CHAPTER 1

INTRODUCTION

1.1 Statement of problems

Cancer has been one of the leading causes of death around the world. Not only with low survival rate, especially in advanced cases, but also there are high incidence of side effects resulting from conventional cancer treatment, including surgery, chemotherapy and radiation[(1)]. Lung cancer is one of the most prevalently occurring and the most life-threatening neoplasia in most part of the world. It has an incidence of 1.3 million people worldwide, and accounted for about 18.2% of all cancer death. In Southeast Asia, lung cancer with an estimated 135,852 deaths in 2008 (12.0% of total) is the most common cause of death from cancer, followed by cervix-uteri cancer (9.1%), breast cancer (8.3%) and colorectum cancer (6.0%) [(2)]. In Thailand, the incidence and the mortality associated with liver cancer and lung cancer are predicted to steadily increase. Data from National Cancer Institute (NCI) of Thailand reported that lung cancer was the major cancer of men and the fourth place in women. Unfortunately, lung cancer is the number one cause of cancer-related death in both male and female [(3)].

Lung cancers can be grouped in two major histological types, i.e. non-small cell (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for 75-85% of lung cancer patients and consists of several subtypes, predominantly squamous cell carcinomas, adenocarcinomas and large cell carcinomas, which are treated in the same manner. Small cell lung cancer accounts for 15-25% of lung cancer patients,

often has neuroendocrine components, and is primarily treated with chemotherapy and/or radiotherapy. Many lung cancers constitute histologically mixed tumor types consisting of non-small cell and small cell components. Histological differentiation and staging of lung cancer is mandatory for therapeutic stratification.

Patients with lung cancer often do not exhibit specific symptoms, particularly in early stage disease. Dyspnoea, cough and thoracic pain are early signs, while hemoptysis often indicates advanced disease. Relapsing infectious diseases of the respiratory system in combination with a smoking history suggest a need for further diagnostic investigations, including medical history and physical examination, laboratory tests, chest radiography, thoracic CT or MRI, bronchoscopy and biopsy. For staging, additional CT or MRI of the abdomen and the brain, bone scan, and eventually positron emission tomography are used. Serum tumor marker measurements also potentially have an important role in both diagnosis and staging.

Figures on disease outcome measures are very discouraging as even with the most advanced treatment strategies, 86% of lung cancer patients die within 5 yrs of diagnosis. With early detection and treatment, however, the 5-yr survival rate improves dramatically from 20% in patients with stage III lung cancer to 70% in patients with stage I disease [4, 5]. Therefore, early detection of cancer is the key to the success of treatment. Early diagnosis of lung cancer is limited by the fact that the disease usually develops asymptomatically and the available screening methods or tumor markers do not fulfill the requirements for reliable discrimination between patients with lung cancer and subjects not suffering from the disease.

Lung carcinogenesis is a multistep process, characterized by the stepwise accumulation of genetic and molecular abnormalities after carcinogen exposure, resulting in the selection of clonal cells with uncontrolled growth capacities. Uncontrolled growth of a cell can be summarized as the dysbalance between the activation of oncogenes, which drive the cell to multiply, and the inactivation of tumour suppressor genes. One of the most inactivated tumor suppressor genes in human cancer and the most intensively studied is p53 tumor suppressor gene.

The identification and characterization of the p53 protein relied extensively on immunological methods. Antibodies directed against p53 protein have been valuable tools for investigating the structure-function relationship of wild-type and mutant p53 as well as other p53 related proteins such as p63 and p73. Monoclonal antibodies raised against *xenopus* p53 have been demonstrated to cross-react with human p73 [(6)]. The p53 proteins of both mouse and human origin are detectable with PAb240, reflecting a high sequence homology of the p53 protein between these two species. The epitope for the monoclonal PAb421 antibody [(7, 8)] is also highly conserved between human and mouse [(9)].

