CHAPTER II

BACKGROUND

Hepatocellular carcinoma

Primary hepatocellular carcinoma (HCC) is the most common fatal cancer, causing in excess of 250,000 deaths annually (Patt,1993). It occurs infrequently in the United States and North America (Coog, 1985, Di Bisceglie ,1988) ; but it's the most common malignant tumor in Africa and Southeast Asia (Vatanasapt, 1993). The incidence seems to increasing every year. The difficult in early diagnosis and failure to achieve complete cure are recognized as the possible major contributing factors. More understanding about the cause, etiologic factor and newer technology have become increased. The diagnosis and management are more sufficient but the complete cure is still insufficient today.

1. Classification of Hepatocellular carcinoma (primary liver cancer)

Although, HCC is mostly found in Africa and Asia but with low incidence in USA and North America, the histological features of HCC did not vary with the races and the geographical regions. It has progressively become evident that HCC varies strikingly in its gross features in different parts of the world. Then it was devided to 2 classes: one by mean of gross features, two by mean of histological features. The gross classification of primary liver cancer was first undertaken by Hanot and Gilbert in 1888 and modified in 1901 by Eggel. Later on, Edmondson and Steiner taken the histological features and divided HCC into four

grades namely I - IV according to the degree of differentiation of the tumor. The classification was a slightly modified the version of the WHO classification that was proposed in 1978 and has since obtained popularity in Western countries.

1.1 EGGEL'S Classification (gross features).:

1.1.1 NODULAR FORM : Most of this type occurs as a single solid mass, but may compose of multiple (few to many) nodules, which vary in sizes and are sharply demarcated.

1.1.2 MASSIVE FORM : Tumors penetrate the whole liver lobe. The tumor mass is not well demarcated and is frequently accompanied by a small intrahepatic artery, and tumor thrombosis.

1.1.3 DIFFUSE FORM : Numerous small foci, about as large as the pseudolobules of a cirrhotic liver, are scattered throughout the liver. Each focus is surrounded by connective tissue which is frequently difficult to distinguish from the pseudolobudes of liver cirrhosis.

1.2 EDMONDSON - STEINER Classification (histological features) : Basically, the histological structure of HCC rembles that of the normal liver, in that the tumor parenchyma comprises a liver cell cord - like (trabecular) structure and the stroma consists of a sinusoid - like blood space lined by a single layer of endothelial cells.

1.2.1 GRADE I CARCINOMA : This type of HCC is composed of most differentiated tumor cells with their arrangement figured as a thin trabecular pattern. However, it is not seen as the sole type in any specimen but only occurs in areas predominanted by grade II.

1.2.2 GRADE II CARCINOMA : Tumor cells show a marked resemblance to normal hepatic cells, only nuclei are larger and more hyperchromatic than usual. The cytoplasm, is abundant and acidophilic. The acinar structure is frequently associated with the trabecular pattern .

1.2.3 GRADE III CARCINOMA : The nuclei are usually larger and more hyperchromatic than in grade II . The nuclei occupy greater proportion of the cell. Bile and acinar formation are noted less frequently. Tumor giant cells are most numerous in this type

1.2.4 GRADE IV CARCINOMA : This type of HCC is the most poorly differentiated. The nuclei are intensely hyperchromatic and occupy a great part of the cell. The cytoplasm varies in amount; it is often scanty and contains fewer granules. The growth in the liver is more medullary. The trabecular are difficult to find. Cell masses seems to lie loosely and without cohesion.

2. Pathology of the HCC

The gross appearance of HCC varies from a single, large, dominant nodule or mass, which may be well - circumscribed or infiltrating, to a multicentric tumor. The tumor itself is characteristically soft, a factor that may lead to rupture and intraperitoneal hemorrhage. There may be areas of necrosis or hemorrhage, especially in large tumors. Large nodular tumor are often seen intermediate forms. Most tumors consist of neoplastic hepatocytes arranged in trabeculae, freguently with acinus - like structures. Usually the tumor cells are sufficiently well differentiated to be recognizable as of hepatocyte origin, but a few tumors are highly anaplastic and these generally lack the trabecular organization (Vincent, 1989).

3. Incidence and Epidemiology

Primary hepatocellular carcinoma (HCC) is one of the most common cancers in the world and it is estimated to be responsible for up to 250,000 deaths every year (Chlebowski, 1984, Lincell, 1987, Patt, 1993). It occur infrequently in the United States and North America, with fewer than 10,000 new patients annually (Patt, 1993), accounting for less than 2 % of all malignancies (Chlebowski, 1984, Lincell, 1987, Silverberg, 1988). The age standardized annual incidence is 2.9 per

100,000 men and 1.2 per 100,000 women. A similar low incidence is found in Britain, Canada, Australia, and South America. In Africa and Asia , HCC is the most common malignant tumor in male (Di Bisceglie, 1988, Fu-Sun, 1986, Okuda, K.,1986). The incidence ranges from 34 per 100,000 men of Chinese descent in Singapore, to 65 per 100,000 men in Zimbabwe, to more than 100 per 100,000 men in Mozambique and Taiwan (Fu - Sun, 1986). Worldwide, the disease occurs predominately in men over 30 years of age (Di Bisceglie, 1987, Okuda, 1986, Vatanasapt, 1993).

From Public Health Statistics, 1990 Thailand, cancer is the third leading cause of death in Thailand while the first and second are heart disease and accident respectively as shown in Table 1. The trend in the rates of liver mortality seems to be increasing every year (figure 1). The most important cancers in males and females in terms of age standardized incidence rates are shown for the four registries in figures 2 - 5. There is a very marked regional variation, thus, with the highest incidence in the Northeast; the age- standardized incidence rate of liver cancer in Khon Kaen is the highest in the world. The type of liver cancer is mostly cholangiocarcinoma (Parkin, 1992, Vatanasapt, 1993). In Bangkok, Chiangmai and Songkla, liver cancer ranks the second cancer of men , on the other hand, it is the first most important cancer in Khon Kaen. For women , liver cencer is less frequent in Chiang Mai, Bangkok and Songkla but it is the first most important cancer in Khon Kaen, too. The incident rates of liver cancer in Khon Kaen are much higher than elsewhere in Thailand in both men and women. The rational estimates of liver cancer as leading cancer are shown as age standardized rates in Figure 6. The estimated numbers of new cases in Thailand in the year 1990 indicated that liver cancer has the highest numbers of new cases are shown in figure 7. The estimated incidence of liver cancer in Thailand is very high : 40.5 per 100,000 in males with an estimated 8,000 new cases from 29,950 all cancer cases every year

Table 1 Leading causes of deaths, Number and rates per 100,000 population, 1986-1990.

Cause of death	2529 (1986)		2530 (1987)		2531 (1988)		2532 (1989)		2533 (1990)	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate
Total	218,025	414.1	232,968	434.6	231,227	424.0	246,570	444.7	252,512	448.2
Disease of the heart	19,681	37.4	22,987	42.7	24,286	44.5	27,452	49.5	28,924	51.3
Accident and poisoning	13,052	24.8	14,009	26.1	16,491	30.2	19,482	35.1	23,634	41.9
Malignant neoplasm,all forms	14,709	27.9	16,905	31.5	18,284	33.5	20,385	36.8	22,154	39.3
Suiside, homicide and injury	8,329	15.8	8,401	15.7	8,980	16.5	9,034	16.3	8,621	15.3
Hypertension and cerebrovascular disease	6,541	12.4	6,863	12.8	7,240	13.3	7,966	14.4	8,445	15.0
Disease of liver and pancrease	7,039	13.4	7,536	14.1	5,134	9.4	7,738	14.0	7,520	13.3
Pneumonia and lung disease	4,448	8.4	5,096	9.5	5,502	10.1	6,168	11.1	5,902	10.5
Nephritis, nephrotic syndrome and nephrosis	2,923	5.6	3,508	6.5	3,589	6.6	3,957	7.1	4,254	7.6
Tuberculosis, all forms	5,169	9.8	5,471	10.2	4,449	8.2	4,218	7.6	3,937	7.0
Paralysis, all types	2,882	5.5	3,421	6.4	3,360	6.2	3,347	6.0	3,341	5.9
Others	133,252	253.1	138,861	259.0	93,866	172.1	136,823	246.8	135,780	241.0

From: Public Health Statistics A.D. 1990.

Note : 1. The data obtained from death certificate.

