CHAPTER II

LITERATURE REVIEWS

Cancer is now regarded as an acquired genetic disease due to multiple alterations of genes that affect cell growth of differentiation. Genetic host factors can interact with environmental carcinogens, i.e., carcinogens in the diet, tobacco smoke and ambient air due to environmental or occupational sources, to place an individual at a greater or lesser risk of a particular cancer than another individual.

Apoptosis also plays an important role in development in cellular and tissue homeostasis (33, 35). Defects in the regulation of apoptosis may cause the accumulation of virtually immortal cells and can lead to many human disorders, including cancer (31, 38). Apoptotic cell death is orchestrated by the activation of a cascade of enzymes called caspases (39). Two distinct but converging pathways for caspase activation have been delineated: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. These two pathways have an independent group of initiator caspases but use the same group of effector caspases, primarily caspase-3, -6 and -7, that execute the final cell death program (40, 41).

Abnormalities in the control of programmed cell death or apoptosis play an important role in tumorigenesis. Specifically the inhibitor of apoptosis proteins (IAPs) are a family of anti-apoptotic proteins that inhibit initiator (caspase-9) and effector caspases (caspases-3 and -7) and thereby prevent apoptosis. To date, eight

human inhibitors of apoptosis protein have been identified: NAIP, c-IAP1 (MIHB, HIAP-2), c-IAP2 (HIAP-1, MIHC, API2), XIAP (hILP, MIHA, ILP-1), survivin, BRUCE (apollon), ILP-2 and livin (ML-IAP, KIAP) (42). Each contains one or more baculovirus IAP repeat domains, which are necessary to bind specifically to a terminal effector cell-death protease (for instance, caspases-3 and -7). Binding results substantially reduced caspase activity and reduced cell death in response to diverse apoptotic stimuli. Among these IAP members, survivin and livin are highly expressed in cancer cells and transformed cells, but show little or no expression in normal differentiated tissues (21, 24, 43-49). These anti-apoptotic genes might contribute to the development and progression of cancer.

1. Survivin gene

Survivin is a multifunctional protein that inhibits apoptosis, regulates cell division and enhances angiogenesis. Survivin belongs to the family of inhibitor of apoptosis proteins. The protein contains one copy of the baculovirus IAP repeat (BIR) domain, a domain essential for apoptosis inhibition. At 16.5 kDa, survivin is the smallest member of the IAP family. The survivin gene localizes to chromosomal region 17q25. It encodes a 1.9-kb transcript, which contains 4 exons resulting in a 142-amino acid protein. Alternative splicing of the human survivin gene can give rise to five different mRNA isoforms (50-52) wild-type survivin, survivin-2B, survivin-Ex3, survivin 3B and survivin 2a.

2. Expression characteristics of survivin

An important characteristic of survivin expression is the developmentally regulated pattern. Survivin is highly expressed in embryonic tissue (53), but is almost undetectable in many adult differentiated tissues (43). However, upon

malignant cell transformation, survivin expression is dramatically increased. In a genome-wide search for abundantly expressed genes in cancerous tissue versus normal tissue, survivin was identified as the fourth highest expressed gene in the most common human cancers (54). In addition, survivin overexpression can be detected in almost any form of human cancer (18). Survivin alternatively spliced forms survivin-2B and survivin-Ex3 display a similar expression pattern as full-length survivin (55). Survivin plays an important role in mitosis and its expression is associated with the division of cells. Since tumor cells proliferate rapidly, the elevated expression of survivin in tumors was initially addressed to the high number of dividing cells.

Survivin can be found in different subcellular compartments. Using either immunohistochemistry or subcellular fractionation, two main pools of survivin have been located, i.e., in the nucleus and cytoplasm. Following subcellular fractionation, the approximate ratio of cytoplasmic to nuclear survivin was 6:1 in HeLa cells (56). These different pools of survivin were found to be immunochemically different and to be independently modulated during the cell cycle progression. Recently, another pool of survivin was identified in mitochondria from tumor cells (57). Following cell death stimulation, this pool of survivin was released into the cytoplasm, where it was found to inhibit cell death.

3. Survivin as a tumor marker

The characteristic of ideal diagnostic tumor marker is that it is absent from normal or benign tissue but expressed in all cancers, especially in early or small cancers. Furthermore, it should be detected in a readily available body fluid such as serum or urine thus obviating the need for an invasive procedure (57). Survivin is an

attractive cancer diagnostic marker as it is almost universally upregulated in malignancy. Furthermore, as mentioned above, this increased expression is found in a number of preinvasive lesions with a high predisposition of progressing to malignancy. The rational for investigating survivin as a prognostic marker in malignancy is based on its ability to inhibit apoptosis, promote proliferation and enhance angiogenesis. Because of its involvement in these processes, survivin is likely to be causally involved in tumor progression and consequently, increased levels would be expected to predict aggressive disease. Indeed, multiple reports have shown that high tumor expression levels of survivin are associated with adverse outcome in patients with different types of cancer for example lung cancer (non-small cell) (23, 58, 59) neuroblastoma (60) lymphoma (large cell) (61) sarcoma (62) glioma (20) and cancers of colorectal (24) ovary (25, 27) endometrial (27) esophageal (22, 63) prostate and bladder (21, 26).

