CHAPTER I

INTRODUCTION

Cholangiocarcinoma

Cholangiocarcinoma is a highly malignant epithelial neoplasm that arises within the intrahepatic and extrahepatic biliary tract (Khan S.A. 2005). Cholangiocarcinoma has become a serious threat to public health due to increasing worldwide incidence and mortality rates associated with lack of early detection and limited therapeutic options. At diagnosis, most patients are presented with an already advanced disease, possibly with undetected metastasis, resulting in less than 12 months survival. Currently the only effective treatment for CCA is operation. Even for those with operable tumors, the recurrence rate was extremely high, with a 5-year survival rate of less than 5% (Shaib YH. 2004, Khan SA. 2005). The annual age-standardized incidence rate in Europe is less than 1.5 cases per 100,000 population. Unfortunately, Thailand has the highest incidence rate of this type of cancer in the world (Parkin DM. 2002) (Figure 1). The mechanism of CCA initiation is not well understood. However, the pathogenesis of this disease has been strongly associated with chronic inflammation and cellular injury within bile ducts, as well as partial obstruction of bile flow, manifested by various high-risk conditions such as PSC (primary *Sclerosing chloangitis*), hepatolithiasis and infestation by liver fluke (*Ophisthorchis viverrini* or *Clonorchis sinensis*) (De Groen P.C. 1999). Moreover, dysplasia is an intermediate step in the development of biliary tract cancer. Recently it has been shown that cytokines such as TNF-alpha, released in the biliary microenvironment during the process of inflammation, are responsible for the malignant transformation (Fava G. 2007).

