CHAPTER I

INTRODUCTION

1. Carcinogenesis

Carcinogenesis is the process by which normal cells are transformed into cancer cells. This process is caused by the mutation of the genetic material which governs proliferation and cell death in normal cells. This results in uncontrolled cell division and the evolution of those mutated cells by natural selection in the body. The uncontrolled and often rapid proliferation of cells can lead to cancer. The gene mutations can have various causes like radiation or chemicals also called carcinogens. The latter possess the ability to damage the genome or to disrupt various cellular metabolic processes.

A carcinogen refers to any substance or radiation that is an agent directly involved in the promotion of cancer or in the increase of its propagation and can be classified as genotoxic or non-genotoxic. Genotoxins cause irreversible genetic damage or mutations by binding to deoxyribonucleic acid (DNA). Genotoxins include chemical agents like N-nitroso-N-methylurea (MNU) or non-chemical agents such as ultraviolet light and ionizing radiation. Certain viruses can also act as carcinogens by interacting with DNA. Non-genotoxins include hormones and some organic compounds do not directly affect DNA but act in other ways to promote cell growth (Longe, 2005).

Recently, the role of mitochondria in carcinogenesis has been the object of numerous investigations, in part because of their prominent role in apoptosis and reactive oxygen species (ROS) production. The mitochondrial genome is particularly susceptible to mutations because of the high level of ROS generation in this organelle and a relatively low level of DNA repair. Mitochondrial dysfunction is a hallmark of almost all diseases. Acquired or inherited mutations of the mitochondrial genome DNA may give rise to mitochondrial diseases. Another class of disorders, in which mitochondrial impairments are initiated by extramitochondrial factors, includes neurodegenerative diseases and syndromes resulting from typical pathological processes, such as hypoxia/ischemia, inflammation, and carcinogenesis. Otto Warburg has be observed abnormally high glycolysis and lactate production in oxygenated cancer cells suggesting that defects in mitochondrial functions might represent the main transformation of malignant cells (Warburg *et al*., 1930). In contrast, normal cells mainly generate energy from oxidative breakdown of pyruvate (oxidative phosphorylation) (Warburg *et al*., 1930). These observations known as the Warburg effect illustrated a fundamental difference between normal and cancer cells that is the ratio of glycolysis to respiration. The metabolic shift from oxidative phosphorylation to aerobic glycolysis (Warburg effect) (Warburg *et al*., 1930) tolerance to hypoxic microenvironment, ability to control ROS levels and avoidance of apoptosis are the hallmarks of cancer cells, greatly contributing to their viability, autonomous growth, migration and chemoresistance (Eapen *et al*., 1983, Burgart *et al*., 1995, Hanahan *et al*., 2000 and Zörning *et al*., 2001). The major function of mitochondria is the generation of ATP, the energy currency of the cell, by oxidative phosphorylation. During this process ROS are produced. It has been reported that ROS are generated

from mitochondria in the electron transport chain (ETC)-inhibited and mitochondrial DNA-damaged cells, which have impaired ETC (Indo *et al*., 2007). ROS can cause oxidative damage of mitochondrial DNA and increase tumorigenicity (Sasaki *et al*., 2008, Dasgupta *et al*, 2008) as well as metastatic ability (Ishikawa *et al*., 2008). Thus mitochondria could play an important role in tumor development. Mitochondrial dysfunction, one of the most notable features of cancer cells, can be caused by molecular defects in mitochondrial or nuclear genes that encode proteins involved in mitochondria biogenesis, respiratory chain assembly and function, maintenance of membrane potential and energy metabolic or signal transducing pathways. Ma and coworkers in 2009 have demonstrated that mitochondria in breast cancer cells have been altered, genetically and functionally. These alterations can send signals to the nucleus, which in turn can regulate the expression of genes involved in energy metabolism and tumorigenic properties (Ma *et al*., 2009). Reungpatthanaphong and coworkers in 2003 have reported that the mitochondria from multidrug resistant cancer cells exhibited defects in their electron transport chain and antioxidant system compared with their corresponding drug sensitive cells (Reungpatthanaphong *et al*., 2003). Indeed the mitochondrial dysfunction might be caused by the excess of superoxide anion ($O_2^{-\bullet}$) concentration (Kothan, 2004).

