, uncoating transcription, translation and the assembly of progeny viruses (Lengyel, 1982; Pestka *et al.*, 1987; Samuel, 1988; Stacheli, 1990). The reaction of IFN is indirectly discarding the virus from infected cell. The mode of antiviral actions of the IFN is initiated by binding specific receptors on virus-carrier cells, resulting in transmembrane signal and proteins synthesis. Different IFN interfere biochemical pathways which may be responsible for inhibiting the replication of different viruses. Over dozen of IFN-induced proteins have been identified. Three of them representing antiviral pathways are protein kinase (eIF-2α protein kinase), 2',5'-Oligo-A synthetase , and Mx protein.

Protein Kinase: The eIF-2α protein kinase mediates antiviral effect by reducing the translation of viral protein synthesis by phosphorylating the α-subunit of initiation factor 2 and there by decreasing the efficiency of initiation of protein synthesis (Jacobsen, 1986).

2',5'-Oligo-A Synthetase: This system exerts its antiviral effect by enzymatically degrading viral RNA, there by reducing it availability as a template for translation in to a viral protein (Greenberg, 1987; Faltynek and Kung, 1988). The 2',5'-oligo-A synthetase is upregulated by IFN and activated by viral dsRNA to convert ATP into a series of small oligoadenylates with unusual 2',5'linkage that bind to a cellular endoribonuclease at the same site as the viral RNA resulting in degradation of the viral RNA .

Mx Protein: The Mx protein, originally described in mouse cells, confers resistance to influenza virus (Arnheiter and Meier, 1990). The mechanism of Mx protein in blocking virus replication may involve an inhibition of transcription.

Together with its IFN-induced proteins, which affect the virus replication, IFN also has immuno-modulatory function affect virus replication. IFN enhanced the expression of MHC, the macrophage activation, the regulation of NK cells and cytotoxic T cells, and the cytokine induction which would be expectd to affect virus multiplication of many types of viruses by inducing a variety of intracellular mechanism.

2.2.6.2 Antitumor Actions

This action has been found since 1960 but the volume has to be high up to 100 times of antiviral used. The content study has to wait until 1970 after Gresser and his colleague reported the satisfactory response to the mouse L1210 leukemia and L-cell (Gresser *et al.*, 1970; Gresser, Maury, and Brouty-Boye, 1972). Interferons exert powerful effects on the growth of tumor through both direct and indirect mechanisms.

The direct antiproliferative effects primarily exert by

1. Cytostatic mechanism resulting in increasing the length of their cell multiplication cycle.

2. IFNs modulate the levels of cellular 2',5'-oligo-A synthetase (Well and Mullucci, 1985) and oncogenes such as c-myc, c-fos, and c-H-ras (Contente *et al.*, 1990; Takada *et al.*, 1991).

3. Depleting essential metabolites. For example, all three IFNs inhibit the induction of ornithine decarboxylase, decreasing the biosynthesis of putrescene and other essential polyamines (Sekar *et al.*, 1983; Chang *et al.*, 1987; Takada *et al.*, 1991).

4. IFN-ɣ induces the synthesis of indolamine 2,3-dioxygenase, resulting in the degradation of

the amino acid tryptophan (Yasui *et al.*, 1986).

5. Inhibitory of the movement of cell and change of microfilament.

The indirect antitumor effect of IFN is mediated by host cytotoxic effector leukocytes and the immune response. First, the function to the tumor cells as IFN-induced protein, enhanced expression of tumor surface antigen such as MHC (major histocompatibility complex) (Houghton *et al.*, 1984; Schwartz *et al.*, 1985; Towata *et al*, 1993) and tumor-associated antigen (Nanus *et al.*, 1990) resulting in more efficient recognition and killing of tumor cells by cytotoxic leukocytes. Second, the induction of antibodies to the tumor cells, contributing to an enhanced tumor cell lysis mediated by complement and antibody-dependent cell mediated cytotoxic leukocytes. Thrid, induce enhancement of tumor cytotoxicity by macrophage, natural killer lymphocytes, and T-lymphocytes (Nathan *et al.*, 1983; Weigent, Stanton, and Johnson, 1983)

Tumor cell populations demonstrate different degrees of sensivity to the different IFNs. IFNs can also inhibit normal cells such as bone marrow. However, IFNs may exert more potent antiproliferative effects against some malignant cells than against non-malignant cells, particularly when used in synergistic combination of IFN-γ

with either IFN-α and IFN-β (Fleishmann *et al.*,1984).