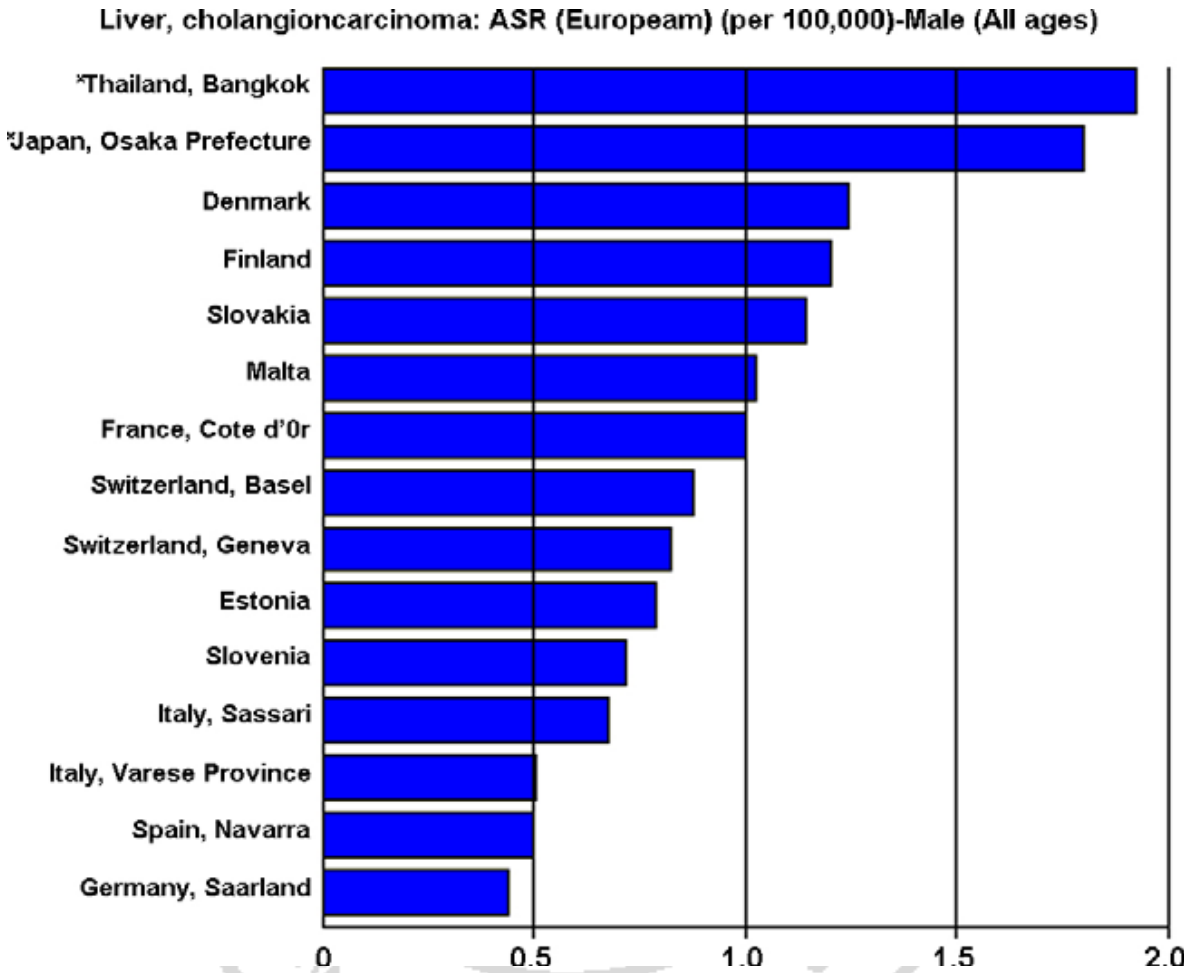

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Figure 1 Age-standardized incidence rates in European and Asian populations by gender.

Apoptosis

Certain gene products that are released during development, or at some specific time of the life cycle, may cause selective cell death. In the course of an organ's lifespan, some cells die either because the organ is returned to a lower level of function, or because of aging (atrophy). Cells respond by apoptosis or programmed cell death to endogenous triggers such as the cytokines of the tumor necrosis factor-alpha (TNF-alpha) family. If DNA damage beyond repair causes cell death, but the cells don't die, the damaged DNA may be replicated and cause cancer (Cross M. 1991).

Apoptosis is a multi-step cascade regulated by proteins that promote or counteract cell death. The induction of apoptosis is a common event for different classes of anti-cancer agents, and it is believed to be one of the main cellular mechanisms by which chemotherapy and radiation therapy kill cancer cells (Kerr JF. 1994). The number of agents that cause apoptosis is large and still growing: for example EGF, FAS (APO I, CD 95) and TNF-alpha.

Apoptosis, through the death receptor ("extrinsic") pathway involves activation of the initiator caspase 8, a process which can be inhibited by FLIP. The mitochondria-mediated ("intrinsic") pathway is activated by different stimuli and conditions (only some of which are shown), leading to the release of pro-apoptotic factors from the mitochondria, and to the activation of caspase 9. This step can be inhibited by the anti-apoptotic members of the Bcl-2 family of apoptosis regulators. Active caspases 8 and 9 convert the pro-form of caspase 3 to an active form, which then initiates apoptotic cell death through activation of the other executioner caspases 6 and 7, along with the degradation of multiple death substrates. Active caspase 3 can be inhibited by IAP family members (Figure 2).