2. Disease of the heart includes heart failure.

3. Disease are put in order according to leading causes in 1990.

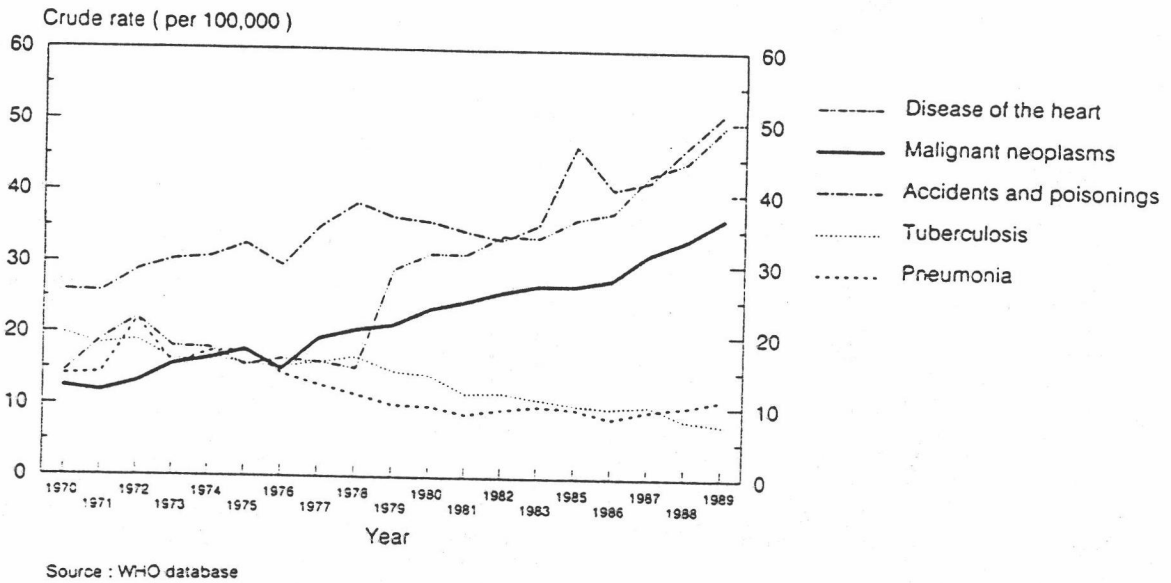

Figure 1. Trends in the rates of mortality from five major causes, Thailand, 1970-1989.

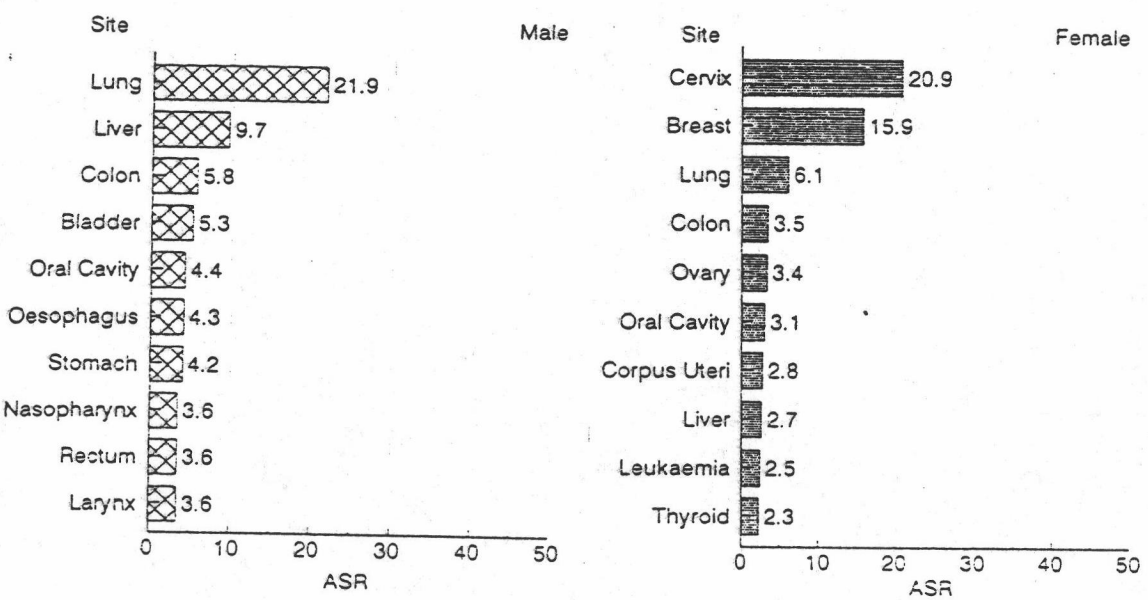

Figure 2. Leading cancers in Bangkok (1988-1990)

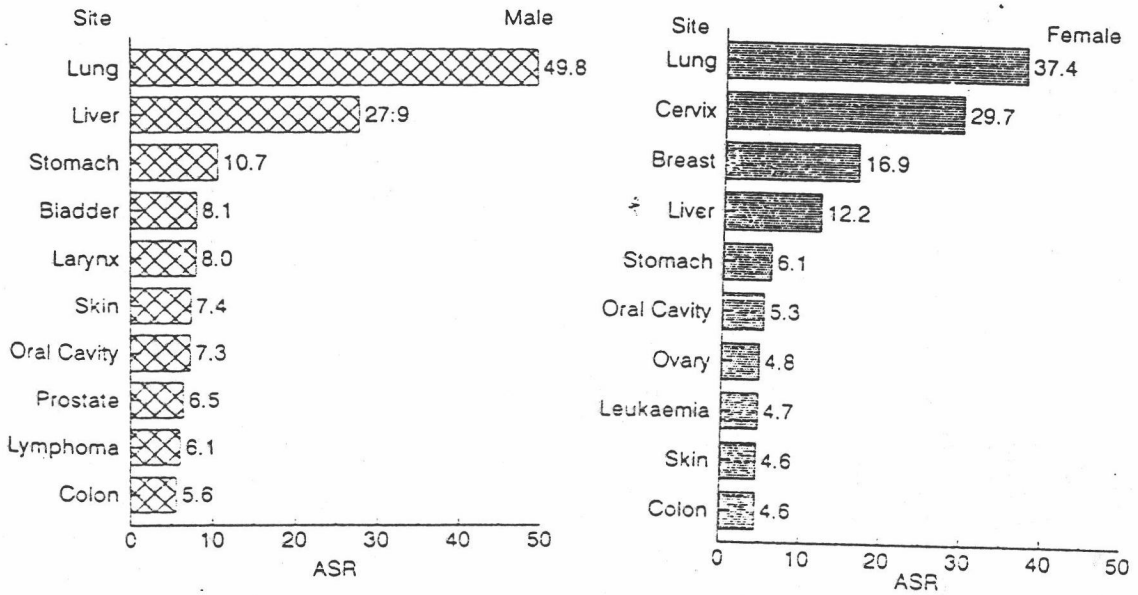

Figure 3. Leading cancers in Chiang Mai (1988-1991)

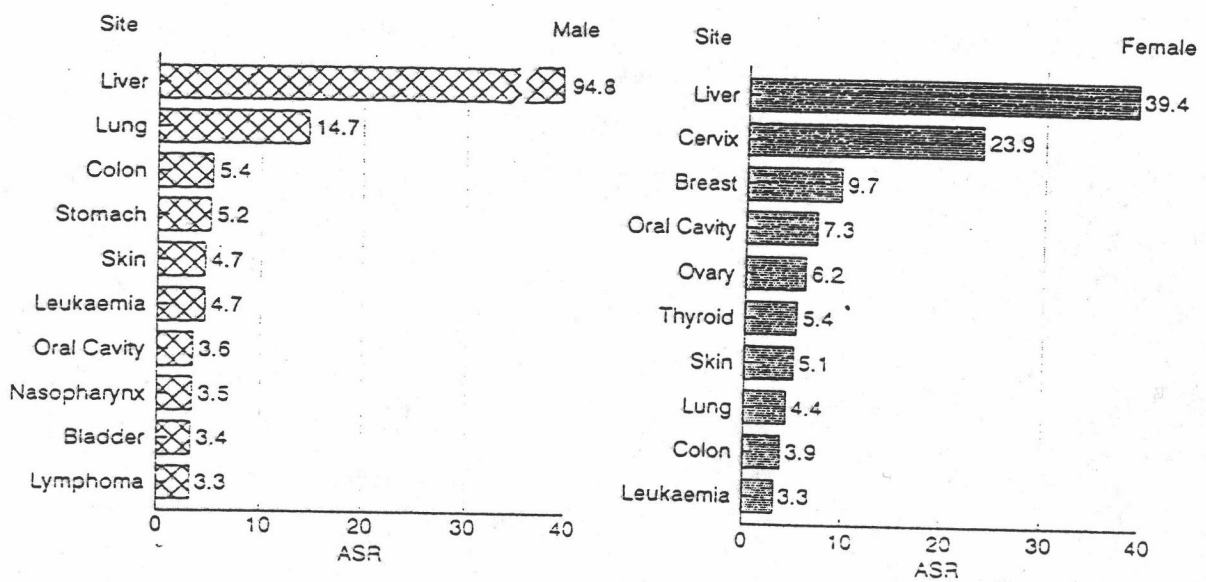

Figure 4. Leading cancers in Khon Kaen (1988-1991)

