CHAPTER I

INTRODUCTION

1.1. Statement and significance of problem

Apoptosis is a physiological cell suicide program that plays a critical role in embryonic development and the maintenance of tissue homeostasis in multicellular organisms (Meier *et al.*, 2000). Dysregulation of apoptosis has been implicated in numerous clinicopathological conditions, including neurodegenerative diseases, autoimmunity and cancer. It is one of the crucial mechanisms which causes tumourigenesis and drives its progression. Thus, proteins involved in apoptosis regulation are of intense biological interest and many are attractive therapeutic targets. Anticancer drugs, including those that target DNA replication, DNA integrity, or cytokinesis, induce apoptosis in susceptible cell types (Kaufmann & Gores, 2000).

Defects in apoptosis pathway are involved in the clinicopathological processes and progression of cancer. Several mechanisms have been described including accumulation of abnormality in immortal cells, induction of genetic instability and oncogene activation. These processes consequently induced resistance to either immune attack or treatment, survival in hypoxic condition and support the detached state for tumor metastasis (Stonner-Liewen & Reed, 2003).

The inhibitor of apoptosis protein (IAP) family was first discovered in baculoviruses (Crook *et al.*, 1993), and has been evolutionarily conserved from viruses to nematodes, flies and several mammalian species (Deveraux & Reed, 1999).

Among the regulators of apoptosis that may participate in cancer, interest has been recently focused on survivin. Survivin is a unique member of the inhibitor of apoptosis (IAP) gene family that inhibits, either directly or indirectly, the activation of the terminal cell death effector, caspases. It is involved in both cell division control at the G_2/M phase and suppression of cell death (Altieri, 2003; Johnson & Howerth, 2004) Its expression also contributes to tumor cell resistance to chemo- and radiotherapy (Asanuma et al., 2000; Zaffaroni et al., 2005).

Survivin was found during embryonic and fetal development but was undetectable in terminally differentiated adult tissues, except for weak signals in placenta, thymus (Ambrosini et al., 1997), proliferative endometrium (Saitoh et al., 1999), secretory endometrium (Konno et al., 2000) and basal colonic epithelium. It is strongly expressed in most of the common human cancers, thus called a universal tumor-associated antigen. There was a correlation between survivin expression and reduced tumor cell apoptosis in vivo, low patient survival rate, accelerated rates of recurrences, and increased resistance to therapy (Blanc-Brude et al., 2003). Furthermore, survivin expression has been demonstrated in several pre-neoplastic lesions, including polyps of the colon, and nearly all cases of Bowen's disease (SCC in situ) and hypertrophic actinic keratosis. Therefore, re-expression of survivin is an early event during stepwise malignant transformation (Zaffaroni et al., 2005).

Early diagnosis holds promise in identifying disease at curable stage. Molecular markers are being developed to identify rare cancer cells against a background of millions of normal cells. The sensitivity of a marker is defined as the minimum amount that can be detected and its specificity for tumor cell detection is the percentage of tests that correctly distinguish samples that contain cancer from

those that do not. Moreover, it also acts as a prognostic marker that helps to establish the patient outcome and response to treatment. These markers can be applied to tissue biopsies, exfoliated cells, saliva and serum (Partridge *et al.*, 2005).

Molecular changes usually occur long before changes can be observed histologically. A common approach that has been used to measure the gene expression at mRNA levels in tissue samples is reverse transcriptase-polymerase chain reaction (RT-PCR). This process can typically detect cancer cells at a ratio of 1 in 10,000 normal cells (Partridge *et al.*, 2005). Furthermore, the changes in the structure or expression of certain self protein, occurring during tumorigenesis may trigger the immune system to perceive tumor-associated epitopes as foreign. Therefore, detection of autoantibody might be a reporter in identifying aberrant transformed cells in tumorigenesis (Tan, 2001).

This study aimed to determine the expression of survivin mRNA in various tumor tissues by semi-quantitative RT-PCR, survivin autoantibody in serum of cancer patients and evaluate the correlation between survivin mRNA, survivin autoantibody and the clinicopathological parameters.

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1.2. Literature reviews

1.2.1. Apoptosis pathway

The most common and well-defined form of programmed cell death (PCD) is apoptosis, which is a physiological 'cell-suicide' programme that is essential for embryonic development, immune-system function and the maintenance of tissue homeostasis in multicellular organisms (Ellis *et al.*, 1991; Jacobson *et al.*, 1997; Kerr *et al.*, 1972).

Apoptosis in mammalian cells is mediated by a family of cysteine proteases known as the caspases. To keep the apoptotic programme under control, caspases are initially expressed in cells as inactive procaspase precursors. When initiator caspases, such as caspase-8 and caspase-9 are activated by oligomerization, they cleave the precursor forms of effector caspases, such as caspase-3, caspase-6 and caspase-7. Activated effector caspases in turn cleave a specific set of cellular substrates, resulting in the well-known constellation of biochemical and morphological changes that are associated with the apoptotic phenotype. There are two apoptotic pathways including the extrinsic and intrinsic by which caspase activation is triggered (Figure 1.1). The extrinsic pathway is activated by the engagement of death receptors on the cell surface. Binding of ligands such as FASL and tumour necrosis factor (TNF) to FAS and the TNF receptor (TNFR), respectively, induces the formation of the death induced signaling complex (DISC). DISC in turn recruits caspase-8 and promotes the cascade of procaspase activation that follows. The intrinsic pathway is triggered by various extracellular and intracellular stresses, such as growth-factor withdrawal, hypoxia, DNA damage and oncogene induction. Signals that are transduced in response to these stresses converge mainly on the mitochondria. A series of