The CM5 polyclonal antibody (CM5pAb) from rabbits immunised with mouse wild-type p53 was originally developed for an immunohistochemical analysis of mouse p53 expression [(10)]. It has high affinity for mouse p53 and low affinity for human p53 and is also very useful in immunoblotting of mouse p53. CM5pAb recognizes several antibody epitopes of the mouse p53 protein, including the epitope for PAb240 [(9)]. Using an anti-mouse p53 CM5pAb to perform immunoblotting of human lung tumor tissues, we unexpectedly found that the CM5pAb cross-reacted with an unknown human protein with an apparent molecular weight of 95 kDa. The

CM5-reactive protein was found to overexpress in almost all of human lung tumor tissues, with only minimal expression in normal adjacent lung tissues. The induction of this unknown protein in tumor tissues indicated its crucial role in lung tumorigenesis and thus might become a novel biomarker in aiding the screening of lung cancer. Therefore, the objectives of this study were to identify and characterize this protein.

1.2 Literature review

1.2.1 Cancer

Cancer is a disease of dysregulation of cell growth which capable of invading adjacent tissue and metastasis. The most fundamental characteristic of cells is their ability to reproduce themselves. They do this simply by dividing. One cell becomes two. The two become four and so on. The division of normal cells occurs in a regulated fashion. In most parts of the body, the cells continually divide and form new cells to supply the material for growth or injured cells. For example, when the skin is cut, certain cells divide rapidly until the tissue is healed and the skin is repaired. Afterward, they will go back to their normal rate of division. In contrast, cancer cells divide in a disorganized manner. The result is that they typically pile up into a non-structured mass or tumor. There is a disease that is similar to cancer but not cancer. It can be called a benign tumor. A benign tumor does not spread to the other parts of body, but cancers or malignant are capable of spreading throughout the body by two mechanisms, which are invasion and metastasis. Invasion refers to the direct migration and penetration by cancer cells into neighboring tissues. Metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, to

circulate through the bloodstream, and then to invade normal tissues elsewhere in the body.

Cancer is caused by both internal and external factors. Internal factors are those related to family history, genetic or DNA abnormalities, as well as abnormalities of the immune system. External factors include lifestyle behaviors that can affect health and lead to cancer; these include poor diet, smoking and lack of exercise. But other external risk factors such as hepatitis B, hepatitis C, and excessive exposure to x-ray or ultraviolet radiation can also lead to cancer [(11)].

Molecular basis of cancer is the cell cycle's problem. The cell cycle is a series of ordered events that occur in a cell, initial by formation, and final step by duplication and division into two daughter cells. Cells in human body normally reproduce up to about 50 times, doubling their number with each cell cycle. Stem cells provide a pool of dividing cells to replace those that are died. Interphase, the period of cell divisions, is the most cells that remain for at least 90% of the time in cell cycle. Interphase consists of three phases such as G1 (Gap1), S (Synthesis) and G2 (Gap2) phase. During G1 phase, the cell undergoes rapid growth and metabolic activity, including production of RNA and synthesis of protein. For the cell which to divide and produce an identical its copy, its genome must be duplicated. DNA replication occurs in S phase. In G2 phase, cell growth continues and the cell prepares for division that occurs in M (Mitosis) phase, respectively. In normal cells, during G1 phase there are specific genes that control the speed of the cell cycle. These genes, called tumor suppressor and oncogenes, are mutated (meaning damaged) in cancer cells and can result in uncontrolled reproduction. Additionally, unlike normal cells, cancer cells do not stop reproducing after about 50 divisions. Thus, a

cancer is an uncontrolled proliferation of cells. Cancer may begin because of the accumulation of mutations involving oncogenes, tumor suppressor genes, and DNA repair genes. For example, colon cancer can begin with a defect in a tumor suppressor gene (APC) that allows excessive cell proliferation. The proliferating cells then tend to acquire additional mutations involving DNA repair genes, other tumor suppressor genes, and many other growth-related genes. Overtime, the accumulated damage can yield a highly malignant, metastatic tumor. In other words, creating a cancer cell requires that the brakes on cell growth (tumor suppressor genes) be released at the same time that the accelerators for cell growth (oncogenes) are being activated. Abnormal genes may be inherited at the birth or acquired after birth. The normal operation of our physiological system continuously produces free radicals, which could damage DNA, thereby resulting in gene mutations. DNA may also be damaged by exposure to radiation or chemicals (carcinogens). As a number of mutations do accumulate, that's why a 75-year-old person is a hundred times more likely to develop colon cancer than a 25-year-old because the older person has a longer exposure time to factors that may promote gene mutations linked to cancer.