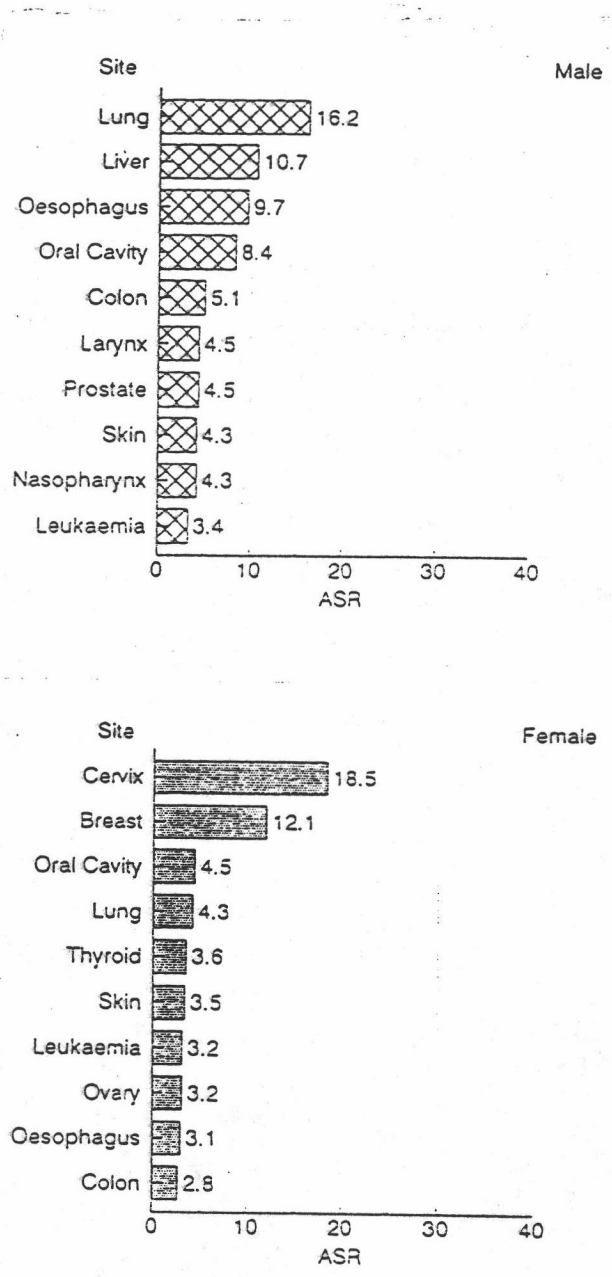

Figure 5. Leading cancers in Songkhla (1988-1991)

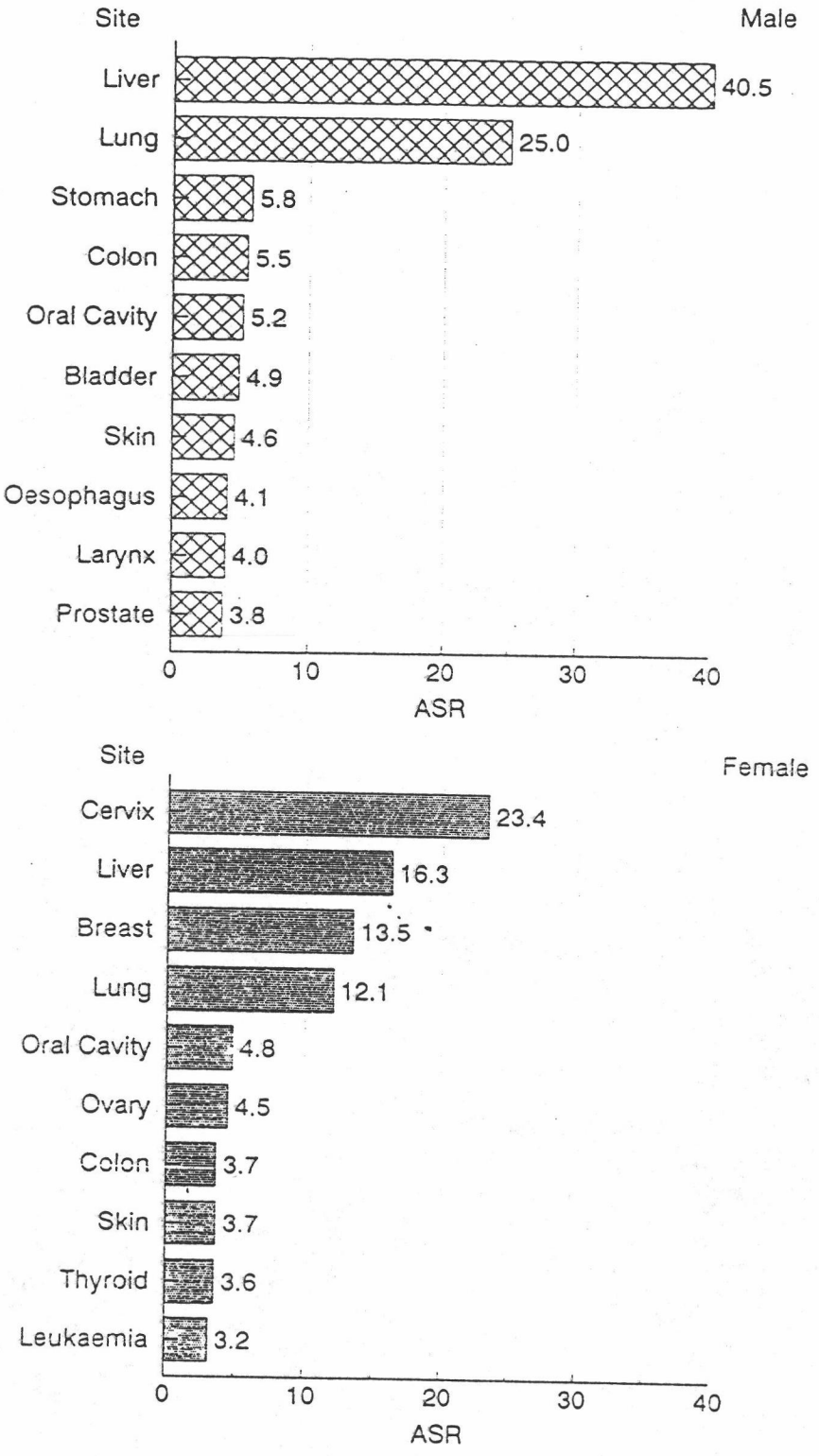

Figure 6. Leading cancers in Thailand (estimate), 1990

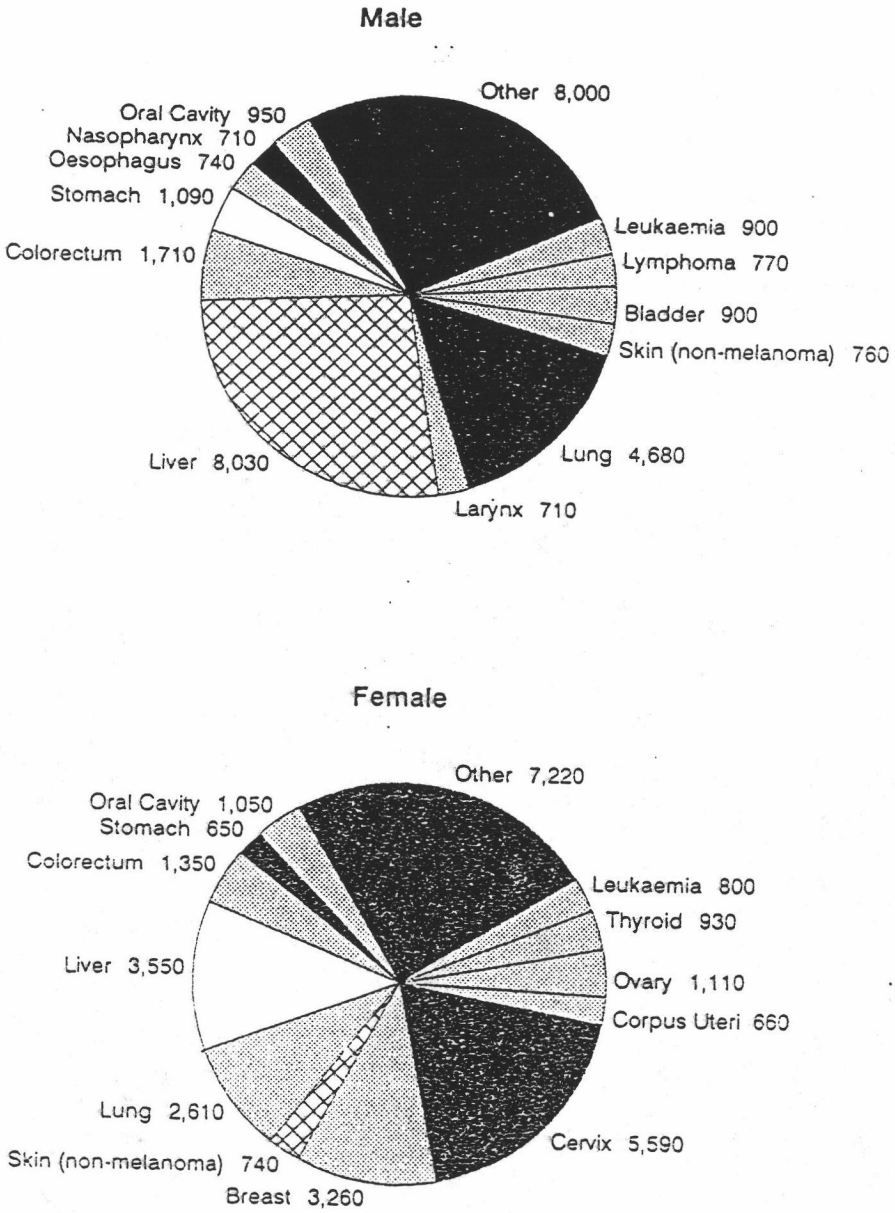

Figure 7. Estimated number of new cancer cases in Thailand (1990)