biochemical events is induced that results in the permeabilization of the outer mitochondrial membrane, the release of cytochrome c and other proapoptotic molecules, the formation of the apoptosome which a large protein complex that contains cytochrome c, apoptotic protease activating factor 1 (APAF1) and caspase-9 and caspase activation (Budihardjo *et al.*, 1999). Among these processes, only the permeabilization step is regulated, in that anti-apoptotic members of the Bcl-2 family can stop the march towards apoptotic death. Once cytochrome c is released, however, the downstream cascade of caspase activation is irreversible (Okada & Mak, 2004).

The proteins that directly control the intrinsic, extrinsic, and other less understood caspase activation pathways often exist as families that can be recognized based on their amino acid sequence and/or structural similarity. Moreover, interactions among these proteins are commonly mediated by domains that are intimately associated with apoptosis regulation, including caspase-associated recruitment domains (CARDs), death domains (DDs), death effector domains (DEDs), Bcl-2 homology (BH) domains of Bcl-2 family proteins, NB-ARC domains representing the nucleotide-binding oligomerization domains of CED4/Apaf-1 family proteins, and baculovirus inhibitor of apoptosis proteins (IAP) repeat (BIR) domains of IAP family proteins (Reed, 2000).
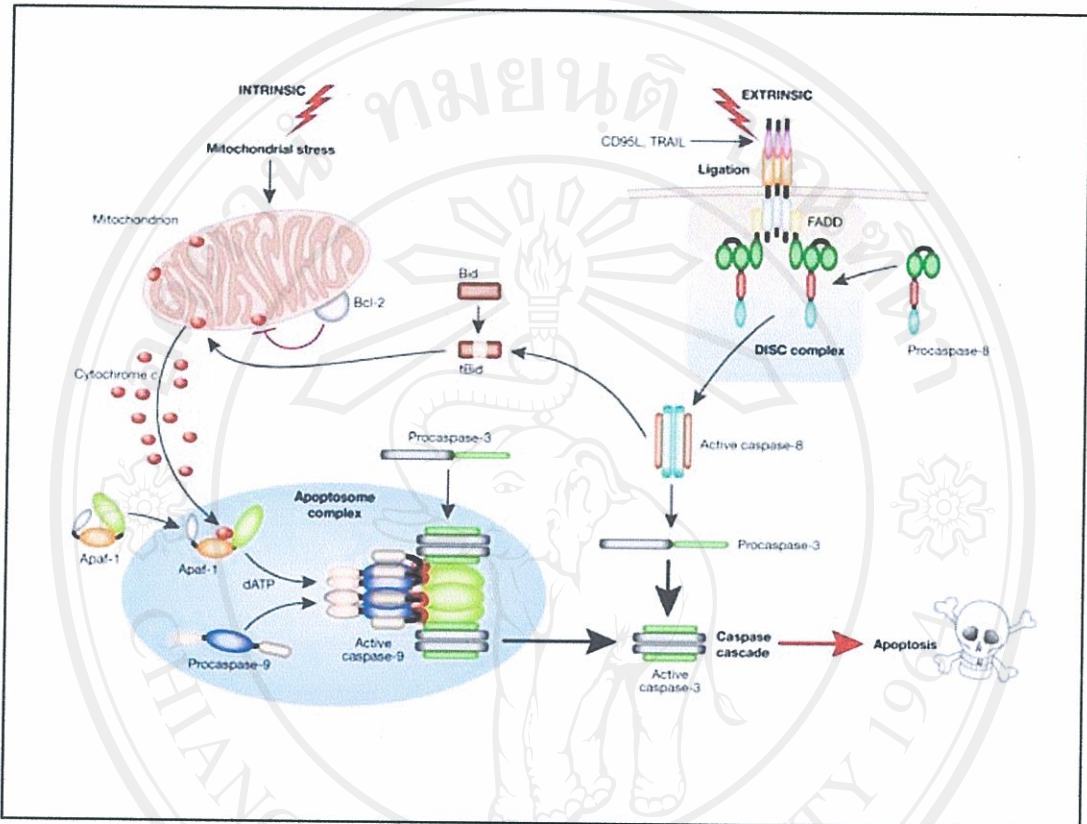
Figure 1.1. Apoptosis: the 'extrinsic' and 'intrinsic' pathways to caspase activation. Two major apoptotic pathways are illustrated: one activated via death receptor activation (extrinsic) and the other by stress-inducing stimuli (intrinsic) (reproduced from *http://www.nature.com/embor/journal/v5/n7/fig_tab/7400191_fl.html accessed 28 October 2006*).

1.2.2. Cell death defects and tumorigenesis

The impaired balance between cell proliferation and their proclivity to undergo apoptosis may be crucial for the development of many types of cancer (Kerr *et al.*, 1994; Lin *et al.*, 2002). Failure to undergo apoptosis in conjunction with unrepaired damage leads to enhanced mutation, including chromosomal alterations, and can be a cause of the genomic instability that is a general characteristic of cancer progression (Williams *et al.*, 1998).