2. Normal and cancer cell biochemistry and physiology

2.1 Cellular energetic state

Cellular homeostasis requires permanent energy production and consumption. Adenosine triphosphate (ATP) is the major energy component for the cell. Its synthesis occurs mainly in mitochondria where the oxidative phosphorylations realize

the coupling between oxygen consumption and phosphorylation of adenosine diphosphate. The energy stored in ATP can then be used to drive various energy-requiring processes, including biosynthesis, locomotion or transportation of molecules across cell membranes. Normal cells obtain the energy they require from anaerobic or aerobic respiration. Aerobic respiration requires oxygen in order to proceed, whereas anaerobic respiration can proceed in the absence of oxygen. Aerobic respiration in eukaryotes consists of four processes: glycolysis, pyruvate decarboxylation, the citric acid cycle and finally oxidative phosphorylation which occurs in the mitochondria. (Voet D and Vote JG, 1995).Glycolysis is the first pathway in both aerobic and anaerobic respiration and this reaction take place in the cytosol of cells. Glycolysis is the anaerobic metabolic pathway by which molecule glucose is oxidized to form two molecules pyruvate. In aerobic respiration glycolysis is followed by a pyruvate decarboxylation reaction to convert pyruvate to acetyl-CoA used in the citric acid cycle. The citric acid cycle, or tricarboxylic acid cycle (TCA) or Krebs cycle, is a ten step cycle in which one molecule of guanosine triphosphate (GTP), three molecules of nicotinamide adenine dinucleotide (reduced form) (NADH), one molecule of flavin adenine dinucleotide (reduced form) ($FADH_2$). Oxidative phosphorylation is the final step in aerobic respiration, one that occurs in the mitochondria. During oxidative phosphorylation electron are transferred from NADH or $FADH_2$, created in glycolysis, fatty acid metabolism and the Krebs cycle, to molecular oxygen via a series of protein complexes located in the inner mitochondrial membrane. Protons are pumped from the mitochondrial matrix into the intermembrane space as a result of this flow of electrons. This generates a pH gradient and transmembrane electrical potential across the mitochondrial membrane. This is a form of potential energy

which is referred to as a proton-motive force. The protons flow back into the mitochondrial matrix via the large protein complex ATP synthase. It has been reported that cancer cells derive most of their energy for growth from anaerobic glycolysis in the cytoplasm where glucose is converted to lactate and that this is due to some impairment of the mitochondria in these cells (Warburg *et al*., 1930).The by-product of the anaerobic glycolysis is increased levels of lactic acid. The large amount of lactic acid produced by cancer cells is then transported to the liver. This conversion of glucose to lactate creates a lower, more acidic pH in cancerous tissue. The hallmarks of cancer growth, increased glycolysis and lactate production in cancer, have raised attention recently due to novel observations suggesting a wide spectrum of oxidative phosphorylation deficits and decreased availability of ATP associated with cancer cell expansion. The reason for the predominance of glycolysis, rather than the more energy efficient process of oxidative phosphorylation within the mitochondria is not clear. There is postulation that the mitochondria are somehow damaged by the process that causes the cell to become cancer and unable to convert pyruvate to ATP (Warburg *et al*., 1930).

2.2 Cellular redox state

2.2.1 Cellular oxidants and antioxidants

Cellular oxidants, derivatives of oxygen, which are often called reactive oxygen species (ROS), are constantly produced in our cells. Among cellular ROS, the most aggressive entities are superoxides and hydroxyl radicals. There are a few main sources of ROS in our body. ROS are generated by mitochondria (Figure 1) via the release of electrons from the electron transport chain and the reduction of

oxygen molecules to superoxide anion ($O_2^{-\bullet}$). Superoxide anion, through the reaction catalyzed by superoxide dismutase (SOD), are transformed into the much less reactive hydrogen peroxide (H_2O_2). However, when hydrogen peroxide interacts with ions of transition metals such as iron or copper, the most reactive ROS, hydroxyl radicals ($OH^{\bullet}$) are formed (Fenton reaction). Other sources of ROS, located in the endoplasmic reticulum, are cytochrome P450 complexes (Figure 2), which generate superoxides to metabolize toxic hydrophobic compounds (Shenkman, 1993). Important sources of ROS are phagocytes (Figure. 2), which produce superoxides, hydrogen peroxide and hydroxyl radicals to kill infectious microorganisms (Babior, 1978) and cancer cells (Halliwell and Cutteridge, 1999 and Alexander, 1983).