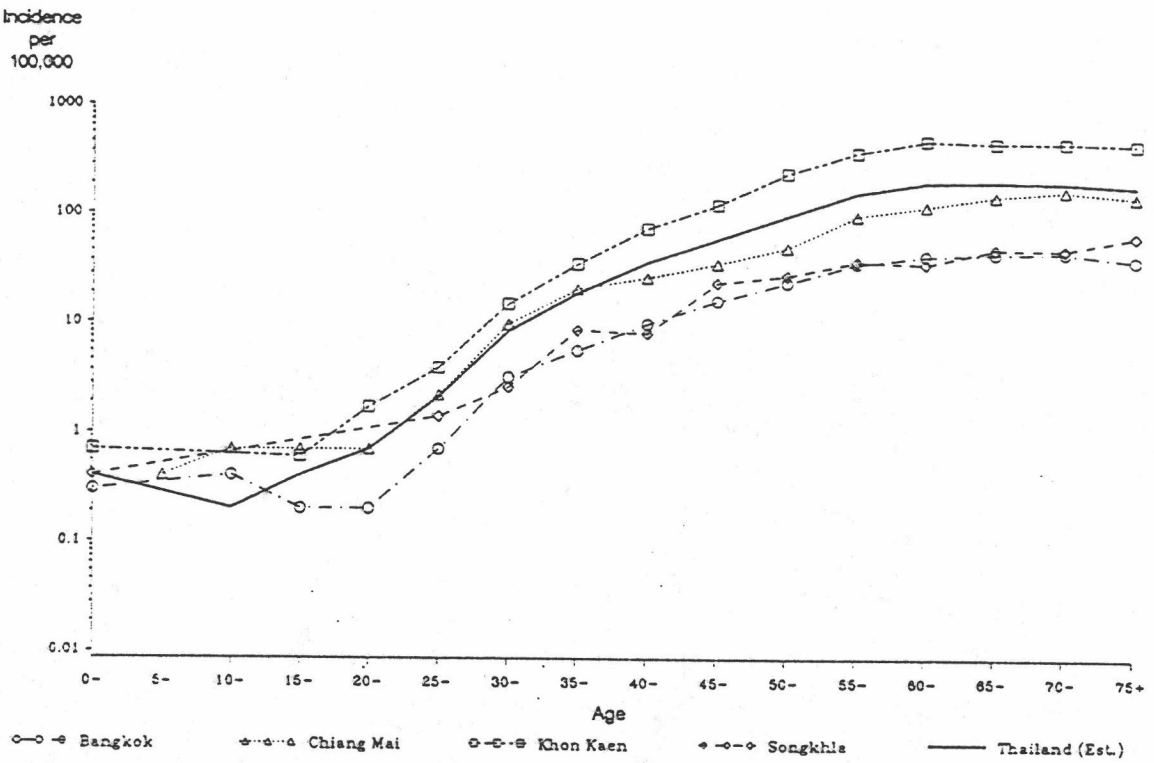

Figure 8 Age-specific incidence rates of liver cancer - Male

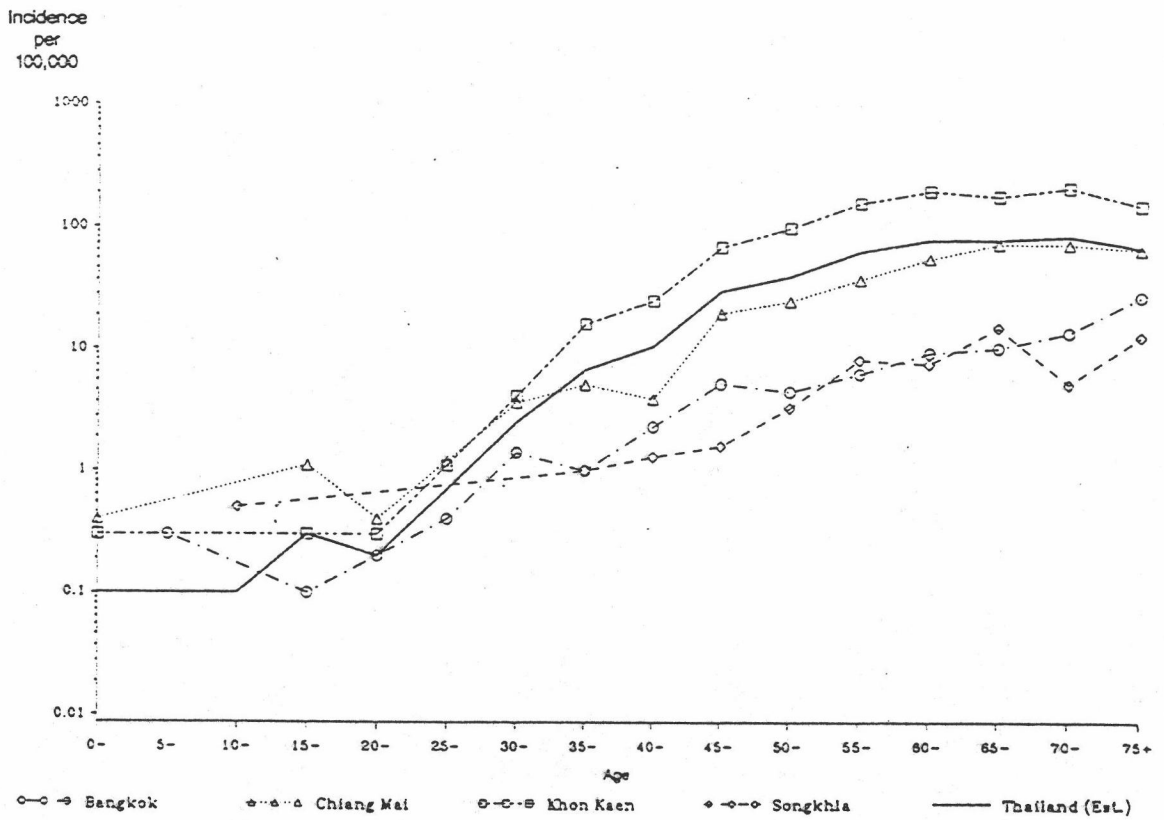

Figure 9. Age-specific incidence rates of liver cancer - Female

and 16.3 per 100,000 in females with an estimated 3,500 new cases from all cancer cases, 29,950 every year. The frequency of malignancy in the country was estimated to have 11,581 new cases in 1990 (the M/F ratio is 2.3:1) (Vatanasapt, 1993). The average age of the patients is 35-65 years (see figure 8-9). The death rates for liver cancer in males are 6.6 per 100,000 (crude rate: CR) and 10.6 per 100,000 (age standardized mortality rate: ASMR). Death rate in females are 3.0 for CR and 4.3 for ASMR per 100,000 (Table 2 and 3).

4. Etiology

Genetic factors, alcoholism, viral infection, cirrhosis, dietary carcinogens, malnutrition, parasites and chemicals such as aflatoxin have all been implicated as etiological agents (Lincell, 1987).