Due to the sensitivity of the intrinsic pathway, tumors arise more often through the intrinsic pathway than the extrinsic pathway. In the intrinsic pathway, a very common cause of tumor genesis is mutation of the p53 protein (Johnstone *et al.*, 2002). Besides regulating apoptosis, p53 also regulates the check points in the cell cycle, DNA repair, senescence and genomic integrity (Schmitt *et al.*, 2002). Any mutation that causes p53 to lose any of its function will induce tumorigenesis by letting the cell grow indefinitely without any regulation. Another important factor in tumorigenesis is the balance between the Bcl-2 family proteins, which possesses both pro- and anti-apoptotic members, and the inhibitor of apoptosis (IAP) family proteins. Bcl-2 proteins are thought to regulate mitochondrial permeability by either decreasing or enhancing the release of mitochondrial proteins, particularly cytochrome *c*, therefore modulating the intrinsic pathway of apoptosis. In contrast, IAP family proteins possess an inhibitory function and act downstream by preventing activation of caspase 9 in apoptosome and inhibiting the activity of effector caspases (Altieri, 2003). In a tumor cell, a mutation of *Bcl-2* gene that results in increased expression will suppress the normal function of the proapoptotic proteins, BAX and BAK (Johnstone *et al.*, 2002). On the other hand, if a mutation on the *BAX* or *BAK* genes

causes a downregulation of expression then the cell will also lose its ability to regulate apoptosis, again causing tumorigenesis (Johnstone *et al.*, 2002). Several abnormalities in apoptosis regulatory mechanisms leading to resistance to caspase-dependent cell death were reported in tumor cells from patients with acute leukemias. (Earnshaw *et al.*, 1999; Joza *et al.*, 2001; Nicholson, 1999).

It is now generally accepted that inhibition of apoptosis plays a role in the carcinogenic process. Studies of colon specimens harvested at various points along the adenoma-to-carcinoma transition have demonstrated that the rate of apoptosis during short-term culture ex vivo is relatively high in normal colonic epithelium, intermediate in adenomas and low in carcinomas (Bedi *et al.*, 1995).

1.2.3. The inhibitor of apoptosis protein family (IAP)

The inhibitor of apoptosis proteins (IAPs) are a family of key regulators of apoptosis which inhibit the activity of the caspase cascade. These proteins block both the mitochondrial-dependent and-independent apoptotic pathways (Carson *et al.*, 2002; Jia *et al.*, 2003). They also participate in the regulation of cell cycle and intracellular signal transduction. The IAPs family was found in virus, yeast, nematodes, fruit flies and mammalian. All IAP family members share one or more signature motifs termed baculovirus IAP repeat (BIR) which consists of a conserved sequence of about 70 amino acids and carboxyl terminal is RING finger. Other structural motifs found in certain IAPs include a caspase recruitment domain (CARD), an ubiquitin-conjugating domain and a nucleotide binding P-loop motif (Deveraux & Reed, 1999). There are several IAP proteins in humans which have been identified including X-chromosome linked IAP (XIAP), cellular inhibitor of apoptosis 1 and 2 (cIAP1, cIAP2), neuronal apoptosis inhibitor protein (NAIP), livin and survivin. The potential and well-known members of this family are XIAP, c-IAP1, c-IAP2 and survivin (Figure 1.2) (Wrzesien-Kus *et al.*, 2004).
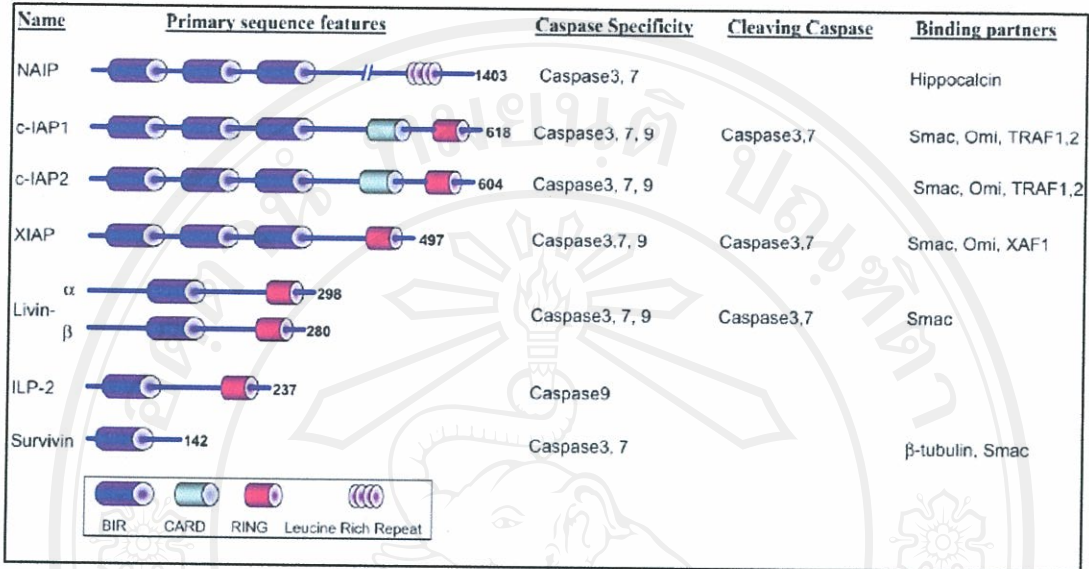
Name	Primary sequence features		Caspase Specificity	Cleaving Caspase	Binding partners
NAIP		1403	Caspase3, 7		Hippocalcin
c-IAP1		618	Caspase3, 7, 9	Caspase3,7	Smac, Omi, TRAF1,2
c-IAP2		604	Caspase3, 7, 9		Smac, Omi, TRAF1,2
XIAP		497	Caspase3,7, 9	Caspase3,7	Smac, Omi, XAF1
Livin- α		298	Caspase3, 7, 9	Caspase3,7	Smac
Livin- β		280			
ILP-2		237	Caspase9		
Survivin		142	Caspase3, 7		β-tubulin, Smac