1.2.2 Lung cancer

Lung cancer is major public health problem and cause of death both in developed and developing countries. It has been recognized that lung cancer arises as the consequence of an accumulation of multiple somatic genetic alterations involving critical genes, chromosome rearrangements, microsatellite instability, deregulated expression of telomerase and angiogenesis. Lung cancer is a highly lethal disease. The outcome of lung cancer has not changed considerably despite substantial

therapeutic progress, due to the tendency of detection at a late stage. The 1 year relative survival rate increased from 34% in 1975 to 41% in 1997, due to improvements in surgical techniques [12]. Survival at 5 years, measured by the Surveillance, Epidemiology, and End results program (SEER) in the United States, was 16% when recorded at the population level [13]. The average survival in Europe was 10% and in developing countries 8.9% [14]. Smoking is responsible for at least 80 percent of all lung cancer cases, which means that lung cancer is highly preventable. Other risk factors include exposure to secondhand smoke, asbestos and air pollution.

Nonsmokers who breathe in the smoke of others (secondhand tobacco smoke or environmental tobacco smoke (ETS)) are also at increased risk of lung cancer. Numerous researchers studied the association between ETS and lung cancer and reported that a nonsmoking spouse of a smoker had a significant dose-response increase in risk of lung cancer according to exposure to ETS [15]. The International Agency for Research on Cancer (IARC) estimated an increased risk of developing lung cancer from ETS of 30% in males and 20% in females when compare with males and females not exposed to ETS. In addition, nonsmokers who were exposed to ETS at the workplace found a statistically significant increase in risk of 12-19% [16].

Ambient air pollution is a complex mixture of substances, chiefly due to combustion of fossil fuels, motor vehicles, bonfires, road dust and dust storms. There has been an accumulation of evidence from different types of studies showing that ambient air pollution has an effect on lung carcinogenesis. The increased risk of lung cancer from ambient air pollution may be similar to the risks from passive smoking, i.e. 20-30% [17].

Lung carcinogenesis, like any other type of cancer, is a multistep process, characterized by the stepwise accumulation of genetic and molecular abnormalities after carcinogen exposure, resulting in the selection of clonal cells with uncontrolled growth capacities. Uncontrolled growth of a cell can be summarized as the dysbalance between the activation of oncogenes, which drive the cell to multiply and migrate, and the inactivation of tumour suppressor genes.

1.2.3 p53 Tumor suppressor gene

Tumor suppressor gene or anti-oncogene is a gene that protects a cell from one step on the path to cancer. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes. Tumor suppressors are genes that either slow down cell division, inducing DNA repair or triggering cell death (a process known as apoptosis or programmed cell death). These processes are all interconnected. Throughout the cell cycle there are DNA damage checkpoints; if there is damage, DNA replication is paused while the damage is repaired. In the event that the damage cannot be repaired, the cell initiates apoptosis. The analogy is often made between tumor suppressor and the brake on a car. Just as the brake keeps the car from going too fast, tumor suppressor keeps the cell from dividing too quickly. When a tumor suppressor gene is mutated or inactivated (meaning turned off; also referred to as “loss of function”), cells can grow out of control and lead to cancer. One of the most intensively studied tumor suppressor gene is p53. The p53 was first identified thirty years ago as a cellular partner of simian virus 40 large T-antigen, the oncoprotein of this tumor virus [18, 19]. The first decade of p53 research involved cloning of p53 DNA and the realization that

p53 is not an oncogene, but a tumor suppressor that is frequently mutated in human cancer. In the second decade of research, the function of p53 was uncovered: it is a transcription factor induced by stress, which can promote cell cycle arrest, apoptosis and senescence. In the third decade after its discovery new functions of this protein were revealed, including the regulation of metabolic pathways and cytokines that are required for embryo implantation. The fourth decade of research may involve identification of new p53-based drugs to treat cancer[19].