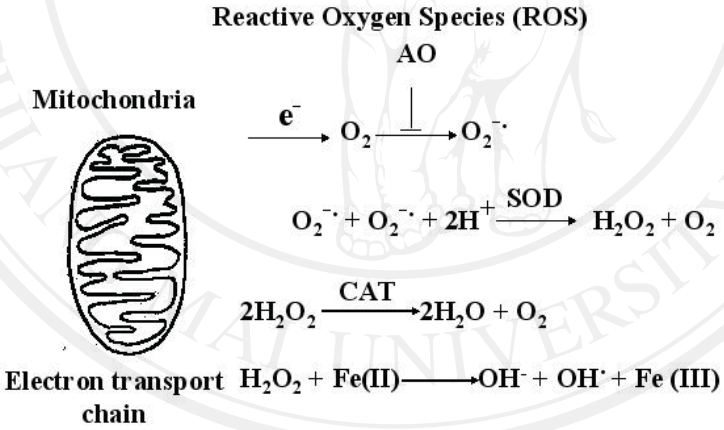

Figure 1. The generation of ROS by mitochondria. Electrons released from mitochondria reduce oxygen molecules, thereby producing such ROS as superoxides ($O_2^{-\bullet}$). Superoxide dismutase (SOD) catalyzes H_2O_2 formation from superoxides. H_2O_2 might be deactivated by catalase (CAT). However, when H_2O_2 reacts with iron or copper ions, hydroxyl radicals ($OH^{\bullet}$), the most reactive form of ROS, are produced. Excessive anti-oxidants (AO) can inhibit production of $O_2^{-\bullet}$ and other ROS. (modified from Salganik, 2001)

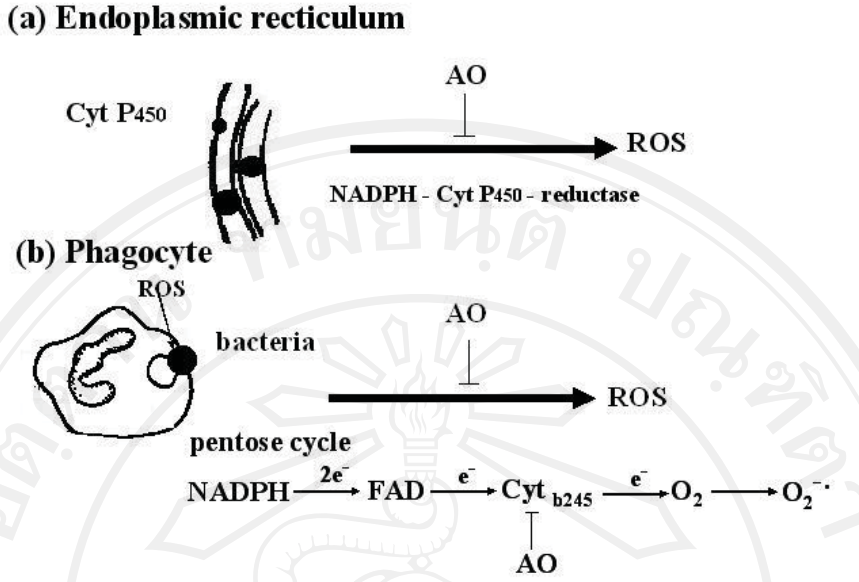

Figure 2. (a) ROS generated by microsomal mono-oxygenases, which have cytochrome P450 as a central link. Oxidation is the way to transform hydrophobic toxic substances, drugs, steroids etc., and thereby remove them. Excessive anti-oxidants (AO) can inhibit this protective function. (b) ROS generated by phagocytes kill infectious microorganisms and cancer cells. Excessive antioxidants can inhibit this protective mechanism. (modified from Salganik, 2001)

Production of ROS is essential for a number of biochemical reactions involved in the synthesis of prostaglandins, hydroxylation of proline and lysine, oxidation of xanthine and other physiological oxidative processes (Halliwell and Cutteridge, 1999). Numerous data demonstrate that ROS are capable of oxidizing cell constituents such as DNA, proteins, and lipids, thereby incurring oxidative damage to cell structures. Excessive oxidation leads to impairment of cell functions and development of morbid conditions (Halliwell and Cutteridge, 1999, Aims *et al*., 1993). Besides ROS, cells also generate reactive nitrogen species (NOS) such as nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and peroxynitrite ($ONOO^{\bullet}$) (Moncada, 1991). Nitric oxide and

nitrogen dioxide carry out a number of physiological functions. Excessive $NO^{\bullet}$, $NO_2^{\bullet}$ and $ONOO^{\bullet}$ damage cell constituents.