BIR   CARD   RING   Leucine Rich Repeat

Figure 1.2. IAP family members. The primary sequence feature is indicated for the different IAP members. In addition, the molecular interaction either with caspases and other cellular targets are shown (Nachmias *et al.*, 2004).

1.2.4. Survivin: Structure & Function

Survivin is an intracellular protein, termed baculoviral IAP repeat containing protein-5 (BIRC-5) that belongs to the inhibitor of apoptosis (IAP) gene family. Survivin was discovered in 1997 by hybridization screening of a human genomic library with a factor Xa receptor, effector cell protease receptor-1 (EPR-1) (Ambrosini et al., 1997). Transfection of Hela cells with EPR-1 cDNA resulted in a suppressed expression of endogenous survivin and loss of cell viability, due to apoptosis induction. It has been suggested that EPR-1 may act as a natural anti-sense to survivin (Ambrosini et al., 1997).

The survivin gene spans 15 kb, on the telomeric position on chromosome 17, to band q25. It comprises three introns and four exons. The coding strand of survivin contains an open reading frame of 426 nucleotides, and encodes a protein of 142 amino acids, with a molecular weight of approximately 16.5 kDa (Figure 1.3). The structure of the survivin protein is intimately linked with its function as an inhibitor of apoptosis. The amino terminal portion of survivin consists of three alpha helices (residues 14-21, 31-41, 68-80) and 3 beta-sheets (residues 43-45, 55-58, 61-64), which closely resemble the BIR domain that is conserved in the IAP family with a highly hydrophobic center that includes a C2HC motif coordinating a zinc ion (Chantalat et al., 2000). This core structure includes a number of highly conserved cysteine and histidine residues, which are required for anti-apoptotic activity. Unlike other IAPs, survivin contains only one BIR domain and no RING finger motif. Crystal structure analysis of survivin revealed an extensive dimerization interface along the hydrophobic on each BIR domains of survivin monomer. (Chantalat et al., 2000).

Besides the four dominant exons (1, 2, 3, and 4), human survivin gene contains three hidden exons (2 α, 2B and 3B) (Li & Ling, 2006). Alternative splicing of its pre-mRNA produces four different mRNAs, which encode four distinct proteins; survivin, survivin-2B, survivin-ΔEx3, and survivin-3B (Badran *et al.*, 2004; Mahotka *et al.*, 1999) (Figure 1.4). Survivin (142 aa) is derived from exons 1–4; survivin-2B (167 aa) has an additional 23 aa derived from a 69-bp cryptic exon (2B) within intron 2, which is spliced into survivin mRNA in frame between exons 2 and 3; survivin-ΔEx3 (137 aa) is derived from exons 1, 2, and 4, a frameshift read-through variant due to exon 3 escape; and the recently reported survivin-3B (120 aa) consists of the N-terminal 113 aa of survivin (coded by exons 1, 2, and 3) plus seven new aa sequences at C-terminal tail encoded by a DNA sequence (exon 3B) from intron 3 of survivin. In addition, Caldas *et al.* (2005) identified and characterized a novel survivin isoform called survivin 2α. This protein contains the coding sequences from exon 1 and exon 2.

Survivin has two main functions; one function as a chromosomal passenger protein (Carvalho *et al.*, 2003) and the other function as an inhibitor of apoptosis (Adida *et al.*, 1998). Survivin 2B has been shown to be a proapoptotic protein that sensitizes resistant leukemia cells to chemotherapy in a p53-dependent fashion (Zhu *et al.*, 2004). Survivin-ΔEx3 functions as an anti-apoptotic protein and is upregulated in malignancies (Mahotka *et al.*, 1999). No function has yet been described for survivin-3B where as the functional assays show that survivin 2α attenuates the anti apoptotic activity of survivin (Caldas *et al.*, 2005). In addition, survivin 2α is expressed at high levels in several malignant cell lines and primary tumor.

The presence of transcripts of survivin isoforms has been correlated with cancer progression. For example, using reverse transcription-PCR, Mahotka *et al.* (1999) found that wild type survivin and survivin-ΔEx3 were expressed in cell lines derived from renal carcinomas but not in normal renal cells (Mahotka *et al.*, 1999). Differential expression of survivin-2B was also reported in gastric carcinomas and, in this case, negatively correlated with disease progression (Krieg *et al.*, 2002; Meng *et al.*, 2004). Further reports have linked the expression of survivin isoforms with poor patient prognosis (Li, 2005).
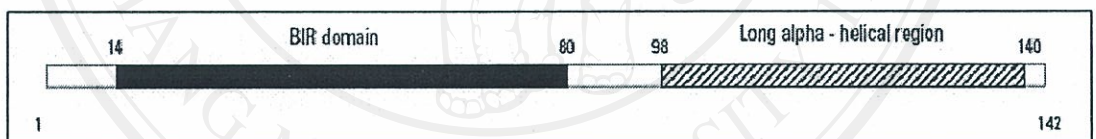