The p53 gene is located on chromosome 17q13 and encodes a nuclear 393-amino acid nuclear transcription factor that is implicated in the regulation of normal cell growth and apoptosis. Human p53 protein can be divided into five domains, each corresponding to specific functions. 1) The amino-terminus part 1-39 contains the acidic transactivation domain and the MDM2 protein binding site. 2) Region 40 - 100 contains series repeated proline residues that are conserved in the majority of p53. 3) The central region (101 – 306) contains the DNA binding domain. It is the target of 90% of p53 mutations found in human cancers. 4) The oligomerization domain (307 – 355) consists of a beta-strand, followed by an alpha-helix necessary for dimerization, as p53 is composed of a dimer of two dimers. A nuclear export signal is localized in this oligomerization domain. 5) The carboxy-terminus of p53 (356 – 393) contains 3 nuclear localization signals and a non-specific DNA binding domain that bind to damaged DNA. This region is also involved in downregulation of DNA binding of the central domain [20-22]. The p53 has several biological effects involving cell-cycle arrest, DNA replication and repair, proliferation, apoptosis, angiogenesis inhibition, and cellular stress response [23, 24].

The native or wild type of p53 is believed to control cell division by regulating the entry into the S phase. This controlling effect of p53 may be lost by deletion of the gene or production of a competing mutant protein. An example is given by a well molecular characterized colon cancer. Seventy-five to eighty percent of colon carcinomas show deletion in one p53 allele and a point mutation in the other allele; thus no wild type of p53 protein is expressed in these tumors. Allelic deletion of p53 occurs only rarely in adenomas (10%), suggesting that p53 inactivation may be a relatively late event in colon carcinogenesis (reviewed in (25)). In addition, up to 70% of breast cancers also have detected p53 [26, 27]. Mutations of p53 produce proteins that inactivate the wild type of p53 protein and allow cells to move through the cell cycle and contribute to the autonomous growth of cancer. A number of different mutations of p53 have been found in human cancers. Most point mutations are localized in four regions of the protein (amino acid residues 117- 142, 171-181, 134-158 and 270-286); three "hot spots" affect residue 175, 248 and 273. Monoclonal antibodies to mutated p53 proteins have been developed. The wild type of p53 is normally present in very small amounts that are not detected by immunohistochmistry, whereas the mutant protein accumulates to easily detectable amounts. Overexpression of the mutant proteins has been detected in up to 70% of primary colorectal cancers [28]. Overexpression of p53 in breast cancers is associated with poor prognosis [29, 30]. Circulated antibodies to mutant p53 proteins have been found in sera from patients with breast and lung cancer and B-cell lymphomas [31]. This antibody response may be useful in this subset of patients for monitoring for relapse.

1.2.4 Response of p53 to stress signals

Cells are continuously subjected to DNA lesions arising both from environmental conditions and from the intrinsic metabolism of a cell. Such lesions can lead to mutations and large-scale genome alterations that may be deleterious for cellular function. To maintain genomic stability cell cycle checkpoints exist that can detect errors during DNA replication. If errors are encountered, cell division is paused and repair mechanisms and/or cell death ensues. The p53 tumor suppressor protein plays an important role in this process [(32)]. By being part of a signal transduction process, p53 relays information leading to cellular responses such as cell cycle arrest and apoptosis, resulting from DNA lesions. P53 activity is regulated mainly at the protein level. In response to DNA lesions, p53 is rescued from targeted degradation, which leads to a strong increase in the amount of the otherwise short-lived tumor suppressor protein, and the protein is intensively modified [(33)]. Cells deficient in p53 fail to undergo apoptosis or cell cycle arrest in response to DNA damage which increases the rates of tumorigenicity and genomic instability in these animals [(34-37)]. DNA damage leads to genotoxic stress.

A key player in the regulation of p53 is the Mdm2 protein. Mdm2 is the product of an oncogene, whose excess activity facilitates several types of human cancer [(38)]. Mdm2 exhibits a unique relationship with p53. On the one hand, the Mdm2 protein binds to p53 and inactivates it. The binding occurs within the p53 transactivation domain, interfering with recruitment of basal transcription machinery components [(39)]. Moreover, Mdm2 can actively repress transcription when bound to p53 [(40)]. Importantly, Mdm2 binding can also lead to complete elimination of p53 through proteolytic degradation [(41)]. On the other hand, p53 binds specifically to the *mdm2*

gene and stimulates its transcription [42]. This duality defines a negative feedback loop (Figure1.1), which probably serves to keep p53 in tight check and to terminate the p53 signal once the triggering stress has been effectively dealt with.