Table 1. Principal cellular anti-oxidants that scavenge or inactivate excessive ROS and thereby protect cells from oxidative damage.

Anti-oxidant Defense		
ROS Scavenging Agents	**ROS Protective enzymes**	**Sequestration of transition metal ions**
• Glutathione • Uric acid • Ascorbic acid • Albumin	• Superoxide dismutase • Catalase • Glutathione peroxidase • Glutathione reductase	• Transferrin • Ferritin • Metallothioneins • Cerulloplasmin

An array of powerful cellular anti-oxidants protects cells from excessive oxidation (Table 1). Among the endogenous anti-oxidants that scavenge ROS are glutathione, ubiquinol, bilirubin, uric acid, albumin and others. Potent anti-oxidant enzymes such as superoxide dismutase and catalase protect cells from oxidative damage by inactivating ROS. Metallothioneins, ferritin, transferrin and ceruloplasmin eliminate ions of transition metals, which are capable of catalyzing the formation of hydroxyl radicals through the Fenton reaction (Halliwell B, 1996 and 2000).

There is large evidence that endogenous anti-oxidants do not completely remove ROS in animal and human cells. This raises the question of why, despite the existence of a powerful cellular system of anti-oxidants, the short-living ROS are not

removed entirely and are permanently present in cells. The reasonable explanation for this phenomenon is that continuously produced ROS are needed to perform some important biological functions (Halliwell and Cutteridge, 1999). Seemingly, the cells are tuned to remove excessive ROS and to leave the required level of oxidants.

Superoxide dismutase (SOD) is one of the major cellular enzymes that protects against toxic effects of ROS. The first SOD was discovered by McCord and coworker in 1969 (McCord *et al*.,1969). Three members of SOD family have been identified in eukaryotes. They are copper-zinc containing SOD (CuZn-SOD), manganese containing SOD (Mn-SOD), and extracellar SOD (Ec-SOD). SOD catalyzes the dismutation of superoxide ($O_2^{\cdot-}$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Mn-SOD represents the major antioxidant enzyme in mitochondria and is encoded by a nuclear gene mapping to locus bq25.3, and its function is often lost in tumor cells due to deletions and /or point mutations (Foulkus *et al*., 1993 and Re *et al*., 2003). The Mn-SOD mRNA migrates to the cytosol and Mn-SOD protein is made in ribosome as a precursor form consisting of 233 amino acids. The Mn-SOD precursor is transported post-translationally into the mitochondrial matrix. The mitochondrial target sequence (MTS) is clipped by a protease in an energy-dependent manner, mature protein for human and most eukaryotic Mn-SOD resides in the mitochondrial matrix (Wispe, 1989). Most Mn-SOD from eukaryotes are homotetramers with four subunits and a total molecular weight of 88 kDa. Manipulation of cellular Mn-SOD levels includes; (i) induction or suppression of endogenous Mn-SOD gene expression by chemicals, heating, radiation, or cytokines such as TNF-α, (ii) direct introduction of exogenous Mn-SOD protein by

liposomal SOD or conjugated enzymes such as polyethylene glycol-SOD (Peg-SOD), (iii) transfection or transduction of exogenous Mn-SOD cDNA by vector such as plasmids or adenovirus and (iv) inhibition of endogenous Mn-SOD expression by antisene or RNAi techniques. Mn-SOD is necessary for aerobic life and modulates intracellular signal pathways and gene expression. The overexpression of catalytically active Mn-SOD reportedly inhibits the proliferation of a wide variety of cancer types (Oberly, 2005), strongly suggesting for Mn-SOD a role as a tumor suppressor protein. The high level of Mn-SOD have been associated with poor survival in multiple malignancies including glioblastoma (Ria *et al*., 2002), gastric cancer (Kim *et al*., 2007) and colorectal carcinoma (Nozoe *et al*., 2003). Increased Mn-SOD protects normal tissue against oxidative stress (Epperlt, 2002). However, overexpression of Mn-SOD exceeding physiological conditions can lead to the accumulation of ROS and oxidative stress, which may contribute to tumor metastasis and angiogenesis (Zhang, 2002). On the other hand, the accumulation of ROS by overexpressing Mn-SOD and inhibition of H_2O_2 removal may beneficial to tumor therapy (Oberley 2001). Increasing Mn-SOD expression can suppress tumor cell growth *in vitro* and tumor formation in nude mice in a large variety of cancer types (Oberley 2001). Mn-SOD transgenic mice showed resistance to chemical induced tumor formation and oxidative stress (Zho, 2001).