Figure 1.3. Representation of survivin protein structure. Survivin is a 142 amino acid protein containing a BIR domain (black bar) at the amino terminal region and a long alpha-helical region (striped bar) at the carboxyl terminus (Chiou *et al.*, 2003)
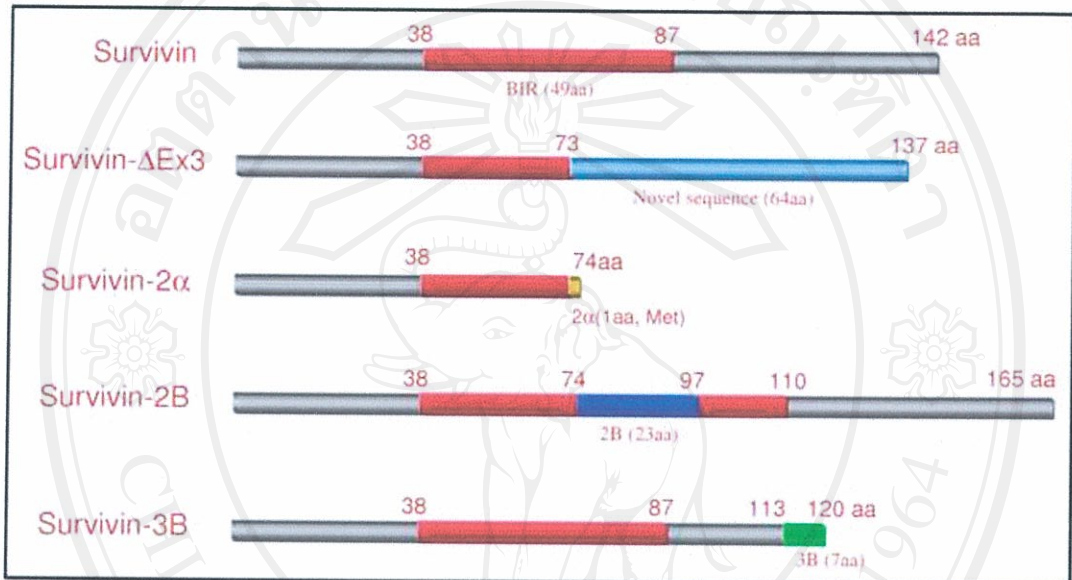
Figure 1.4. Diagram of the protein structures for the survivin splice variants. While survivin and survivin-3B possess the intact baculovirus IAP repeat (BIR) domain, the BIR domains are truncated in survivin-ΔEx3 and survivin-2α, and disrupted by the 2b exon (coding 23 aa) in the survivin-2B protein. aa, amino acid(s) (Li & Ling, 2006).

1.2.5. Survivin in apoptosis control and cell cycle regulation

Survivin is a bifunctional protein that involved in both control of apoptosis and regulation of cell division (Johnson & Howerth, 2004). It blocks a common downstream part of both apoptosis pathways, by directly inhibiting terminal effector caspase-3 and caspase-7, and by interfering with caspase-9 activity/processing (Figure 1.5). In addition, survivin counteracts apoptotic stimuli induced by interleukin (IL)-3, Fas (CD95), Bax, tumor necrosis factor (TNF) α, caspases, anticancer drugs, and X-irradiation (Asanuma *et al.*, 2000; Tamm *et al.*, 1998).

Survivin is unique among other IAP proteins because it exhibits cell cycle-regulated. It is prominently expressed at mitosis G_2/M phase in a transcriptionally-controlled pathway (Ambrosini *et al.*, 1997) and rapid decrease in expression following cell cycle arrest (O'Driscoll *et al.*, 2003). At the beginning of mitosis, phosphorylation of survivin on Thr34 by p34^{cdc2}-cyclin B1 is necessary to preserve cell viability and function (Verneris *et al.*, 2000).