2.2.7 Clinical indication for IFNs

IFN-α has been approved for treatment hairy cell leukemia and AIDS-related Kaposi's sarcoma by Food and Drug Administration (FDA) since 1986. IFN-α is now available for general clinical used. Most of them are recombinant IFN-α-2a (Roferon-A, Hoffmann-LaRoche, Basel, Switzerland), recombinant IFN-α-2b (Intron-A, Schering Coorporation, Keniworth, NJ, USA) and human lymphoblastoid IFN (Wellferon, Wellcome Foundation, London, England).

The majority of experience with IFN-α has been gained with early chronic myelogenous leukemia (CML), low-grade non-Hodgkin's lymphoma and cutaneous T-cell lymphoma with response rates 40 percent except for the CML which revealed 80 percent (Koeller, 1989; Barson *et al.*, 1991).

Recombinant IFN-γ (Actimmune, Genetech, South San Francisco, California, USA) is lately in 1990 approved by FAD for chronic granulomatous disease. This disease is an inherited immune disease mostly manifest as severe recurrent infection of skin, lymph nodes, liver, lungs and bones due to the disfunction of phagocytes.

Formerly, the only treatment was antibiotics. For clinical studies, 128 patients with chronic granulomatous disease treated with IFN-ɣ showed 70 percent reduction of serious infection in both X-linked and autosomal recessive traits types compared with the placebo group (Gallin *et al.*, 1991).

Surprisingly, IFN-α appears to have activity in wide range of malignancies as summarized in Table 6. Responses have been most notable in certain hematological malignancy. Results with solid tumor was not an encouraging one. However, the majority of these trails have focused on patients with advanced disease rather than absolute inactively specific tumor types. Some solid tumors, namely malignant melanoma and renal cell carcinoma, have shown sensitivity to IFN-α in the phase II trail. In both cases, the overall response rates appear around 20 to 25 percent. This activity was approved to be real effect and encouraging for future trail. Robert (1987) and Berek *et al.*(1985) reported that intraperitoneal administration of IFN-α resulted in complete response in 5 out of 11 women with ovarian cancer. This trail clearly demonstrated the effect of tumor burden which is parallelly related to the degree of responses. The response cases were residual disease which tumor size less than 5 mm. In contrast, non of the patients with large bulky disease achieved a

significant response. Sach *et al.*(1985) and Nair *et al.* (1985) reported that IFN-α did not show therapeutic benefit in patients with HCC which is hepatitis virus infected malignancy while Duke *et al.*(1985) studied of human lymphoblastoid IFN-α inhibitory thymidine uptake in PLC/PRF/5 hepatoma cell *in vitro*. In addition, in a large clinical trial from Hong Kong, 75 patients with HCC were randomized to receive IFN-α or doxorubicin. The result showed that IFN treatment was associated with significant increased in major and minor response (22 percent and 0 percent of IFN and doxorubicin trials, respectively). This controversial exists as to whether IFN-α has antitumor activity against HCC. The actual efficacy of IFN-α should be assessed. The aim of this study was to evaluate the demonstrated effect of IFN-α with special emphasis on morphological alteration of hepatoma cell line observed with electron microscopy. The result from this study is expected to reconfirm the effect of IFN-α leading to improvement of HCC treatment.

Table 6 Activity of alpha interferon against various malignancies in Phase II studies.

Active	Hairy cell leukemia
	Chronic myelogenous leukemia
	Non-Hodgkin's lymphoma
	Myelosis fungoides
	Cutaneous T-cell lyphoma
	Kaposi's sarcoma
	Multiple myeloma
	Malignant melanoma
	Renal cell carcinoma
	Bladder cancer(intravesical)
	Ovarian cancer(intraperitoneal)
Inactive	Breast cancer
	Colon cancer
	Non-small cell lung cancer
	Prostate cancer
	Acute myelogenous leukemia
Further study required	Chronic lymphocytic leukemia
	Acute lymphocytic leukemia
	Preleukemia
	Hodgkin's disease
	Osteogenic sarcoma