2.2.2 The beneficial functions of reactive oxygen species (ROS)

Indeed, ROS play a crucial role in a few lifesaving biological mechanisms. Phagocytic cells protect us from dangerous microorganisms, killing them by producing an avalanche of ROS. When neutrophils and other phagocytic cells

engulf bacteria, they greatly increase consumption of oxygen ("respiratory burst"), which is rapidly transformed to ROS that kill the dangerous intruders. NADPH supplies electrons required for the reduction of oxygen and the formation of ROS (Figure 6). In turn, $NADP^+$ receives electrons from the pentose cycle pathway by NADPH oxidase through cytochrome b_{245} (Rossi and Zatti, 1980). Importantly, by a burst of ROS, phagocytes kill not only invading bacteria (Babior, 1978, Rossi and Zatti, 1980), but also cancer cells (Halliwell and Cutteridge, 1999, Alexander 1983). Excessive antioxidants scavenge these beneficial ROS and can thereby interfere with the protective functions of phagocytes (Cedro *et al.*, 1994).

Detoxification reactions, ensured by the cytochrome P450 family, are dependent on the integrity of the microsomal ROS-generating system. NADPH and NADH supply reducing equivalents for the reduction of cytochrome b_5 and cytochrome P450 (Figure 2). The latter oxidizes hydrophobic toxic substances, steroids and drugs, transforming them into hydrophilic matter, which are removed from the body. In view of the pivotal role of ROS in the functioning of the cytochrome P450 complex, it is reasonable to suggest that excessive antioxidants could interfere with this important cell function.

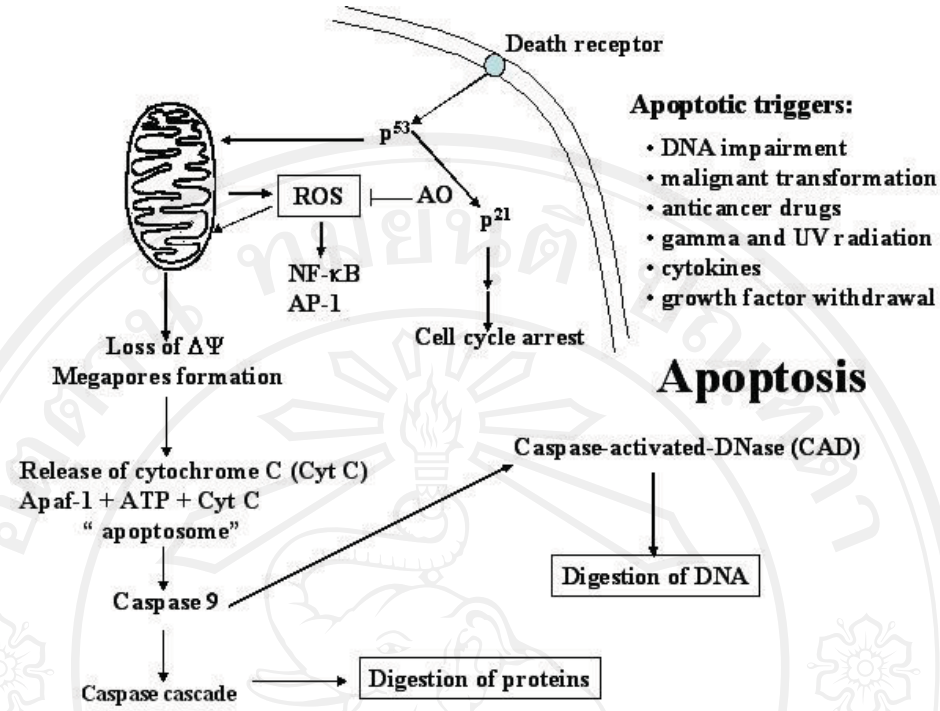

Figure 3. Schematic representation of apoptosis. ROS generated by mitochondria are essential mediators of apoptosis. Together with cytochrome C, Apaf-1, and ATP, released from mitochondria, ROS activate proteolytic enzymes, termed caspases, which promote deoxyribonuclease, and thereby destroy targeted "bad" cells. (modified from Salganik, 2001)