Survivin localizes to various components of the mitotic apparatus. These include centrosomes, also called microtubule-organizing centers, microtubules of the metaphase and anaphase spindle, and the remnants of the mitotic apparatus, i.e. midbodies, at telophase (Li *et al.*, 1999; Li *et al.*, 1998). Recently, a subcellular pool of survivin has been shown to localize to kinetochores of metaphase chromosomes and the central spindle midzone (Skoufias *et al.*, 2000), potentially associating with Aurora B kinase (Wheatley *et al.*, 2001). The various subcellular pools of survivin are immunochemically distinct, potentially reflecting post-translational modifications modulating epitope accessibility and intracellular trafficking of the mature protein (Fortugno *et al.*, 2002) (Figure 1.6).
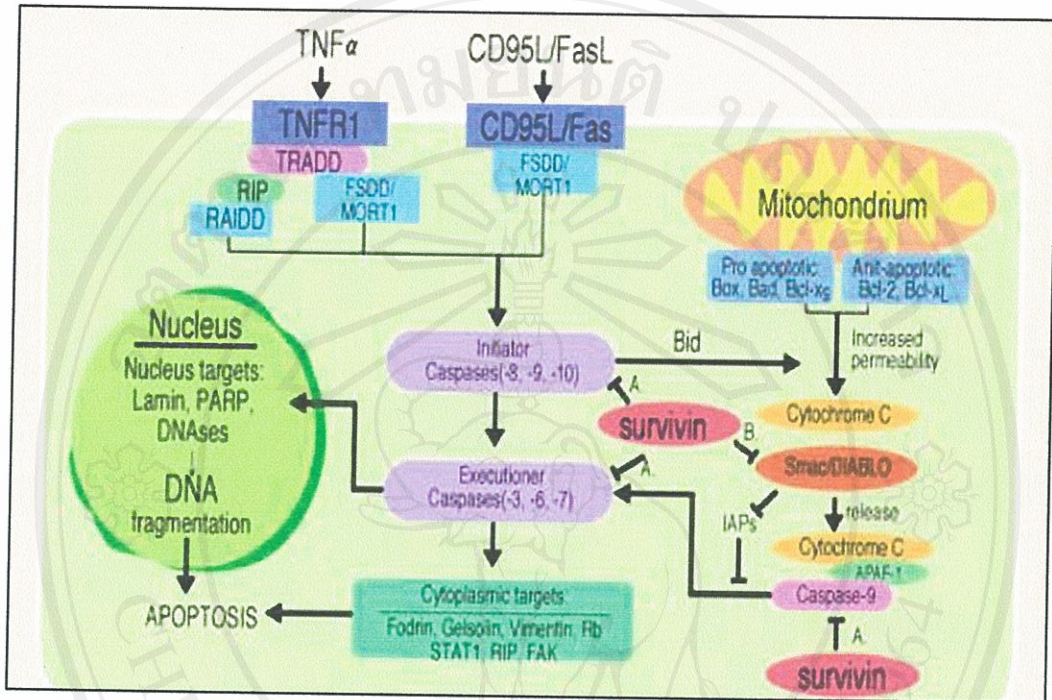
Figure 1.5. The role of survivin in apoptosis pathways. Survivin (red) may regulate apoptosis by either directly or indirectly inhibiting the activity of caspases: A. Directly inhibiting the caspases responsible for induction and execution of apoptosis. B. Indirectly inhibiting caspase function by regulating Smac/Diablo (Adapted from Chiou *et al.*, 2003).
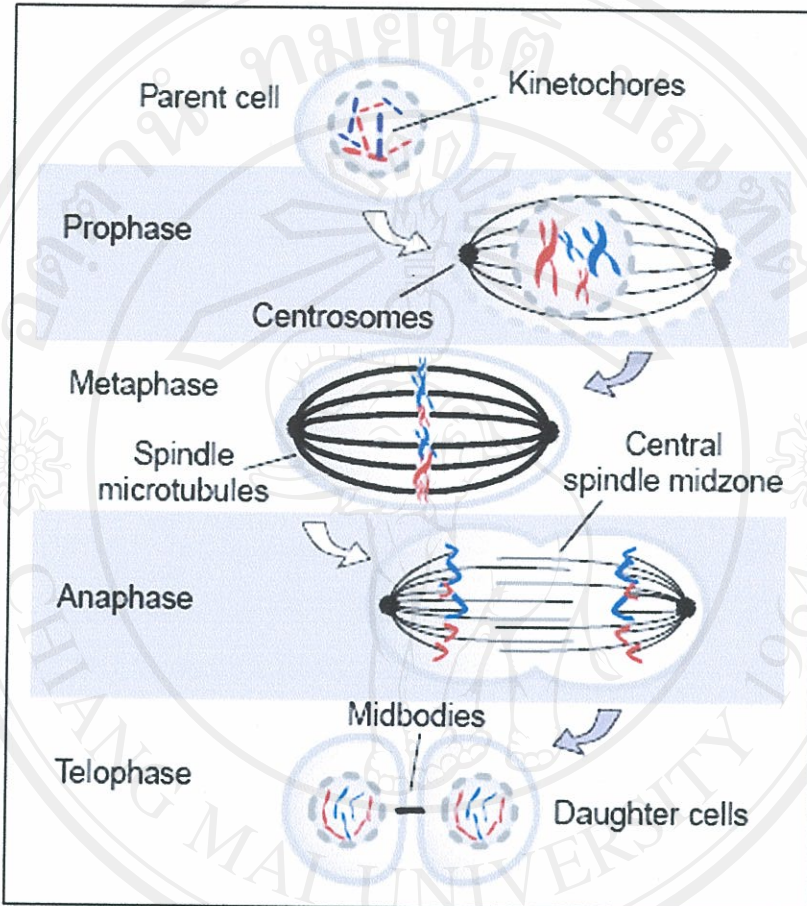
Figure 1.6. Subcellular localization of survivin during mitosis. Survivin localizes to kinetochores, centrosomes (microtubule-organizing centers), spindle microtubules, central spindle midzone and midbodies. Approximately 80% of the total cellular survivin content in mitotic cells is bound to centrosomes and microtubules of the metaphase and anaphase spindle (Altieri, 2001).