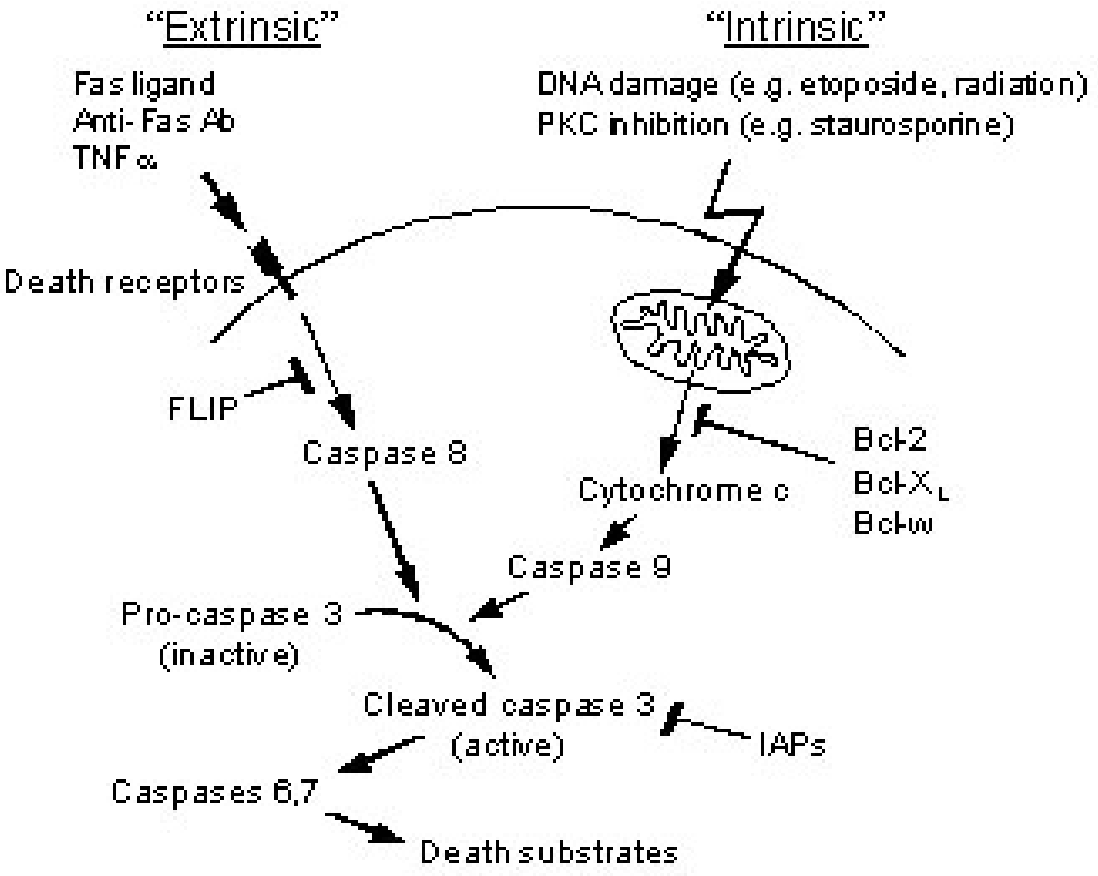

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Figure 2 Schematic overview of the major apoptosis pathways and their inhibitors. Only the most relevant steps and interactions within and between different pathways are depicted.

Tumor Necrosis Factor alpha (TNF-alpha)

Tumor necrosis factor alpha (TNF-alpha) is a potent cytokine (Tracey KJaC. 1993). It orchestrates many cellular responses, including inflammation and apoptosis (programmed cell death) (Chang, H.Y.a.Y. 2000). It is produced by many cell types, including macrophages, monocytes, lymphocytes, keratinocytes and fibroblasts, in response to inflammation, infection, injury and other environmental challenges. Lower expression is known for a variety of other cells, including fibroblasts, smooth muscle cells, and tumor cells (Bazzoni F BB. 1996). TNF-alpha is a dimer with a molecular weight of 40,000 (gel filtration) that drops to 18,000 after sodium dodecyl sulfate (SDS) gel filtration (Haranaka, K. 1985). The gene for TNF-alpha has been localized to the short arm of chromosome 6 (Wingfield P. 1987). The precursor mRNA encodes 233 amino acids, and contains 4 exons. TNF-alpha contains 233 amino acids and has an approximate molecular weight of 25.64 kDa. It has an extracellular C terminus, cleaved by ADAM17 to release a soluble 17 kDa product. There was notable correlation between mouse recombinant TNF-alpha (157 amino acids) and human recombinant TNF-alpha: 76% homology in the respective sequence (157 amino acids). Recombinant TNF injected in humans is rapidly cleared from the serum (half-life: 15-30 minutes) and is no longer detectable in the serum 12 hours after injection (Bazzoni F BB. 1996).