ROS are essential mediators of apoptosis (Figure 3), which eliminate cancer and other cells that threaten our health (Kerr, 1994, Blackstone and Green, 1999, Slater *et al.*, 1995, Johnson et al., 1996, Kroemer *et al.*, 1997, Hickman, 1992). Excessive antioxidants interfere with this highly important protective mechanism (Verhaegen *et al.*, 1995, McGovan *et al.*, 1996, Labriola and Linvingston, 1999, Salganik, 2001). It seems plausible that ROS generation is prevented from being entirely suppressed by endogenous anti-oxidants because of their important beneficial functions. Seemingly, endogenous anti-oxidants might be regulated to scavenge ROS

to a certain level, but not more. The remaining oxidants are required for carrying out apoptosis, phagocytosis, detoxification and other biochemical reactions. Oxidative modification of DNA is also not entirely repaired in healthy animals, despite the existence of potent enzymatic machinery involved in DNA repair (Nickolloff and Hoekstra, 1998).

2.3 Cellular oxidative stress

Endogenously produced reactive oxygen species are essential to life, being involved in several biological functions. However, when the level of anti-oxidants becomes severely depleted or when overproduced, these reactive species become highly harmful, causing oxidative stress through the oxidation of biomolecules, leading to cellular damages that may become irreversible and cause cell death. Oxidative stress is caused by an imbalance between the production of ROS and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, myocardial infarction, Alzheimer's disease and cancer (Albers et al., 2000, Junquerira VBC, 2004).

2.4 Growth/ death

The term cell growth is used in the contexts of cell development and cell division. When used in the context of cell division, it refers to growth of cell population, where one cell grows and divides to produce two daughter cells. Cell

population goes through a type of exponential growth doubling of each new generation. Thus, each generation of cells should be twice as numerous as the previous generation. However, the number of generations only gives a maximum figure as not all cells survive in each generation. Many of the signal molecules that convey information to cells during the control of cellular differentiation or growth are called growth factors. The protein mammalian target of rapamycin (mTOR) is a serine/threonine kinase that regulates translation and cell division (Sulić *et al*., 2005). Nutrient availability influences mTOR so that when cells are not able to grow to normal size they will not undergo cell division.

The process of cell division, called cell cycle, has four major phases. The first phase, called G_1, is marked by synthesis of various enzymes that are required for DNA replication. The second phase defined as the S phase, where DNA replication produces two identical sets of chromosomes. The third phase called G_2 is characterized by protein synthesis, mainly involving the production of microtubules, which are required during the process of division, called mitosis. The fourth phase, M, consists of nuclear division and cytoplasmic division, accompanied by the formation of a new cell membrane. This is the physical division of mother and daughter cells. The M phase has been broken down into several distinct phases, sequentially known as prophase, prometaphase, metaphase, anaphase and telophase leading to cytokinesis.

Cell division is a physiological process that occurs in almost all tissues and under many circumstances. Under normal condition, the balance between proliferation and programmed cell death, usually in the form of apoptosis, is maintained by tightly regulating both processes to ensure the integrity of organs and

tissues. A series of growth disorders can occur at the cellular level and these underpins much of the subsequent course in cancer, in which a group of cells display uncontrolled growth and division beyond the normal limits, invasion, and sometime metastasis. Mutations in DNA that lead to cancer disrupt these orderly processes by disrupting the program regulating the processes.

In 1990, Clark proposed a cell death classification scheme that contains three type of death: apoptotic or type I, autophagic or type II and necrotic or type III (Clarke, 1990). Apoptotic cell death which proceeds by cell shrinkage, chromatin condensation, nucleosomal DNA degradation and fragmentation of cell into so-called apoptotic bodies. Activation of the caspase family of cystein protease gives rise to these characteristic morphological features of apoptosis. The remnants of the cell are removed by the lysosomes of phagocytes or neighboring cells often heterophagocytosis. Type II or autophagic cell death is characterized by the appearance of double membrane cytoplasmic vesicle engulfing bulk cytoplasm and/or cytoplasmic organelles such as mitochondria and endoplasmic recticulum. Autophagic vesicles and their contents are destroyed by lysosomal system of the same cell. In the necrotic cell death, the most prominent features of which are cytoplasmic swelling, membrane rupturing and organelle breakdown, involving remarkably few nuclear change (Festjene *et al*., 2006). In apoptosis and autophagy the remains of cells are disposed of by phagocytosis without an inflammatory response, in contrast to the massive cellular disintegration and subsequent inflammation accompanying necrotic programmed cell death (Lockshin *et al*., 2004, Edinger *et al*., 2004 and Krysho *et al*., 2008). Morphologic features of more than one type of cell death