4.1 Genetic factors

There is considerable evidence that the incidence of spontaneous HCC in mice varies with their genetic background (Lincell, 1987). Study of the changing incidence of a neoplasm in migrant populations could provide insight into whether genetic or environmental factors are mainly responsible for the tumor. In the case of HCC, few careful analyses of this sort have been undertaken, and these do not support a genetically determined susceptibility to the tumor. American Blacks, although originating form the areas of high incidence of HCC in Africa, have the same low prevalence of the tumor as the white population, whose life-style and habits they have adopted and with whom they have shared a common environment for generations. The exposure to carcinogens in infancy or childhood may determine tumor formation much later in life, eventhough they have migrated during their own life - time to low incidence area. Although, familial recurrences of HCC are occationally described, most of the affected family members have been shown to be infected with the hepatitis B virus that not clearly indicated familial factor. Human HCC occurs more commonly in males than females in virtually all

Males	Numbers death	0-14	15-24	25-34	35-44	45-54	55-64	65-74	75-	Crude rate	A.S.R.
Oral cavity	38	0.0	0.0	0.0	0.2	0.4	1.0	0.8	2.1	0.1	0.2
Other pharynx	158	0.0	0.1	0.0	0.5	1.7	3.2	7.2	7.2	0.6	1.0
Nasopharynx	4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.4	0.0	0.0
Oesophagus	122	0.0	0.0	0.0	0.2	0.9	3.8	4.1	9.3	0.5	0.8
Stomach	150	0.0	0.0	0.1	0.6	1.8	3.0	4.9	8.5	0.6	1.0
Small intestine	64	0.0	0.0	0.1	0.3	0.7	1.2	2.1	3.0	0.2	0.4
Colon	275	0.0	0.2	0.3	1.0	2.6	5.5	10.4	14.8	1.0	1.6
Rectum	8	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0
Liver	1775	0.1	0.3	2.0	9.7	24.8	42.0	40.8	36.9	6.6	10.6
Gallbladder etc.	67	0.0	0.0	0.0	0.3	0.9	1.0	2.7	4.7	0.2	0.4
Pancreas	56	0.0	0.0	0.0	0.2	0.4	1.3	2.4	3.8	0.2	0.4
Peritoneum	20	0.0	0.0	0.0	0.1	0.3	0.3	1.0	1.3	0.1	0.1
Nose, sinuses etc.	26	0.0	0.0	0.1	0.1	0.2	0.6	0.8	0.4	0.1	0.1
Larynx	42	0.0	0.0	0.0	0.0	0.3	1.0	2.1	3.4	0.2	0.3
Lung	893	0.0	0.4	0.6	1.4	7.6	20.8	39.8	56.4	3.3	6.0
Pleura	2	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0
Other thoracic organs	4	0.0	0.0	0.0	0.0	0.1	0.0	0.2	0.0	0.0	0.0
Bone	80	0.0	0.1	0.0	0.2	0.8	2.2	2.2	3.0	0.3	0.5
Connective tissue	14	0.0	0.0	0.0	0.0	0.1	0.5	0.3	0.8	0.1	0.1
Skin (non-melanoma)	4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
Breast	3	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
Prostate	32	0.0	0.0	0.0	0.0	0.1	0.6	1.8	4.7	0.1	0.2
Testis	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
Penis etc.	6	0.0	0.0	0.0	0.0	0.1	0.1	0.3	0.0	0.0	0.0
Bladder	52	0.0	0.0	0.0	0.1	0.4	0.6	2.4	7.2	0.2	0.4
Kidney etc.	23	0.0	0.0	0.0	0.0	0.3	0.2	1.4	0.8	0.1	0.1
Eye	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
Brain, nervous system	193	0.4	0.4	0.4	0.6	1.7	2.6	3.0	3.8	0.7	1.0
Thyroid	9	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.1
Other endocrine	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Non-Hodgkin lymphoma	106	0.0	0.2	0.4	0.5	0.8	1.6	2.1	4.2	0.4	0.6
Leukaemia	291	0.7	0.9	0.6	0.9	1.6	3.3	3.5	6.4	1.1	1.3
Primary site uncertain	5029	0.8	1.9	5.1	18.4	64.0	129.1	132.2	119.5	18.7	30.5
ALL SITES	9551	2.2	4.7	10.1	35.5	112.6	226.3	268.6	303.4	35.6	58.1

Source: WHO mortality database

Table 2. Thailand, mortality, 1987, age-specific death rates (male)

Females	Numbers death	0-14	15-24	25-34	35-44	45-54	55-64	65-74	75-	Crude rate	A.S.R.
Oral cavity	23	0.0	0.0	0.0	0.0	0.2	0.4	0.4	1.7	0.1	0.1
Other pharynx	76	0.0	0.0	0.1	0.3	0.6	1.6	2.3	2.8	0.3	0.4
Nasopharynx	4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.3	0.0	0.0
Oesophagus	36	0.0	0.0	0.0	0.1	0.3	0.9	1.4	1.1	0.1	0.2
Stomach	130	0.0	0.0	0.3	0.6	1.2	2.0	3.9	5.1	0.5	0.7
Small intestine	37	0.0	0.0	0.0	0.1	0.4	0.9	1.0	0.6	0.1	0.2
Colon	200	0.0	0.1	0.2	0.7	1.9	4.4	4.6	8.0	0.7	1.1
Rectum	4	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
Liver	797	0.1	0.3	1.0	4.4	9.8	17.2	16.7	11.7	3.0	4.3
Gallbladder etc.	49	0.0	0.0	0.0	0.2	0.3	0.9	1.8	2.0	0.2	0.3
Pancreas	21	0.0	0.0	0.0	0.1	0.1	0.4	0.7	1.7	0.1	0.1
Peritoneum	26	0.0	0.0	0.0	0.1	0.3	0.3	0.8	1.7	0.1	0.1
Nose, sinuses etc.	21	0.0	0.0	0.0	0.2	0.2	0.2	0.3	0.9	0.1	0.1
Larynx	9	0.0	0.0	0.0	0.1	0.0	0.1	0.4	0.3	0.0	0.1
Lung	376	0.0	0.1	0.4	0.9	4.2	7.3	12.5	12.0	1.4	2.1
Pleura	2	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Other thoracic organs	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bone	70	0.0	0.1	0.1	0.4	0.6	1.7	1.8	0.3	0.3	0.4
Connective tissue	7	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0
Skin (non-melanoma)	7	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.0
Breast	210	0.0	0.0	0.3	1.3	3.7	3.2	3.7	2.6	0.8	1.1
Uterus unspecified	143	0.0	0.0	0.2	0.5	2.4	3.4	2.0	2.0	0.5	0.8
Cervix uteri	126	0.0	0.0	0.1	0.7	1.7	2.8	2.2	2.0	0.5	0.7
Corpus uteri	286	0.0	0.1	0.5	1.5	4.0	6.4	3.9	4.0	1.1	1.5
Ovary etc.	57	0.0	0.1	0.1	0.4	0.8	1.1	0.7	0.9	0.2	0.3
Other female genital	12	0.0	0.0	0.0	0.1	0.1	0.4	0.0	0.0	0.0	0.1
Bladder	10	0.0	0.0	0.0	0.0	0.2	0.1	0.1	1.1	0.0	0.1
Kidney etc.	10	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.3	0.0	0.0
Eye	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brain, nervous system	161	0.3	0.2	0.4	0.9	1.3	2.2	2.4	1.4	0.6	0.8
Thyroid	12	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.3	0.0	0.1
Non-Hodgkin lymphoma	50	0.0	0.1	0.1	0.2	0.3	0.7	1.5	0.6	0.2	0.3
Leukaemia	220	0.4	0.6	0.4	0.9	2.2	2.4	2.3	2.3	0.8	1.0
Primary site uncertain	4158	0.9	2.0	5.6	18.8	54.8	83.1	82.1	67.2	15.5	22.2
ALL SITES	7354	1.8	3.7	10.2	33.7	92.7	144.9	150.4	134.8	27.5	39.2

Source: WHO mortality database

Table 3. Thailand, mortality, 1987, age-specific death rates (female)

populations. The high incidence of HCC in males could be genetically determined. Thus males have a higher prevalence of chronic HBV infection and they also are more likely to be exposed to chemical carcinogens, both dietary, because of their generally greater food in take, and also industrial carcinogens. Furthermore, there is both clinical and experimental evidence that the male hormonal environment may be important in tumor progression in HCC. The higher incidence of human HCC in males could have an indirect genetic basis in the form of an increased susceptibility to one or more of the relevant environmental carcinogens. Thus, genetic factors related to sex might play both a direct and indirect role iin the etiology of the tumor

In conclusion, there is no clearcut evidence that genetic factors play a significant direct role in the etiology of human HCC. However, they may play an important indirect role by increasing susceptibility to one or more of the environmental etiological agents which are thought to be responsible for this tumor.

4.2 Cirrhosis. Cirrhosis is a well-documented risk factor for development of HCC (Zaman, 1985). About 75% of all cases of HCC are associated with cirrhosis, a process of itself could have favoured the formation of the carcinoma (UICC conference, 1982, Vikit, 1984). Interestingly, in many patients with this disease, the presence of cirrhosis was unsuspected before they presented with tumor. The association between cirrhosis and hepatocellular carcinoma has been shown in autopsy studies where 60 % to 90 % of HBs-Ag positive patients with HCC are found to have an accompanying cirrhosis. The magnitude of this risk factor is validated by the finding that 20 % to 40 % of patients dying who have cirrhosis are found to have HCC on autopsy (Di Biscegelie, 1988). The association of liver cirrhosis with HCC varies in frequency depending on the etiology of the cirrhosis such as viral hepatitis, various toxication, chronic alcohol abuse,

malnutrition, hepatic injuries secondary to bile stasis, congestion and parasites (Zaman, 1985).

