CHAPTER V

DISCUSSION

5.1 The effect of IFN-α on the growth of hepatoma cell lines

The effect of IFNs on the growth of hepatoma cell lines was reported oppositly. It has potential to depend on cell lines (Desmyter *et al.*, 1985; Hinoue *et al.*, 1985; Motoo, Hill and Osther, 1985; Duke *et al.*, 1986; Ilan *et al.*, 1988). Most of the reports showed the variable of IFN-sensitive hepatoma cell lines. The PLC/PRF/5 hepatoma cell line is the sensitive one. In the present study, IFN-α suppressed the growth of all three hepatoma cell lines, S102, R12, and HepG2. The inhibitory effect on R12 and HepG2 were less sensitive than S102 hepatoma cell line. The difference in sensitivity to IFN-α treatment among these three hepatoma cell lines seems to depend on the grade of differentiation. The heterogeneity of differentiation of cell may alternatively explain the results level of mutation of cells. But the mutation that allowed the response to IFN-α treatment is the interesting question which needs further investigation.

The inhibitory effect of IFN-α treatment on the cell growth persisted as long as IFN-α was present in the

medium. The IFN-resisted cells were still able to proliferate after IFN-α had been withdrawn but multiplicattion rate was slower than the control. This agreed with the result of Ilan *et al.*(1988), which tested on PLC/PRF/5 cells as a model. The administration of IFN-α as a single agent for immunotherapy of HCC seems to be avaiable in suppressing the tumor growth but it is necessary to give continuously longer period. Beside this several questions are remained to be investigated further. Especially, the maintaining dose and the time of continuing should be awared.

5.2 The modified method for immunogold labelling by immunoelectron microscopy technique

One of the major aim in the study was to follow the change of tumor surface antigens. It is necessary to have the techniques for preparation of immunolabelling that preserved the whole of cellular structure and cell organelles without destroying antigen. For immunoreaction, the model cells, viable hepatoma cells, were kept with antibody (anti-hep MAb) until the reaction was finished. After the immunogold labelling step, the hepatoma cells were fixed with 1% glutaraldehyde, which was found as the most adequate for preservation of surface antigen of hepatoma cells. Glutaraldehyde has superior cross-linking properties, which make an excellent fixative for preservation of ultrastructure but the excessive cross-linking may

reduced antigenicity and antibody access to antigenic site (Polak and Varndell, 1984). After postosmication, the specimens were processed for ultrastructural examination. This process is compatible with Polli *et al.*(1987). There is major disadvantage to pre-embed immunoelectron specimen. This method could not eliminate the poor morphologic preservation of detailed cytoplasmic organelles. However, antibodies easily penetrate or bind to the cells resulting in high efficacy of immunolabelling.

The other key factors in preparing the ultrastructural labelling included the titer and incubation time of primary antibody, washing procedure, dilution of gold-conjugated solution and size of gold partilces. In this study, the primary Ab, anti-hep MAb was incubated at 4°C overnight in order to obtain high labelling of first Ab which still used only one kind of MAbs. When sensitivity of MAbs was increased by using mixed form, the incubation time should be arranged in shorter time than present study. In term of the dilution of the gold-conjugated solution, Polak *et al* (1984) suggested that 1:15 or 1:20 diluton provided excess amounts of gold for labelling. In this study, the dilution of 1:10 and 1:50 were compared. The results showed that 1:10 dilution of gold-conjugated solution gave better staining.