Under stress conditions, the p53 protein accumulates in the cell, binds in its tetrameric form to p53-response elements and induces the transcription of transcription of various genes that are involed in cell-cycle control, apoptosis, DNA repair, differentiation and senescence. The loss of p53 tumor suppressor activity by mutation or deletion of *TP53* or inhibition of p53 allows the proliferation of the cells that are damaged under the stress conditions. This uncontrolled proliferation can lead to tumor development. It is nowadays known that p53 is not functional or functions incorrectly in most human cancers, and that it plays a crucial role in the prevention of tumor development. The general assumption is that the p53 network in normal, non-activated situations is non-functional, but is activated in cells as a response to various signals that take place in the carcinogenic process [43]. The main form of stress that activates p53 is genotoxic stress in the form of DNA double-strand breaks (DSBs) or stalled DNA replication forks [35, 43]. If left unchecked by p53, genotoxic stress can lead to loss of genomic integrity and cancer development, as exemplified by the phenotype of mice lacking the p53 gene and the high frequency of p53 gene inactivation in human cancer [37, 44].

However, some studies found that a cellular stress inhibited p53 function [45]. When the unfolded protein response (UPR) was induced specifically in the endoplasmic reticulum (ER), this type of stress often referred to as ER stress, p53's function was inhibited.

