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AJCHARA AKSOMBOON : THE SYNERGISTIC CYTOTOXICITY OF TNF- α AND PHA STIMULATED PBMC SUPERNATANT AND THEIR ROLES ON CYCLIN D1, E, A; p16 and p21 EXPRESSIONS ON CHOLANGIOCARCINOMA CELL LINE. THESIS ADVISORS : MOLVIBHA VONGSAKUL, Ph.D., SITAYA SIRISINHA, D.M.D., Ph.D., SUKATHIDA UBOL, Ph.D., p123. ISBN 974-662-417-2

Immune surveillance against tumor seems to be the most complicated interaction between host immune responses and tumor natures. The delicate interactions result in either the suppression of tumor growth or the defeat of the host and subsequent tumor growth. The characteristics of tumors which are different among tumors themselves play the important roles; one characteristic is the specific cell cycles. All of the cell cycle machineries i.e., cyclin, cyclin dependent kinase (CDK), and cyclin dependent kinase inhibitors (CDIs) which function in certain phases of cell cycle (G1, S, G2 and M) seem to be distinct for each tumor. Furthermore, these cell cycle machineries are different from their normal counterparts. Apart from tumor characteristics, effectiveness of host immune responses is the other actor in immune surveillance against tumors. Both innate and adaptive immune responses show important roles but at different levels of immune interactions. Cellular immune responses play roles by means of both cell-cell interactions and by releasing various soluble mediators. Thus, *in vivo* immune responses occur as consequences. There are interactions (synergistic or antagonistic) among various factor, the results of which benefit either the tumor or the host.

This study focuses on examining the cell cycle phases of tumors cells, cholangiocarcinoma cell line on their cytotoxic susceptibility to either one soluble mediators, rhTNF- α , or the combination of rhTNF- α and other soluble mediators in PHA-P stimulated peripheral blood mononuclear cells. The presence of cyclins (D1, E and A) and CDIs (p16 and p21) was detected from tumors cell extracts by observing specific monoclonal antibodies stained by western immunoblotting. The cell cycle phases were observed by propidium iodide staining for flow cytometry, while the cytotoxic effects were studied by Trypan Blue dye exclusion, DAPI staining and DNA ladder. This study shows the findings as follows. Firstly, cholangiocarcinoma cell cycle phase of G1/S transition, S phase, or both are the most susceptible to cytotoxic treatment. Secondly, there is synergistic effect of TNF- α and PHA-P supernatant on the cytotoxicity to a certain degree. Thirdly, the observed synergistic cytotoxicity is neutralized by monoclonal antibody to IL-2, IL-12, and IFN- γ .

These findings may suggest that tumor cells' responses to the overall immune interactions are likely to be stage dependent; however, the possible mechanisms are not defined in this study but are nevertheless interesting.