4. Livin (alternatively called ML-IAP or KIAP)

Livin is one of the novel human IAPs family members. High livin expression in neoplasms correlates with more aggressive behavior, such as decreased the response to chemotherapeutic agents and shortened survival time (42). The human livin gene spans 46 kb and localizes to chromosomal region 20q13 (64). It comprises five introns and six exons and produces a protein of 280 amino acids. Structurally, it is composed of a single BIR domain and a COOH-terminal RING finger domain**.** The human livin gene, as a result of two alternatively spliced transcripts, can give rise to two different isoforms of the protein. These isoforms include livin-œ and livin-ß (44). The two mRNA variants are detected in various transformed cell lines. Both isoforms have a significant anti-apoptotic activity in the

Jurkat T cell line after triggering apoptosis via tumor necrosis factor and CD95 receptors (44). Despite very close similarity of the two isoforms, they have different anti-apoptotic properties. The livin-œ protects cells from apoptosis induced by staurosporine. In contrast, apoptosis initiated by etoposide was blocked only by the ß-isoform (46). In recent researches, it was found that the targeted inhibition of livin-ß blocks the growth of HeLa cells in clonogenic survival assays and silencing livin-ß sensitizes those cells to different pro-apoptotic stimuli such as UV irradiation, tumor necrosis factor-œ or etoposide (65). Agliano et al. used reverse transcription polymerase chain reaction (RT-PCR) to investigate the expression ratio of livin isoforms-œ and -ß in tumoral bladder tissues and correlate their expression with the emergence of early relapses in a follow-up of four years (21). Their results showed that only livin-œ is expressed in a proportion of tumors with a high risk of relapse.

5. Expression characteristics of livin

Livin expression was detected in various cancer cells, but little or no expression in normal differentiated tissue with the exception of placenta, normal testes, spinal cord and lymph node (44-46, 49). Cancer reported to have increased level of livin expression included soft tissue sarcoma (62) non-small cell lung carcinoma (58) superficial bladder cancer (21) renal cell carcinoma (32) leukemia, lymphoma, cancer of breast, cervix, prostate, colon (44) and melanomas (49, 66).

6. Livin as a tumor marker

In the majority of tumors investigated for livin expression, high levels of the protein were predictive signs of tumor progression and it could also provide prognostically relevant information (21). In recent studies, it had been found that

livin might be involved in the progression of superficial bladder cancer and can be used as a marker of early recurrence (21), RT-PCR was used to investigate the expression of livin isoforms œ and ß in normal and tumoral bladder tissues and correlated their expression with the emergence of early relapsed in a follow-up of 4 years. The result show that the œ isoform was not expressed in normal bladder tissue, but was expressed in a proportion of tumors with high risk of relapse.

7. Auto-antibody directed against TAAs: a potential ideal tumor marker

In the past 30 years, some other proteins, hormones and enzymes have been used as markers. Notable among these are carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG) and prostatic acid phosphatase (PAP). Most of these markers lack specificity; however, these levels are also increased under benign conditions and during gestation. All of these markers are based on the antigen determination method; the markers are lack of specificity and sensitivity.

The presence of autoantibodies is considered to be a malfunctioning of the immune system. The immune system responds to the presence of antigens, which are unusual proteins or carbohydrates on the surfaces of invading organisms, by producing antibodies against these antigens. The antibodies then bind with the antigens, initiating a process leading to destruction of the organisms. During the process of its development, the immune system learns to distinguish self from non-self. For reasons not entirely clear, the immune system can develop antibodies against normal body constituents, such as proteins and nucleic acids, leading to diseases such as diabetes and lupus. Cancer cells are produced by the body and can present either novel proteins or abnormally large amounts of normal proteins. The

immune response to these proteins can also lead to the production of autoantibodies. There are several mechanisms by which cancer antigens can stimulate an immune response: gene activation or repression, gene mutation and amplified expression of gene products (proteins).

Autoantibody against p53 suppressor protein was one of the first few autoantibodies reported. p53 protein is known as the "guardian of the genome" and its levels rise dramatically in response to DNA damage. High levels of p53 protein are normally only transient while it performs its role of either arresting cell growth until the damaged DNA can be repaired or initiating programmed cell death. When the p53 gene mutates, however, the resultant abnormal protein persists in the blood, leading to the formation of autoantibodies. Since mutated p53 genes are the most common mutated genes in cancers, great interest was shown in using the presence of p53 autoantibodies as a screening tool. Crawford et al. reported for the first time the presence of autoantibodies against p53 in serum of patients with breast cancer. During the past few years, analysis of a large number of serum from patients with various malignancies revealed that the most immunogenic tumors are those of the lung (67-72), ovary (71-74), breast (68, 71, 72, 75), head and neck (71, 72, 76).

In the case of survivin and livin, it has recently been reported that 25 of 63 sera from gastrointestinal cancer patients (39.7%) reacted with purified recombinant survivin using an enzyme-linked immunosorbent assay (ELISA), while 17 of 35 sera from these patients (47%) were reactive in an ELISA using purified recombinant livin (29, 36). Another study showed that livin is expressed in approximately 60% of renal cell carcinoma (RCC) cases demonstrated by immunohistochemically and the anti-livin Ab titer in the sera of RCC patients was significantly higher than that

in healthy volunteers (32), it was found that serum samples from 25 (55.6%) of the 45 patients were positive for the anti-livin autoantibody. Although the immune responses without immunotherapeutic stimulation did not affect patient survival, livin may be recognized as a tumor antigen by the immune system in RCC patients. In addition, Rohayem et al. reported that 11 of 51 sera from lung cancer patients (21.6%) and 4 of 49 sera from colorectal cancer patients (8.2%) reacted with purified recombinant survivin (34). These reports indicate that survivin and livin can induce an antibody response in some patients with various cancers. As with survivin overexpression, livin overexpression in lung cancer may lead to anti-livin antibody responses against the cancer. Currently, there are still very few data concerning antibody responses to livin in lung cancer patients, so this is another reason for conducting the present study.