program can be observed in the same cell (Paglin *et al*., 2001, Edinger *et al*., 2004, Gonzaley-Polo *et al*., 2005 and Liu *et al*., 2007).

Apoptosis, sometimes called "a guardian angel" or "cell policeman," is a cell altruistic suicidal mechanism targeted to selectively eliminate cancerous and other cells that threaten the health and life. The sacrifice of the "bad" cells occurs to save the integrity and life of the whole organism (Kerr, 1994, Blackstone and Green, 1999). Apoptosis is carried out by a multistage chain of reactions in which ROS act as triggers and essential mediators (Kerr, 1994, Blackstone and Green, 1999, Slater *et al*., 1995, Johnson *et al*., 1996). Recently, it became evident that mitochondria play a critical role in apoptosis (Kothan *et al*., 2004). Schematically, apoptotic signals, which arise in cancer cells, promote accumulation of the p53 protein that triggers the release of ROS, cytochrome c and a few other regulators from mitochondria. The latter activate a cascade of proteolytic enzymes, called caspases that digest a number of pivotal cell proteins and promote a caspase-activated deoxyribonuclease (Figure. 3). Cleavage of the critical proteins and DNA results in apoptotic cell death. Importantly, most anticancer drugs and radiation kill cancer cells by inducing apoptosis (Hickman, 1992, Verhaegen *et al*., 1995, McGovan *et al*., 1996, Labriola and Linvingston, 1999). Mutations in the p53 gene make cancer cells resistant to apoptosis and, accordingly, to anticancer drugs (Blackstone and Green, 1999).

Most intracellular short-lived proteins are selectively degraded by the ubiquitin-proteosome pathway, while most long-lived proteins are degraded in lysosomes. The mechanism to delivery cytoplasmic components to the lysosomes is call autophagy. Three types of autophagy have been proposed: macroautophagy,

microautophagy and chaperon-mediated autophagy (Seglen and Bohley, 1992, Dunn, 1994 and Blommaart *et al*., 1997). Among them, macroautophagy is believed to be responsible for the majority of the intracellular protein degradation, particularly of the starvation-induced proteolysis (Mortimore and Poso, 1987). In macroautophagy, cytoplasmic constituents, including organelles such as mitochondria, are first enwrapped by a membrane sac called isolation membrane. Closure of the isolation membrane results in formation of double membrane structures, called autophagosomes, which are are also known as initial autophagic vacuoles. Then, autolysosomes are generated by the fusion of the outer membranes of the autophagosomes and lysosomes. Lysosomal hydrolases degrade the cytoplasm-derived contents of the autophagosome, together with its inner membrane. During nutrient starvation or growth factor deprivation, autophagy is a cell defense mechanism by which intracellular nutrients are release to ensure survival (Kim *et al*., 2007). However, in certain setting, autophagy can lead to cell death by generating a non-apoptotic form of programmed cell death, termed autophagic cell death (Yoshimori, 2007). Autophagy is a self-digestion process that degrades intracellular structures in response to stresses leading to cell survival. When autophagy is prolonged, this could lead to cell death.

Increasing number of studies propose autophagic cell death as the mechanism of action of some anticancer agents. These observations suggest that autophagic cell death induction in cancers may have a therapeutic value. Autophagy and autophagic cell death can be activated in cancer cell lines in response to various agents used in cancer treatment. The treatment of breast carcinoma cell line MCF-7 with estrogen antagonist tamoxifen caused cell death with autophagic characteristics (Bursch *et al*.,