4.3 Diet and nutrition. The impression that diet and nutrition are of significance for liver cancer in human populations has developed because of the high incidence of HCC in residents of Southeast Asia, South America and Africa where this type of tumor coexists with malnutrition. This situation is clouded by the occurence of mycotoxins (such as aflatoxin), nitrosamine and other hepatoxins that are composed of their popular food.

4.4 Chemical hepatocarcinogenesis. Aflatoxins (a group of closely related toxic metabolites produced by certain strains of the Aspergillus fungus) causes carcinoma of the liver in animals. Aflatoxin causes liver cancer in rainbow trout raised on dry food contaminated with Aspergillus (Lutwick, 1979). Estimated minimum aflatoxin intake from foods (corn, beans, peanuts, and sorghum) in areas of Africa and the crude liver cancer rate are shown in Table 4 and these data demonstrate that an increased intake of aflatoxin is associated with hepatoma. There are no reports of a low frequency of hepatoma in areas of high aflatoxin exposure to counterbalance these data. Aflatoxin may act primarily as an immunosuppressive agent causing an increase in hepatitis B virus. To prevent HCC we must solve the major economic problem of changing farming and crop storage technique and food consumption by populations likely to be exposed to aflatoxin.

Table 4 Aflatoxin ingestion and hepatoma incidence

Country	Locale	Aflatoxin intake (ng/kg/day)	Hepatoma rate (per 10^5/year)
Mozambique	Inhambane	222.4	13.0
Swaziland	Lowveld	43.1	9.2
Thailand	Ratburi	45	6.0
Swaziland	Lebombo	15.4	4.3
Kenya	Low altitude	10.0	4.0
Swaziland	Midveld	8.9	3.8
Kenya	Mid altitude	5.9	2.5
Swaziland	Highveld	5.1	2.2
Thailand	Songkla	5.0	2.0
Kenya	High altitude	3.5	1.2

From: Lincell, C.A., and Peers, F.G. 1977.

4.5 Liver fluke infestation. Liver fluke infestation is common in the Northeastern part of Thailand where people consume raw fish. Malignant change of the liver and biliary passages is the most important complication (Viranuvatti, 1984). It is one of the cause of parasitic cirrhosis (due to Schistosoma japonicum) that play the important cause of HCC. HCC has been reported to schistosomiasis, while cause without schistosomiasis (8.5%) had HCC (Nakashima, 1987). Thus, most HCC victims with schistosomiasis probably had hepatitis B virus infection at one time. Nowaday, there is no conclusive evidence as to whether or not schistosomal infection plays a direct role in hepatocarcinogenesis.

4.6 Chronic viral hepatitis infection Although many factors many have a role in this disease, it appears that hepatitis virus (HBV) plays a major role in the pathogenesis of most HCC studied (Beasley, 1991, Hoofnagle, 1987, Robinson, 1986, Shimotohno, 1993). The etiological relationship between hepatitis B virus and HCC were advanced by the demonstration of a human tumor cell line that has HBV DNA integrated into the cellular genome, which replicates hepatitis antigen (HBs Ag). In addition studies with the newly discovered human hepatitis C virus (HCV), a major cause of chronic liver disease, have suggested that this virus also may play an important role in the development of HCC (Beasley, 1991). It has been noted that there is a significantly high incidence HCC in HBs Ag carrier. (Nakashima, 1987). A high percentage of patients with HCC can be shown to have chronic hepatitis B virus infection. Thus, hepatitis B virus proteins are present in the livers of most patients with HCC from areas endemic for hepatitis B virus and HCC, and they are also found in the livers of many patients from low incidence areas. In high incidence rate of HCC, virtually 100% of adults have serological evidence of hepatitis B virus infection and 10% to 15% are chronic HBsAg carriers; in these same areas, HCC occurs at a rate of 20 to 150 per 100,000 a year. In contrast, in the low incidence rate, only 5% to15% of adults have a serological evidence of hepatitis B virus infection and less than 1% are carriers, while the rate of HCC ranges from 1 to 5 per 100,000 a year (Di Bisceglie, 1988, Tao, 1985, Wen, 1985).

In conclusion, among all of the risk factor correlate to HCC, the two most important factors seem to be hepatitis B virus (HBV) and the aflatoxin.

5. Early detection and diagnosis of the HCC

Thus, it has long been known that HCC has related to chronic hepatitis and cirrhosis, this high risk patients must be follow - up to check HCC that may lead to earlier diagnosis (Sheu, 1985). HCC is difficult to detect in its early stage because the symtoms always show in the advanced stage (Okuda, 1986). In

the past decade, screening and regular follow - up in high risk population with sensitive assays of serum alpha - fetoprotein (AFP) levels have been used for early detection of this cancer. Thus, even in advanced HCC, 10% to 15% of patients still had normal AFP levels (Sheu, 1985), AFP surveillance is not sensitive enough in early detection of this cancer. Ultrasonography (US) was the new method in early detection of HCC. It was found to be effective in finding mass lesions in the liver (Sheu, 1985). As real - time US became popular, small HCC came to be found either by chance or intention in patients who have no complicants or signs attributable to HCC (Sheu, 1985, Shinagawa, 1984).

5.1 Signs and symptoms of HCC Most patients complain of right upper quadrant pain or distention and weight loss. The pain is usually dull or aching, but it can be acute and frequently radiates to the right shoulder. Fatique and loss of appetite are common, and unexplained fever may occur. Patients may present with hepatic decompensation and have ascites variceal bleeding, jaundice, or encephalopathy. The findings of firm nodular hepatomegaly and an arterial bruit, combined with a hepatic rub, strongly suggest HCC in an advanced stage. Earlier stages may have hepatomegaly only or have no specific findings (Vincent, 1989).

5.2 Tumor Markers in early diagnosis. The presence of AFP in the serum of patients with HCC has led to its use as a screening method in high-risk populations. Elevated AFP, unfortunately, is not specific for the diagnosis of HCC, and the histologic confirmation is essential. In high risk patients having chronic HBV or cirrhosis, ultrasound and AFP monitoring may lead to earlier diagnoses (Sheu, 1985, Shinagawa, 1984).

In conclusion, the finding of an upper abdominal mass in a high-risk patient, who is HBV positive or who lives in an indigenous area for HCC, should prompt an AFP test, and followed rapidly by an ultrasound. All of the diagnostic method may used to evaluated of HCC for the best result to detected the cancer.

6. Prognosis and Treatment of the HCC

Surgery, radiation, and chemotherapy are common modularity for the treatment of cancer. For HCC these methods fail to provide complete cure (Dvorak, 1991). The reason are that; first, because most irresectable structure , second, it already spread into vital (Novell, 1989). However, surgical resection remains the undisputed treatment of choice in early HCC, curative excision of minute tumor is a few in spite of a very few resection rates from 27% to less than 1% of large tumor (more than 5 cm) (Maraj, 1988, Okuda, 1984, Takayasu, 1987). Transplantation has been performed in patients with unresectable tumor apparently confined to the liver, the long - term survival is poor compared with that for benign conditions due to subsequent growth of occult metastasis (Hobbs, 1987). The prognosis of patients with an irresectable tumor is bleak, survival for more than 6 months being exceptional (Takayasu, 1987). Many tumors have already metastasized by the time of diagnosis. Therefore, although the primary tumor can be removed, metastasis, which tend to be multiple and widespread, do not lend themselves to surgical excision (Dvorak, 1991).

Radiation is helpful for the treatment of localized tumors but cancers vary widely in their sensitivity, and radiation is not generally useful for metastatic stage (Dvorak, 1991). Chemotherapy, is useful as an adjunct, in many causes affords only palliation, not cure (Ihde, 1985).

However, even if radiation and chemotherapy were more widely effective against tumors, they would not be ideal treatments because of the severe morbidity often associated with their use. Because of their relatively low therapeutic ratios, they exert severe toxic effects on many normal tissues when used at the levels that are neccessary to kill tumor cells (Dvorak, 1991).

Early results of direct percutaneous injection of alcohol (Livraghi, 1988) into the tumor tissue under ultrasound guidance appear impressive, but to date the

technique has only been tried on small asymptomatic tumors which somehow tumor unfit for surgery (Livraghi, 1988).

Local ablation such as cryotherapy have a limited role at present in the treatment of superficial tumor deposits at laparotomy (Ravikumar, 1987).

It is not surprising, therefore, that investigators have looked for entirely new approaches to tumor therapy that are more specifically lethal for cancer cells and less toxic for normal cells. The development of monoclonal antibody technology has stimulated this expectation to the "magic bullet", reawakening the hope for that may be one method that able to destroy cancer cells specifically without harming normal cells(Byer, 1988, FitzGerald, 1989, Foon, F.A.,1985, Hellstorm, 1989, Schlom, 1986, Sell, 1985, Sharkey, 1990, Zalutsky, 1989). The details of immunotherapy will be discussed in the next section.