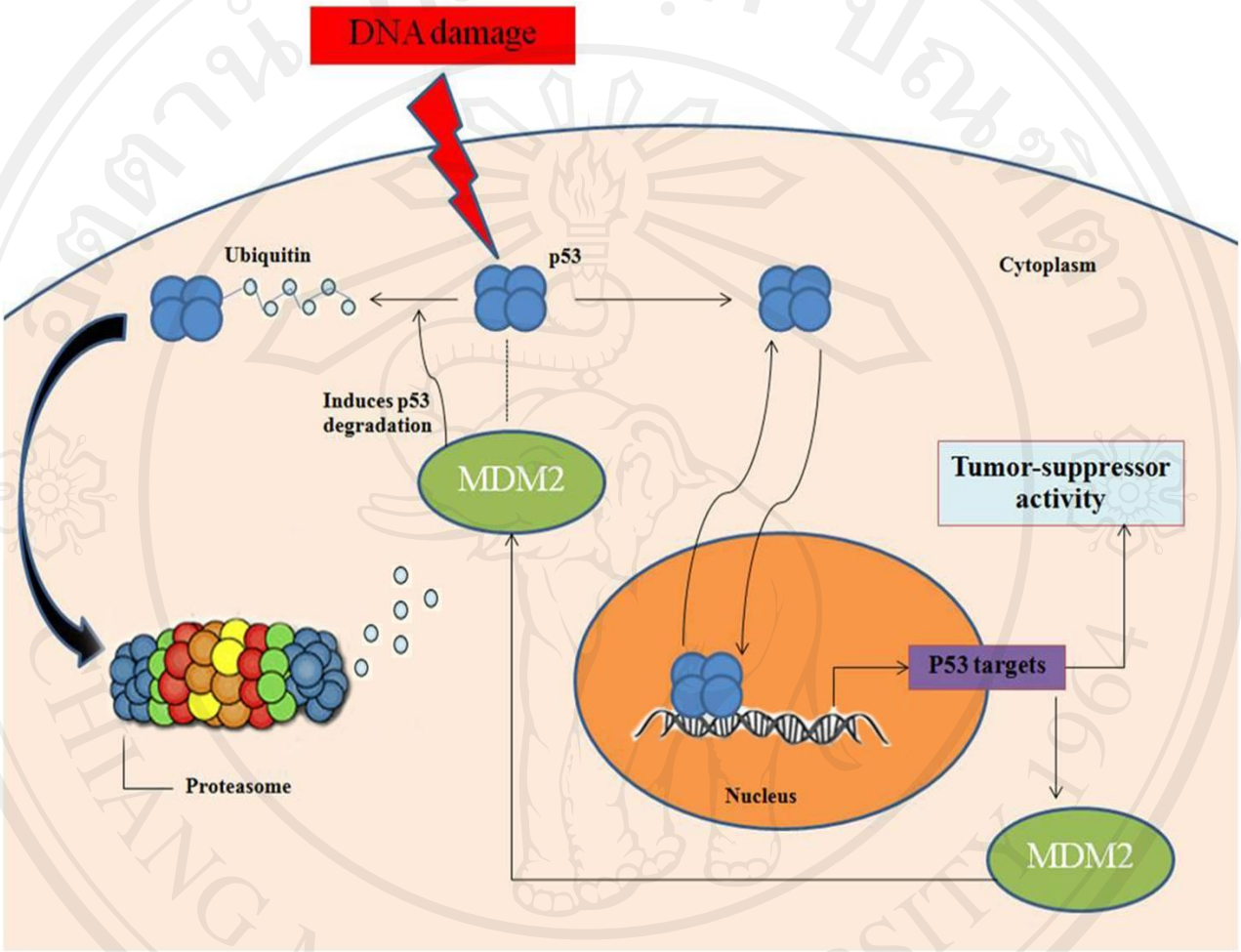

Figure 1.1 Regulation of p53. p53 and MDM2 form an auto-regulatory feedback loop. p53 stimulates the expression of MDM2; MDM2 inhibits p53 acivity because it blocks its transcriptional activity, favours its nuclear export ant stimulates its degradation. Different cellular signals, such as DNA-damage or oncogene activation, induce p53 activation. DNA damage favours p53 phosphorylation, preventing its association with MDM2.

The ER lumen is the site where secretory proteins adopt their native conformation. The folding process is in general quite complicated, because folding of secretory proteins proceeds hand-in-hand with several posttranslational modifications, including most prominently N-linked glycosylation and disulfide bond formation. The accumulation of unfolded proteins above a certain threshold in the ER lumen leads to a response referred to as ER stress [46, 47]. Experimentally, ER stress can be induced by agents that interfere with the function of ER-resident glycosylases (such as tunicamycin), agents that lower calcium levels in the ER (such as thapsigargin and ionomycin), and agents that inhibit disulfide bond formation. In physiological conditions, unfolded proteins accumulate in the ER is induced under conditions of glucose starvation, hypoxia, and increased temperature. Cells have an elaborate quality-control system to ensure that protein folding proceeds normally in the ER under normal situation and in responded to ER stress. This system involves protein chaperones of the heat shock family that can discriminate between native and nonnative folds. When these chaperones become saturated by unfolded proteins, the ER sensors, ATF6, PERK, and IRE1, become activated, leading to increased synthesis of ER-resident chaperones and folding enzymes and transient suppression of new protein synthesis [46]. Together, these changes restore the balance between newly synthesized unfolded proteins entering the ER and folded proteins being secreted [46, 47]. ER stress and, more broadly, conditions that favor protein unfolding could nonspecifically stall DNA replication leading to p53 activation and apoptosis [45]. While, genotoxic stress resulted in an increased of p53 nuclear localization [49], ER stress increased localization of p53 to the cytoplasm [48, 50].

1.2.5 The p53 tumor suppressor gene family

For a long period of time, p53 was believed to be a unique protein, with no obvious relative. This was changed by the discovery of two additional members of the family, p63 and p73 [51]. p63 (3q27.29) and p73 (1p36) recently emerged as sharing overall architectural similarities with p53. Phylogeny indicates that these three genes derive from a common ancestor thus defining a new gene family [52, 53]. Both p63 and p73 have essential roles in development (including that of the skin, the nervous system and female reproductive organs) [54-56] and can under some circumstances, function as tumor suppressors [57, 58]. Two genes have been found to encode proteins that share significant amino acid identity in the transactivation domain (30%), the DNA binding domain (60%) and the oligomerization domain (37%). Curiously, p53, p63 and p73 have closely related DNA-binding domains, bind to similar DNA sequences and can induce the transcription of some of the same genes, but can also induce the transcription of different genes in specific cell types [59]. All three genes can bind to similar DNA consensus sequences in the promoters of many genes and regulate common generic aspects of growth control, survival, DNA repair or differentiation. However, their regulation patterns are distinct. While p53 is ubiquitous, stress-response protein regulated at the post-translational level, p63 and p73 are expressed in a tissue and differentiation-specific manner and are also regulated at the transcriptional level. Furthermore, p53 itself is not a single protein. Over the past few years it has become clear that, through extensive alternative splicing and alternative transcriptional initiation, p53 produces as many as nine different isoforms, containing various combinations of alternative amino-terminal and carboxy-terminal portions of the protein [19]. Notably, deletion of the N-terminus

produces a dominant-negative repressor of p53-regulated genes. Some of the p53 isoforms are observed in different tissues and during different stages of development. The precise roles of each of the reported p53 isoforms remain largely unknown [60]. Nevertheless, there was clinical evidence that the p53 family is frequently overexpressed in lung cancer specimens [61].