TNF-alpha plays a physiological role in host defense, inflammation, and cell differentiation, while it plays a pathological role in condition such as fever, cachexia, septic shock, rheumatoid arthritis and inflammatory bowel disease (Tracey KJ. 1987, Pisetsky DS. 2000). TNF-alpha acts through two receptors: tumor necrosis factor receptor-1 (TNFRI) and tumor necrosis factor receptor-2 (TNFRII) (Vandenabeele P DW. 1995). Both receptors have cysteine-rich extracelllular subdomains and in

addition, TNFRI has an intracellular region of about 80 amino acids, termed the death domain (DD). The cysteine-rich extracellular subdomains are thought to adopt tertiary folds required for intracellular clustering of DD motifs (Ashkenazi A DV. 1998). TNFRI is expressed constitutively in a broad spectrum of different cell types and has been shown to mediate most of the commonly known biological effects of TNF-alpha (Locksley RM KN. 2001). In contrast, expression of TNFRII, molecular weight of 75 kDa, seems to be modulated by various stimuli, and only a few cellular responses can be attributed exclusively to signaling via TNFRII. Mutations in both ligands and receptors of the TNF-alpha superfamily have been found in humans. These include mutations in TNFR, CD95/CD95L, RANK, ectodermal dysplasin (EDA), and CD40L. Missense mutations of TNFRI lead to autosomal dominant periodic fever syndrome that is characterized by unexplained episodes of fever and severe localized inflammation (McDermott MF. 1999). These missense mutations disrupt the conserved extracellular disulphide bonds, causing a decrease in the level of soluble TNFRI in the plasma of patients. Leukocytes from individuals with a Cys52Phe mutation in TNFRI express increased levels of membrane TNFRI and show reduced receptor cleavage after stimulation. The autoinflammatory phenotype results from impaired downregulation of membrane TNFRI and diminished shedding of potentially antagonistic soluble receptor. The existence of TNFRI-associated periodic syndromes (TRAPS) establishes an important class of TNFR mutations (Aganna E. 2001).

The recombinant TNF-alpha, however, causes severe local inflammations. Indeed, the name “tumor necrosis factor” emerged from the factor’s capacity to induce hemorrhagic necrosis in tumors and the killing of tumor cells by activated macrophages. The TNF-alpha was originally thought of as selective antitumor agents, but is now known to have a wider range of actions. In binding to their

receptor present on virtually all cells examined, they activate a large array of cellular genes, along with multiple signal-transduction pathways, kinases and transcription factors.

TNF-alpha and signalling pathway

TNFRI is an important member of the death receptor family that shares the capability of inducing apoptotic cell death (Gilbert LC. 2005, Misseri R. 2005, VandenBerghe T. 2004). TNFRI is widely studied because it is a dual-role receptor: besides apoptosis induction, it also has the ability to transduce cell survival signals (Thakar J. 2006, Gupta S.2002, MacEwan DJ. 2002). Although signaling pathways are already well-defined, the life-death signaling regulation is still poorly understood. The updated signaling pathways of TNFR are depicted in Figure 3. TNFRI signaling pathway was described to function as follows: upon binding of the homotrimer TNF-alpha, TNFRI trimerizes, and the silencer of death domain (SODD) protein is released (Takada H CN. 2003). TNFR-associated death domain (TRADD) binds to the death domain (DD) of TNFRI and recruits the adaptor protein, receptor interacting protein (RIP), TNFR-associated factor 2 (TRAF-2), and Fas-associated death domain (FADD) (Rath PC AB. 1999). In turn, these adaptor proteins recruit key molecules that are responsible for further intracellular signaling. When TNFRI signals apoptosis, FADD binds pro-caspase-8, which is subsequently activated. This activation initiates a protease cascade leading to apoptosis, also involving the mitochondria and caspases as key regulators (Degterev A BM. 2003). The ultimate event in this apoptotic signaling is the activation of endonucleases, like EndoG, resulting in DNA fragmentation. An alternative to that event is TNFRI signal survival, in which TRAF-2 is recruited to the complex that inhibits apoptosis via cytoplasmic inhibitor of apoptosis protein (cIAP). Occupancy of TNFRII results in direct recruitment of TRAF2, which in turn recruits TRAF1 (Baud V. 1999). TRAF2 plays a central