Monoclonal antibody

1. Introduction

Antibodies are a complex proteins that are produced and secreted by B lymphocytes, a type of white blood cell that forms an important component of the body's immune system. Antibodies are detected in many animal species as part of the specific immune response to foreign substances, namely antigens. The specificity of antibody corresponding to antigen epitope, is an advantage tool for analysing the cells, its function, the biochemical agents and medical applications. Each B - cell produces only one single kind of antibody that recognized only one specific epitope monoclonal antibody. However, in natural immune response, there are a virtually unlimited number of different lymphocytes and each of them proliferates rapidly when it detects its corresponding antigen. In the past decade, classic antibodies preparation gained by injecting antigen to animal namely the

polyclonal antibodies. It is aggregated in serum which generates many different that react with the most antigenic sites of the stimulated antigens. However, the supply of this polyclonal antibodies is limited by means of quality control, and contamination of undesirable antibodies, different from monoclonal antibody which recognized a single epitope.

In 1975 Cesar Milstein and Grorges Kohler of the British Medical Research Council Laboratory of Molecular Biology in Cambridge established hybridoma technique and on a way around this technique solved the problems, of limitation that found with classic polyclonal Abs naturally (Kohler, and Milstein, 1975). They fused myeloma tumor cells from a mouse, which have the capacity to grow indefinitely in culture, with mouse B lymphocytes, which have desire memories. This hybrid cell, namely hybridoma cells composed with just the right qualities of each parent. Hybridomas grow in culture and produced virtually unlimited quantities of monoclonal antibodies; MAbs (so named because it produced single specific antibody and drived from a single hybridoma. MAbs introduce many variable application in research because of their great specificity. It is an ideal tools for separating and purifying specific proteins, other cellular components, and tasks that were formerly difficult and impossible with conventional techniques (Olson, 1986). MAbs open the excellent role in understanding the malignant and normal cells antigens, their functions, and the nature of maturity cancer by means of occurrance of the antigens. Clinically, MAbs could be used widely through the purpose for diagnosis, monitoring the stage of disease and therapy of cancer.

2. Molecular structure of antibody

IgG antibodies have Y - like shape and consists of two heavy and two light chains (see Figure 10). The two heavy - chain polypeptides in the Y structure are identical and are approximately 55,000 daltons. The two light chains are also identical and are about 25,000 daltons. Each arm of Fab domain contains a site that

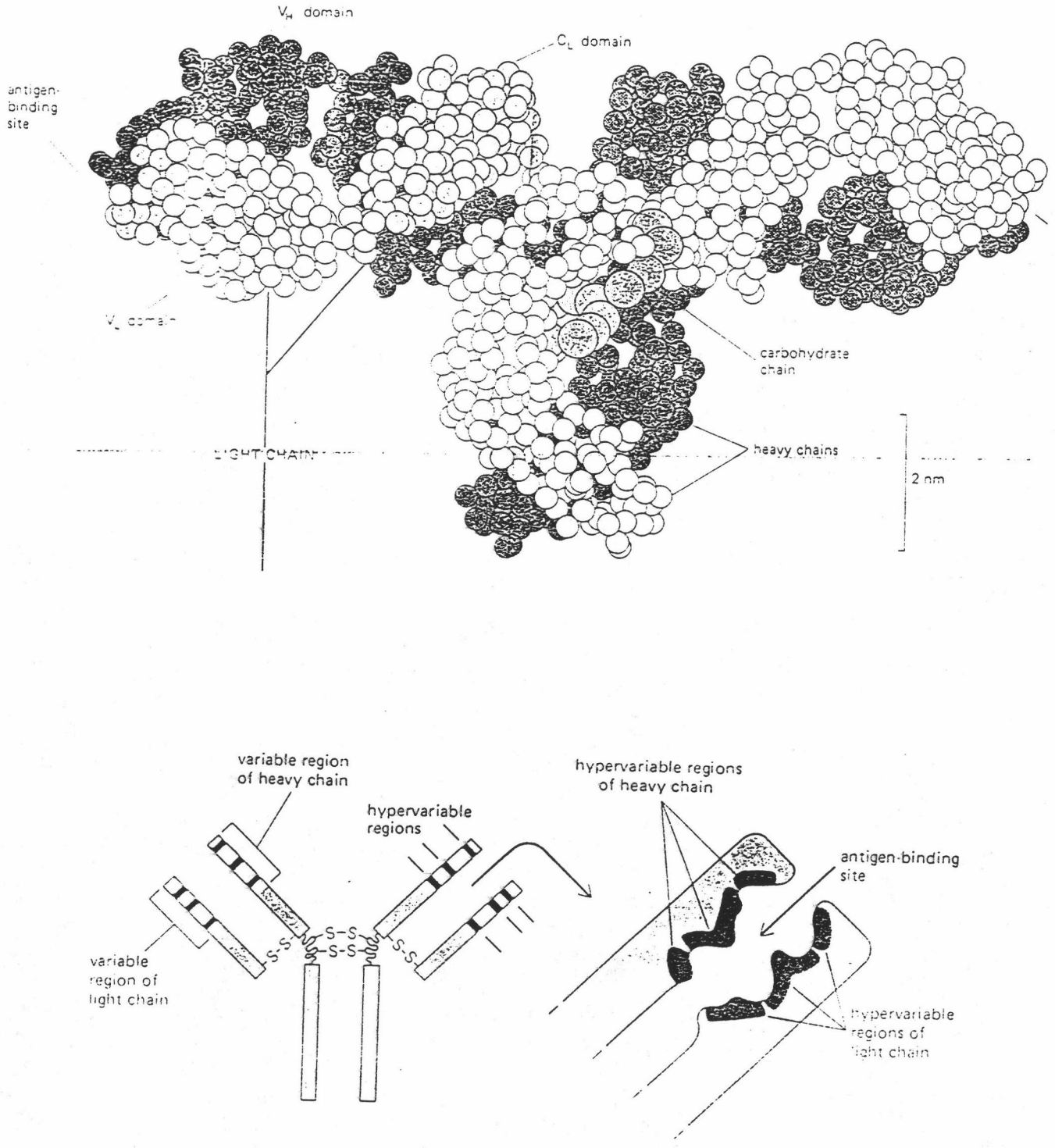

Figure 10. The structure of antibody.

can bind to an antigen which is called hypervariable region. While the Fc domain contains a site for effector cell or second Ab binding. The subunits are linked together covalently through disulphide bonds between the heavy chains. The IgG light chains have composed with about 220 amino acids long and can be divided into variable and constant regions, while heavy chains are about 440 amino acids long and are also divided into one variable region and three constant regions (see figure 10). The different sequences of the IgG; IgG1, IgG2a, IgG2b and IgG3, heavy chains have also shown in different subclass (Harlow, 1988).

3. Anti - hepatoma MAbs

The anti - hepatoma MAbs (Laohathai, 1985) was produced by murine system. Myeloma cells line was NS-I. Splenocytes were that gained from BALB/C mice immunized with Thai human HCC cell lines (Laohathai, 1985).

3.1 The characters of anti - hepatoma MAbs

3.1.1 Class and subclass. Thus, the anti - hepatoma MAbs was produced on the purpose to use for cancer immunotherapy finally. Most of all selected MAbs are IgG2a subclass. Murine MAbs of the IgM and IgG3 subclass may be most potent to induce human complement activation (Dillman, 1989) while the mouse immunoglobulin subclass IgG2a and IgG3 are best to induce antibody dependent cellular cytotoxicity with human effector cells (Dillman, 1989, Hellstorm I, 1988, Ortaldo,1987).

There are many reports support that the tumor growth could be suppressed and killed regard to the specific isotype of MAb (Herlyn, 1982, 1985). Tumoricidal effect which have been attempted with human tumor barring nude mice and the IgG2a isotype significantly inhibited growth of human tumor, while other isotypes showed no effect (Fukuda, 1988, Herlyn, 1982, 1985).

3.1.2 Classification of anti - hepatocellular carcinoma MAbs.

Some of the applied anti - hepatoma monoclonal antibodies (anti- hep MAbs) in this panel were found to react with over 29 human malignant cell lines and cancer tissues (Laohathai, 1985, in press). These MAbs were classified into three groups, according to their reaction to HCC, cancer cell lines and the fetal and new born liver cells as described below; (Table 5)

Type I; This MAbs have potential in recognizing oncofetal development antigen (ODA). Beside of reaction to surface membrane antigens of many HCC and other cancer, it also recognized new born and fetal liver but not the adult liver. Those were used in this study are #16, #20, #27, #40, #43, #44, #57, #58, and #75.

Type II; This group of MAbs have potential in recognizing tumor associated antigen (TAA). It react with many HCC and others cancer cell lines but not the new born and fetal liver. Those were group in this type are #36, #54, and #78.

Type III; This group of MAbs have potential in recognizing tumor specific antigen (TSA). It react only with HCC, namely anti-hepatoma MAb #100.