1.2.6 Antibodies and the analysis of p53 protein

Expression of p53 is constitutively repressed in most tissues and is thus almost undetectable by immunological methods in normal cells. It can, however, be detected in a variety of tumor tissues because of the extended half-life of the wild-type or mutated-type protein. The earlier clinical studies of p53 involved using immunological methods such as immunohistochemistry, western blotting or ELISA to demonstrate the accumulation of p53 protein in tumor tissues [6-9]. A variety of antibodies have been developed for immunological detection of p53. The mouse p53 protein can be detected with monoclonal PAb240 and PAb421 antibodies and with CM5 serum, among others. The monoclonal PAb240 antibody[62] detects both mutated and wild-type proteins under denaturing conditions, e.g. in immunoblotting. The p53 proteins of both mouse and human origin are detectable with PAb240, reflecting the high level of sequence homology of the p53 protein between these species. The epitope for the monoclonal PAb421 antibody[8] is also highly conserved between human and mouse [9]. The CM5 polyclonal serum from rabbits immunised with mouse wild-type p53 was originally developed for an immunohistochemical analysis of mouse p53 expression [10]. It has high affinity for mouse p53 and low affinity for human p53 and is also very useful in immunoblotting. CM5 recognizes

several antibody epitopes of the mouse p53 protein, including the epitope for PAb240[9].

Antibodies are soluble immunoglobulins (Ig) that react specifically with the antigen, of blood plasma. The most antigens refer mainly to proteins and peptides. The common antigen usually exhibits several different epitopes. Monoclonal antibodies are different from polyclonal antibodies. The former are produced by plasma cell clones: the latter are produced by different plasma cells. The result is that monoclonal antibodies are mono-specific. They recognize and bind with only a single epitope of the antigen, while polyclonal ones are found of multiple different epitopes of an antigen. If an animal is immunized with an antigen, a population mix of antibodies is formed, each differing from the others in its epitope specificity. All representatives possess the same antigen specificity, but they are directed against different epitopes of the antigen. A population mix is called a polyclonal antibody. The striking feature of antibody-antigen interaction is its specificity. The forces derive from hydrogen bond, electrostatic interactions between charged side-chains, van der Waals forces and hydrophobic interactions. Today, monoclonal antibodies, homogeneous antibodies from a single clone of B cells, can be prepared in virtually unlimited quantities. This has been useful for studies of immunoglobulin structure and function, for clinical investigations, and for therapy. Occasionally an antibody binds to more than one antigen. This is referred to as cross-reactivity or multispecificity. The antibody is specific for antigen 1, but a different molecule, antigen 2, fits well enough to create a stable binding interaction. This happens because there are a sufficient number of chemical interactions between the antigen and the antibody to create a stable structure, regardless of the total “goodness of fit”.

Consequently, polyclonal antibody is probable for cross-reactions more than monoclonal antibody.

1.2.7 Our previous study involved searching for a p53 structurally-related protein

Mutations in the gene of the tumor suppressor p53 represent the most frequent genetic alterations in human cancer, affecting about 50% of all individual tumors [(63)]. A number of p53-related proteins have been identified, including p63 and p73, and believed to be in the same family. Furthermore, it has been reported that monoclonal antibodies raised against p53 of *xenopus* origin could cross-interact with human p73 [(6)], indicating the conservation of protein structure of the p53 related protein in animal with different evolution. In an attempt to search for a novel cancer biomarker, the CM5 polyclonal antibody (CM5 pAb) raised against p53 of mouse origin was used to perform western blot analysis of tissue lysate prepared from human lung tumors [(64)]. The purpose of this experiment was to identify p53 structurally related protein(s) that could play an important role in promoting or preventing lung cancer and may potentially become a novel cancer biomarker.

The CM5pAb was found to react with an unknown human protein with an apparent molecular weight of 95 kDa. The overexpressions of this unknown protein in tumor versus normal adjacent lung tissues are shown in Figure 1.2. It appeared that the human p53 was not recognizable by the CM5pAb as no band at 53 kDa was detected, although a number of tumor tissues showed a positive band with DO7 anti-human p53 monoclonal antibody (Figure 1.2). The high frequency of overexpression of this unknown protein in lung tumor tissues indicates its crucial role in lung

tumorigenesis and thus a potential valuable biomarker in aiding screening and/or diagnosis of lung cancer.