role in early events, common to TNFRI and TNFRII, that lead to IKK and MAPK (JNK and p38) activation (Natoli G CA. 1997). The major signaling event of TRAF-2 and RIP is the widely studied activation of nuclear factor kappa B (NF-κB) transcription factor via NF-κB–inducing kinase (NIK) and the inhibitor of κB kinase (IKK) complex (Baud V. 1999, Devin A CA. 2000). Both the NF-κB and cFos/cJun transcription factors induce the transcription of anti-apoptotic, proliferative, immunomodulatory, motility and inflammatory genes. NF-κB is the major survival factor in preventing TNF-alpha-induced apoptosis, and inhibition of this transcription factor may improve the efficacy of apoptosis-inducing cancer therapies (Devin A CA. 2000). NF-κB activation in many human malignancies is aberrant or constitutive, and its role in the regulation of apoptosis-proliferation balance in tumor cells indicates a role in oncogenesis (Dolcet X LD. 2005).

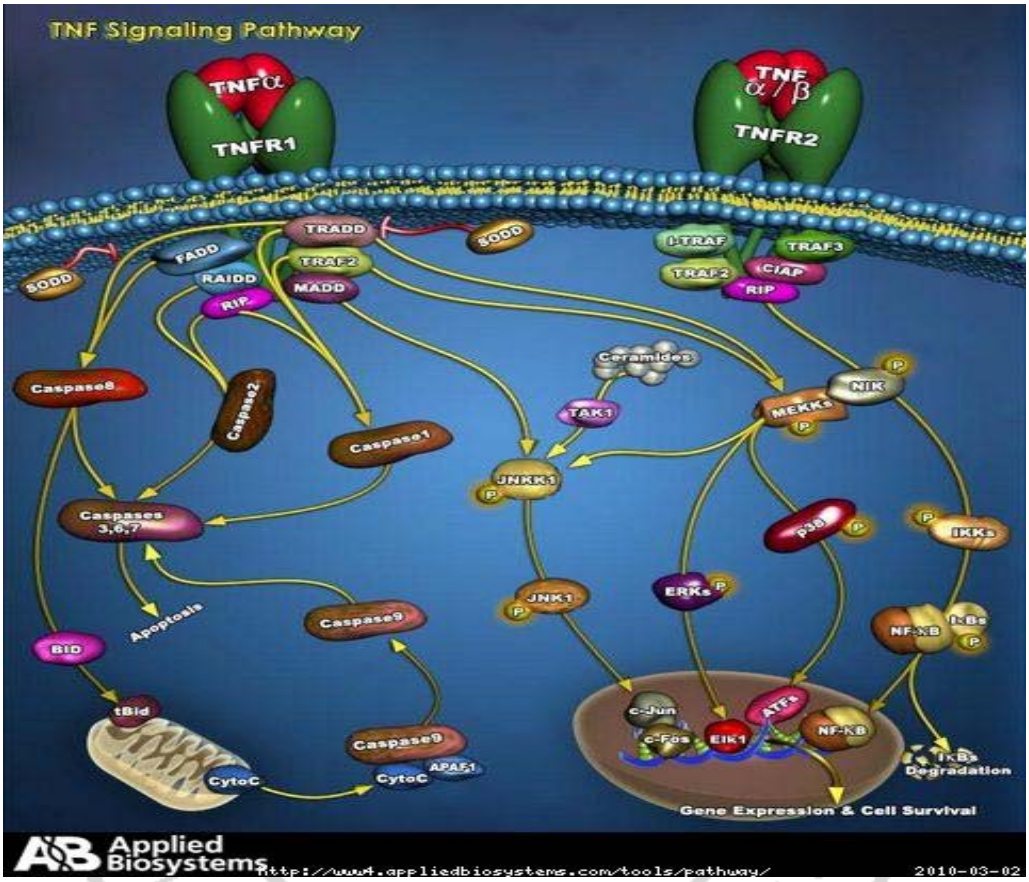

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Figure 3 Signal transduction pathway initiated by trimeric TNF-alpha binding to its receptor, TNFR to initiate receptor clustering and signal transduction.