1.2.6. Survivin expression in normal and cancer tissues

Survivin is highly expressed during embryonic development and may be important in tissue homeostasis and differentiation. The gene is quiescent in most terminally differentiated tissues. Among the IAP family members, survivin exhibits the most restricted expression in adult tissues (Adida *et al.*, 1998; Adida *et al.*, 1998). Overexpression of survivin was observed in most of the common human cancers by analysis of its transcript and protein including lung (Monzo *et al.*, 1999) , breast (Tanaka *et al.*, 2000), colon (Kawasaki *et al.*, 1998), stomach (Lu *et al.*, 1998), esophageal (Kato *et al.*, 2001), pancreas (Satoh *et al.*, 2001), liver (Ito *et al.*, 2000), bladder (Swana *et al.*, 1999), uterus (Saitoh *et al.*, 1999), ovary (Yoshida *et al.*, 2001), leukaemias (Adida *et al.*, 2000; Kamihira *et al.*, 2001), neuroblastoma (Adida *et al.*, 1998; Islam *et al.*, 2000), brain tumors, soft tissue sarcomas, and other skin cancers (Altieri, 2003). The incidence of survivin expression was 34%–100% in these cancer tissues (Yamamoto & Tanigawa, 2001). The up-regulation of survivin at the transcriptional level in human tumors has been confirmed in genome-wide searches, which indicated survivin as the fourth top "transcriptome" in cancers of various histology (Velculescu *et al.*, 1999). At least for some tumors types molecular abnormalities have been described that may account for the increased expression of survivin in cancer as compared to normal tissues. Specifically, a gain of 17q25 containing the survivin locus represents a frequent genetic abnormality in neuroblastoma (Plantaz *et al.*, 1997). Moreover, in most ovarian cancers, survivin exon 1, which is silenced by methylation in normal ovarian epithelium, becomes unmethylated, and consequently, transcriptionally active (Hattori *et al.*, 2001).

In the majority of solid tumors, high levels of survivin protein were predictive of tumor progression, either in terms of disease free survival or overall survival, thus providing prognostic relevant information (Altieri, 2003). In colorectal (Kawasaki *et al.*, 1998) and breast cancer (Tanaka *et al.*, 2000), survivin-positive patients had decreased AI and worse survival rates than survivin-negative patients. In bladder cancer, the positive rates of survivin expression increased along with malignancy, and the mean time to first recurrence in patients with survivin-negative tumors was greater than that in patients with survivin-positive tumors (Swana *et al.*, 1999). Furthermore, survivin expression was correlated with poor prognosis in non-small-cell lung cancer (Monzo *et al.*, 1999) and esophageal cancer (Kato *et al.*, 2001).

1.2.7. Survivin autoantibody

Evidence for an immune response to cancer in humans was demonstrated by the identification of autoantibodies against a number of intracellular and surface antigen in patients with different types of tumor (Ben-Mahrez *et al.*, 1990; Crawford *et al.*, 1982). Such antibodies could have diagnostic and prognostic utility. Enhanced survivin expression in tumor cells led to anti-survivin response as demonstrated by the presence of survivin autoantibody in patients with lung (21.6%) and colorectal cancer (8.2%) (Rohayem *et al.*, 2000). An ELISA using purified recombinant survivin was positive for anti-survivin antibodies in 39.7% (25 of 63) sera from gastrointestinal patients and 58.1% (18 of 31) sera from lung patients (Yagihashi *et al.*, 2005; Yagihashi *et al.*, 2001). Avidin capture of *in vivo* biotinylated recombinant survivin protein expressed in *E. coli* was employed herein this ELISA system. The method is

rapid and does not require high yield purification of the recombinant antigen according to Winkler *et al,* (1997).

1.2.8. The molecular basis and molecular markers for diagnosis and prognosis in colorectal, liver and lung cancer

Cancer is a genetic disorder that is caused by alterations of the important growth-controlling genes (Karp, 1999). At present, cancer is a crucial public health problem of the world as well as in Thailand. It is the leading cause of death. The top three cancer sites contributing to the overall burden in Thai men was liver cancer, followed by lung and colorectal cancer. On the other hand, cervical cancer was the most common cancer in Thai women, followed by breast cancer, liver cancer and lung cancer, respectively (Martin & Patel, 1996).

Colorectal cancer is the third most common cancer and the second most common cause of death from cancer in many industrialized countries. It arises from the epithelial cells outlining the lumen of the colon and rectum. The cancer is thus called a colorectal adenocarcinoma that regard to the most histological type of all colorectal cancer. There are other forms of colorectal cancer such as squamous cell carcinoma, sarcoma, lymphoma, and carcinoid tumors, which are very rare and altogether constitute less than 2% of all this cancer. In histologic grading, as in the system of Broders (Broders, 1926), most investigator use a numbering system from 1 to 4, with large numbers indicating less differentiation, or a series of modifying terms designating tumors as well, moderately or poorly differentiated which poorly differentiated carcinomas will carry a less favorable prognosis than do more differentiated carcinomas (Dukes, 1950). Besides grade of tumor, clinicopathological

features including lymphatic, vascular and perineural infiltration, and presence or absence of an inflammatory response related to prognosis as well (Fleming *et al.*, 1997).

Several studies indicated that certain genetic factors, environmental factors, or dietary factors were probably involved in the etiology of colorectal cancer. The progression of events leading to the transformation of colonic epithelial cells into a cancer is multistep process that involving the inactivation and activation of a variety of well defined tumor suppressor genes, oncogenes and DNA mismatch repair genes (Kinzler & Vogelstein, 1998).