1996). Estradiol and the autophagy inhibitor 3-methyladenine (3-MA) were able to block death, underlining the active and autophagic nature of the tamoxifen-induced cell death. Similarly, arsenic trioxide treatment of malignant glioma cell lines induced G2/M arrest and autophagic cell death (Kanzawa *et al*., 2003). Radiation treatment induced autophagic cell death in breast, prostate and colon cancer cell lines (Paglin *et al*., 2001). Glioblastoma multiforme cell lines also responsed to ionizing radiation by halting cell proliferation and by increasing their autophagic activity (Yao *et al*., 2003). Many cellular stresses can cause induction of autophagy such as endoplasmic reticulum stress or mitochondrial dysfunction. (Marino, 2004). Oxidative stress has been shown to induce autophagy under starvation and ischemia/reperfusion conditions (Matsui *et al*., 2007, Scherz-Shouval *et al*., 2007). Oxidative stress induces autophagic cell death independent of apoptosis in transformed and cancer cells (Chen *et al*., 2008).ROS induced autophagic cell death in transformed and cancer cells but failed to induce autophagic cell death in normal cells (Chen *et al*, 2008).ROS are generated from the mitochondria and other sources, and can oxidize a wide range of cell constituents, including lipids, proteins and DNA, thus damaging cell structures and compromising function (Nathan, 2003). When antioxidant mechanisms are overwhelmed by ROS and subsequent oxidative stress occurs, cell damage and cell death result (Nathan, 2003).

3. Flavonoids

Flavonoids are a particular class of polyphenolic compounds naturally occurring and widely distributed in plants. Flavonoids are a group of chemical compounds which have low molecular weight phenylbenzopyrones. They are usually

subdivided into seven groups: flavonols, flavones, flavanones, flavanols, flavanonol, isoflavone and anthocyanidins (Pietta, 2000).

Numerous observations of flavonoids both *in vivo* and *in vitro* demonstrate a wide variety of physiological and biological effects. Flavonoids are strong anti-oxidants and free radical scavengers with various degrees of ellicacy. Other biological functions, such as anti-inflammatory, anti-allergic, anti-viral, anti-carcinogenic, anti-proliferative and anti-mutagens activities have long been recognized (Lamson and Brignall , 2000, Nijveldt *et al*., 2001) .

Various compounds characterized from natural ant-ioxidants such as vitamins and flavonoids, have been tested for their potential to cure disease by reducing oxidative stress (Rice-Eleva *et al*., 1995, Poppel and Berg, 1997). Quercetin (3,3',4',5,7-pentahydroxyflavone), which is a flavonoid that is commonly found in plants, has been reported to have biological, pharmacological, and medical applications (Morel *et al*., 1993, Cook and Samman., 1996 , Hollman *et al*., 1999). Although the multiple activities of quercetin were believed to arise from its anti-oxidant properties, it was recently suggested that quercetin behaves as a cytotoxic agents and as a mutagens (Scambia *et al*., 1991, Caltagirone *et al*., 2000, Aligiannis *et al*., 2001). A recent study demonstrated that quercetin can act as both anti--oxidant and pro-oxidant, depending on the concentration and source of free radicals in the cell (Lee *et al*., 2003). Several reports have demonstrated that flavonoids can be considered as therapeutic agents. Quercetin has strong growth inhibitory effects on several human cancer cell lines (Scambia *et al*., 1991 and 1994). A combination with quercetin increases the efficacy of chemotherapeutic drugs in the treatment of breast cancers (Akbas *et al*., 2004).

Recent studies have demonstrated the mechanism of flavonoids on cancer cells. Wang and coworkers in 2001 demonstrated flavonoid-induced apoptosis in leukemia HL-60 cells. The flavonoids induced loss of mitochondrial membrane potential, increase of reactive oxygen species (ROS) production, release of mitochondrial cytochrome c into the cytosol and subsequent induction of procaspase-9 processing (Wang *et al*., 2001). Kothan and coworkers in 2004 showed that quercetin cytotoxicity took place at the mitochondrial level, impairing the mitochondrial energetic state followed by an induction of apoptosis and inhibition of cancer cell growth (Kothan *et al*., 2004). Liu and coworkers in 2004 have demonstrated Woodfordin I was able to suppress the proliferation and induce apoptosis in human myelogenous leukemia k562 cells. Wooderin I treatment caused a rapid and sustained loss of mitochondrial membrane potential, transient generation of ROS, transient elevation of intracellular Ca^{2+} concentration and cytosolic accumulation of cytochrome c (Liu *et al*., 2004).

4. Objectives

The aims of the study are:

1. To characterize the biochemical and physiological changes of gastric mucosal cell carcinogenesis
2. To identify the role of Mn-SOD in gastric mucosal cancer cell