3.2 The production of anti - hepatoma MAbs

There are two distinct methods in producing MAb from the hybridoma line (Zola, 1987). One is cultured in tissue culture flask or jar. This can escape from the contamination of microorganisms, but it causes a high cost while gain only 5 ml of MAb from 1000ml of culture medium. The method is the method that expand the hybridoma in the abdominal cavity of the same species of the myeloma. It accumulated in ascites from which has to be purified by affinity chromatography column of rough separation by ammonium sulfate precipitation. The purity IgG2a was stabilized at - 70 c in phosphate buffer with thimerosal. The details of preparation was described in the material and methods.

Table 5. Classification of anti-hepatoma MAbs.

	Anti-hepatoma MAbs												
	ODA									TAA			TSA
Cell line	#16	#20	#27	#40	#43	#44	#57	#58	#75	#36	#54	#78	#100
HCC: S102	3+	3+	4+	2	3	2+	3+	4+	2+	3+	3+	3+	3+
R12	3+	3+	3+	1	2	3+	3+	3+	3+	2	4	1	0
HepG2	3+	3+	3	1+	1+	3+	3+	3+	2+	1	3+	0	2+
GI cancer:													
pancrease CA (HS766T)	1+	1+	2	0	0	0	1	2	0	0	2	0	0
colon CA (SW116RT)	3+	3+	2+	2	2	3+	3+	2+	2+	1	3+	3	0
gastric CA (Kato-3)	2+	1+	2	0	0	1	2+	2	1+	0	3+	0	0
Others CA:													
lung CA (PC-10)	1+	2+	3	2+	3	1+	1+	2	1+	3	4+	3	0
melanoma (Maranski)	3+	3+	3	0	1+	1+	3+	2	2+	0	3+	1+	0
sarcoma (U-205)	3+	3+	0	0	0	2+	3+	0	2+	0	0	2	0
Oncofetal liver:													
new born liver (NL1798)	3+	3+	3	1	0	1	2+	1+	1	0	0	0	0
fetal liver (NL 1813)	3+	3+	3+	0	1	2+	3+	3+	2+	0	0	0	0
ODA													

: By ELISA technique

Anti-tumor activity of monoclonal antibody

The understanding on this activity is not yet completely understood. At this moment the known process could be described as follow;

1. Antibody-dependent cellular cytotoxicity (ADCC): Tumor target cells coated with IgG antibody can be destroyed following interaction with several types of effector cells that bearing receptors for the Fc portion of these antibodies (Levy, 1979). ADCC effector cell populations include granulocytes, monocytes-macrophages (Herlyn, 1985), lymphocytes-NK (natural killer) cells (Dunk, 1987, Hata, 1991) and polymorphonuclear leucocyte (PMN) (Gresham, 1988). The actual mechanism of tumor cell lysis by ADCC is not known (Stites, 1984).

2. Complement-mediated cytotoxicity (CMC): The interaction of the tumor cell with some of the antibodies will activate the complement system, leading to the lesions in the cell membrane and eventual lysis. In general, variation in susceptibility to lysis may be influenced by 1) the ability of the tumor cell to induce high-affinity cytotoxic antibodies both the IgM and IgG; 2) the distribution and density of the antigens present on the cell membrane; 3) the ability of the tumor cell to repair complement-mediated lesions in its membrane; or 4) the ability of the antibody to reach the appropriate cell surface antigens. There is some report that have also shown that complement is not require for this tumor cell destruction (Shulz, 1983). In recently, this mechanism is thought that is less effective tumoricidal effect.

3. Direct effect of MAb itself to the vital epitope on cell surface of tumor cell such as growth factor receptors (Sato, 1989) or necessary receptor of cellular attachment and tumor rejecting antigen (TRA) (Shimizu, 1991, Maki, 1990).

4. Modified MAb: The specificity of MAbs was widly excepted without question this instrùcture the endeavor to be used MAbs as a targeting vehicle by conjugating with radioactive isotopes (Epenetos, 1984), toxins (Tjandra, 1988), or chemotherapeutic agents to the tumor cells (Corvalan, 1988).

Monoclonal antibody and cancer immunotherapy

MAbs provide an important tool in finding tumor markers. MAbs by the nature of picking out a single antigenic epitope on a cell surface antigen, provided a key (i) functions in giving an easy diagnosis such the immunohistochemistry and immunoassays (Kosmas, 1989) (ii) with radioactive isotopes for *in vivo* localization studies by external body immunoscintigraphy (Kosmas, 1989) and (iii) targetted therapy, immunotherapy, by conjugating with radioactive isotopes (Epenetos, 1984, Tjandra, 1988), toxins (Tjandra, 1988), cytotoxic chemotherapeutic agents (Corvalan, 1988). Recently conjugated with non-mammalian enzymes for tumor site activation of non-toxic prodrugs (Bagshawe, 1989, Novell, 1989, Senter, 1990) and drug (Tjandra, 1988). Eventhough the successfulness in using for the diagnosis were widely, the benefit of MAbs in immunotherapy is on the process to discovery. Despite tremendous advances over the past several decades, the current therapies - surgery, radiation, and chemotherapy fail to cure many of the most important human cancers (Hu C-P, 1986). Therefore, entirely new approaches to overcome the tumor that are more specific, lethal for cancer cells and less toxic to normal cells, are looking for the establishment of MAb technology is one that promissing the possibility in having this magic bullet that destroy cancer cells specifically by without any harm to normal cells (Byers, 1988, Foon, 1985, FitzGerald, 1989, FitzGerald, 1989, Hellstorm, 1989, Osborn, 1990, Schlom, 1986, Sell, 1985, Sharkey, 1990, Zalutsky MR 1989). In 1980, the first description of treatment with a MAb was reported (Nadler, 1980). At that time MAbs are highly regarded as candidates for tumor therapy because of the exquisite specificity to desire antigen but the understanding about immune mechanism, the specificity of MAbs itselfs, and the tumor Ag were still immature to carry out the complete success as the expectation. There have been many reports in which MAbs have been used as

carriers of toxic (FitzGerald, 1989) or chemotherapeutic agent (Baldwin, 1985) to target them to tumor masses (Baldwin, 1985, Matsui, 1985) or in chimeric antibody form. An alternative approach for immunotherapy would be to use The MAbs alone, which are able to destroy the tumor cell has to bind the tumor rejecting antigens and negative tumor growth receptors for preferentially expressed on tumor cells (Sato, 1989), or use the MAbs that have potential in offering the activation to the antibody dependent cells such as T cells and macrophage mediated the cytotoxicity, ADCC or ADMC forms (Fukuda, 1988, Hellstorm, 1986, Lieberman, 1991, Smans, 1991) or complement dependent cytotoxicity (Hellstorm, 1986)

Antibody therapy for cancer in humans has been attempted extensively. (Baldwin, 1985, Dillman, 1989, Schlon, 1986). Preliminary the interesting results were demonstrated trancient reductions with melanoma (Houghton, 1985, Oldham, 1984), neuroblastoma (Kenshed, 1986), gastrointestinal cancer (Dillman, 1989, Sears, 1985) and hepatoma. (Fukuda, 1988, Order, 1985). Lymphoma and leukemia trials showed sharp drops in cell counts, but therapeutic effects last for only a few hours (Denardo, 1988, Dillman, 1984, Foon, 1984, Glenn, 1985, Meeker, 1985). The MAb in vivo experiments demonstrated clearly prolonged the survival of athymic mice which had been inoculated with a human liver carcinoma cell line. In addition, the MAb significantly supressed the human hepatoma line transplanted S.C. into nude mice (Fukuda, 1988). Moreover, MAbs were combined with toxin (Blakey, 1988), drug (Embleton, 1986, Patt, 1993) and radioisotope (Kosmas, 1990, Markham, 1986, Munz, 1986, Zhang, 1991) for enhanced the effect to imaging diagnosis and therapy of cancers. This attempts presented a set of difficulties that is different from those associated with surgery, radiation or chemotherapy (Dvorak, 1991).

The major problem is that MAbs these used in clinical studies were murine MAbs. The lack of efficiency in most of these applications has been attributed to

the innate " foreignness" of the mouse protein, which has oftens caused adverse reactions in the host, such as allergic response, hepatic distunctions and immune complex formation in the kidney (Larrick, 1984, Larrick, 1986, Thompson, 1988). In earlier studies bronchospasm was the cheif side effect noted with infusion of mouse- based antibodies, and urticaria was also common. Repeated challenge sometimes lead to the development of anemia (Dillman, 1986). However this side effect are easily control, the big problem of using mouse MAb for clinical trails is the occurrance of human anti-mouse antibody (HAMA) which limited the repeated dose in completing the treatment. Fortunately, this problem would be smartly solved with the development of chimeric antibody technique (Koda, 1990). Another endeavor also has to be put on the correct specificity of antibody, the recognition to the tumor rejecting antigens or the antigen that stimulate the negative process of cell in living.