5.3 The process of lethal effect and the alterations of surface antigen caused by IFN-α treatment

In recent clinical trial with interferon therapy of hepatocellular carcinoma, Lai *et al.* (1990) reported IFN-α induced significant tumor regression. In this study, the observation of viability and cell growth inhibition, it was proved that IFN-α has been significantly destructive on the hepatoma cell. It has clearly shown changes of cytoplasm and nucleus. The inclusion body like-particles in cytoplasm and segmented nuclei were reproducible changes. It is importance to characterize the mechanism involved at the ultrastructural levels in order to understand the process of these changes in relation to the pattern alteration of surface antigen. This investigation was performed at level of TEM

S102 cell line, a human cell line originating from Thai hepatoma, generally demonstrated a cellular morphology similar to that of normal hepatocyte, but with smaller amount of organelles. Thakerngpol *et al.*(1983) reported that the amount of cytoplasmic organelles was related with the morphological grade of differentiation. Cytoplasmic organelles were decreased in the well differentiated tumor but moderately decreased in the moderately differentiated type. Hepatoma cells of poorly differentiated type had few cytoplasmic organelles. This agreed with the study of Backman *et al.*(1991), $HepG_2$ cell line, the other human

cell line, which is dedifferentiated type expressed mainly as decreased in number of organelles.

Ultrastructural appearence of S102 hepatoma cells after IFN treatment revealed time relating specific changes. The alteration of swollen mitochondria was detected within 6 hours after treatment. Subsequently, 1 day after IFN-α treatment demonstrated significant alteration of cytoplasmic organelles such as slightly increased in number of RER and electron translucent non-membrane bounded structures including swollen mitochondria. The increasing of RER suggested the condition of increased in cellular metabolism. Electron translucent structures have not been reported before, the explaination needed further investigation. According to the time of occurrence and the location in cytoplasm, it is possible to be an inclusion body-like particle which observed by light microscopy. However, in this study, it is not explainable whether this changes had any relation to induce nuclei-segmented changes and cell death.

Glycogens in general hepatoma cells were commonly seen (Tanikawa, 1985). However, 3 days IFN-treated S102 cells interestingly showed increasing in glycogens with clusters. Sakura *et al.*(1989) also found this evidence after treated IFN-α on a colon carcinoma cell line. Functionally, the glycogen may relate to the process of the liberation of cytotoxic agent or may be an evidence

that occurred in disturbance of cell division.

The segmented nuclei was a significant alteration after IFN-α treatment which might reflect cell damage. Iwasaka *et al.*(1987) studied the treatment of cervical cancer cell line with IFN-β showing multilobular nuclei and occasional multinucleation and concluded that it is an abnormal cell division due to the cytostatic action of IFN.

The ultrastructure of IFN-α resisted hepatoma cells, was predominantly found with hypertrophic SER. This may reflect the drug resistant mechanism of cells (Trump, 1975). In addition, dilated RER may indicate that these cells were returning to normal structure as observation in normal hepatocytes.

It has been shown that IFN-α enhanced the expression of surface antigen such as HLA (Balkwill *et al.*, 1987) and tumor-associated antigen (Greiner *et al.*, 1984). Our data also agreed with these mention. The antigen expression was significant on 3 day after IFN treatment. By some means, IFN-α enhanced the presentation of antigen on tumor cell membrane. The Mab used in this study was anti-hepatoma #27 MAb, which was proved to have tumoricidal effect. For S102 hepatoma cells and anti-hep #27 MAb, IFN-α may increased the host immune response system.

The general morphological type of cell death can divide into two types, apoptosis and necrosis. Apoptosis is considered to be an active process of gene-directed cellular destruction. Recently, there were several reports that suggested apoptosis could be induced by irradiation (Sellins and Cohen, 1987; Stephens *et al.*,1991), hyperthermia (Takano, Harmon and Kerr,1991), toxins (Morimoto and Bonaavida, 1992) or cytokine (Larrick and Wright, 1990). Kerr (1972) reported the formation of apoptotic bodies involved marked condensation of both nucleus and cytoplasm. The initial morphological event was identified that cell is already condensed and the nuclear chromatin aggregated in dense mass beneath the nuclear envelope. Fully developed apoptotic bodies show closely packed organelles. In this study, nuclear changes with the pattern of chromatin aggregattion at nuclear envelope was commonly seen, however, packing organelle was not found. This result is uncertain to conclude that IFN-α treatment of hepatoma cells induced apoptosis. It would be more certain if it is proved at the DNA level.