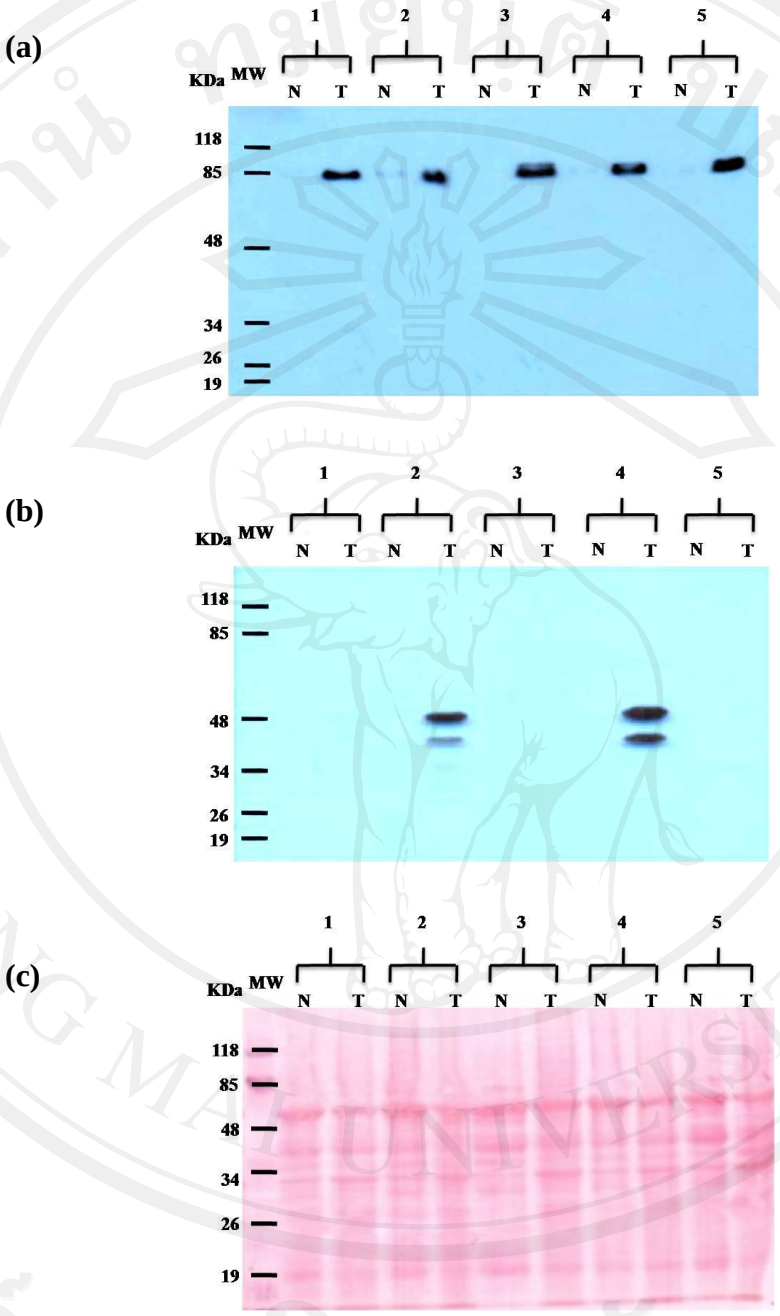

Figure 1.2 Expression of CM5-reactive proteins (a) and p53 (b) in lung tumor tissues and the corresponding adjacent normal tissues determined by western blot analysis using CM5pAb and anti-human p53 mAb (DO7), respectively [64]. The Ponceau S staining of PVDF membrane after chemiluminescence detection was used as an internal control indicating level of total protein loaded in each lane (c). T, tumor tissue; N, normal tissue and MW, protein molecular weight marker.

1.3 Objectives

1. To identify the human protein that reacted with CM5pAb (Rabbit polyclonal antibody raised against p53 protein of mouse origin)
2. To characterize the identified protein in term of its response to stress signals including genotoxic stress and ER stress in comparison to p53 tumor suppressor