Liver cancer is one of the common malignancies worldwide. In Thailand, liver cancer is the most common in men (age standardized incidence rate;ASR = 37.4 × 10^5) and the third most common in women (ASR = 16.3 × 10^5) (Vatanasapt *et al.*, 2002). Malignant tumors of the liver are primarily adenocarcinomas with two major cell types including hepatocellular and cholangiocarcinoma. Hepatocellular carcinoma (HCC) arises from the main cells of the liver. It is the most common form of liver cancer and the third leading cause of cancer deaths worldwide (Jarnagin *et al.*, 2002). The other type is called cholangiocarcinoma or bile duct cancer, because it starts in the cells lining the bile ducts. Cholangiocarcinoma is more common in women. Hepatocarcinogenesis is a continuous and slowly unfolding process that leads from the initial occurrence of epigenetic and/or genetic alterations in one or a few hepatocytes to the acquisition of a neoplastic genotype/phenotype. Prognostic factors in HCC conventionally consist of staging with the tumor node metastasis system (TNM) and grading by tumor cellular differentiation. In HCC, DNA ploidy, the proliferating activity of tumor cells, tumor suppressor and promoter genes, cell cycle

controllers, proteinases that degrade extracellular matrix, adhesion molecules, angiogenic factors, and metabolic genes, have been regarded as biomarkers for the malignant phenotype of HCC, and are related to prognosis and therapeutic outcomes (Korn, 2001).

Last, but not least, lung cancer is one of the leading causes of cancer death throughout the world. Non-small cell lung carcinoma (NSCLC) accounts for approximately 75% of the cases and represents a heterogeneous group of cancers consisting mainly of squamous cell, adeno- and large cell carcinoma. The remaining 25% of the cases are characterized by cells of a smaller size, hence the name small cell lung cancer (SCLC) (Niklinski *et al.*, 2001). In Thailand, a high incidence of lung cancer was found in women in northern Thailand with a high proportion of adenocarcinomas (Vatanasapt *et al.*, 2002).

Although TNM stage is the most significant prognostic parameter to be considered, the variability of survival within staging groups requires additional parameters influencing outcome, independent of stage factors (Fielding *et al.*, 1992; Mountain, 1995; Niklinski & Furman, 1995). However, the prognosis of patients with lung cancer has improved only minimally, with overall survival rates between 10 and 15% (Greenlee *et al.*, 2000). Thus, the poor survival following lung cancer may be ascribed to its biological aggressiveness and a relative lack of symptoms attributable to early disease, combined with the rather poor sensitivity of the classical approaches for early detection (Niklinski *et al.*, 2001).

It is known that cancers can be caused by genetic alterations that turn on oncogenes or turn off tumor suppressor genes. Several studies indicated that the molecular alterations, the most well known genetic mutations including translocated

promoter region-*MET* (TPR-*MET*) rearrangement, alteration of APC gene and mutations of p53 and K-ras (Massion & Carbone, 2003; Niklinski *et al.*, 2001; Partridge *et al.*, 2005). Alterations in gene sequences, expression levels and protein structure or function have been associated with every type of cancer. These 'molecular markers' can be useful in detecting cancer, determining prognosis and monitoring disease progression or therapeutic response.

Recently, there has been great interest in survivin as a diagnostic marker and potential drug target because of its predominantly cancer-specific expression in adult human organ tissues. In colorectal, the aberrant expression of survivin appears to be associated with Bcl-2 expression, tumor development, and poor patient survival rates (Li, 2003). During colorectal tumorigenesis, survivin protein expression is significantly and progressively increased during the transition from low dysplasia adenoma to high dysplasia carcinoma (Kawasaki *et al.*, 2001; Lin *et al.*, 2003). In addition, a high levels of survivin were also detected in carcinoma of liver (Ikeguchi *et al.*, 2002). This increase correlates with reduced tumor cell apoptosis, increased tumor cell proliferation, poorer differentiation, and advanced pathologic T stage. These studies indicate that survivin is associated with biologically and histologically aggressive tumor features, and may play an important role in accelerating HCC tumor progression (Morinaga *et al.*, 2004). In lung cancer, Monzo *et al.* (1999) reported that high survivin mRNA expression correlated with poor survival in NSCLC patients, but not with any clinicopathologic factors (Monzo *et al.*, 1999). Ikehara *et al.* (2002) similarly reported that the expression of survivin protein in tumor cells was a poor prognostic factor in patients with small adenocarcinomas of the lung (<2 cm in diameter), again being independently of tumor stage (Ikehara *et al.*, 2002). These

studies suggest that survivin is a useful prognostic marker of cancer and a potential target for cancer treatment.

Survivin expression in colorectal, liver and lung cancer have been studied extensively by the methods involved both the mRNA and protein analysis. However, to our knowledge, none of them was carried out in Thai patients. There were race differences in the extent of disease at diagnosis of bladder cancer between Blacks and Whites (O'Driscoll *et al.*, 2003). Normally, not only the genetic factors but also the environmental factors make up the molecular heterogeneity among different ethnic groups. The knowledge of incidence and extent of survivin expression may provide useful information for proper management of the Thai patients.

1.3. Objectives

1.3.1   To determine the expression of survivin mRNA in tumor tissues of lung,
         liver and colorectal cancer patients by semi-quantitative RT-PCR

1.3.2   To measure survivin autoantibody in serum of cancer patients by avidin
         capture ELISA

1.3.3   To evaluate the correlation between survivin mRNA, survivin autoantibody
         and the clinicopathological parameters