CHAPTER II

LITERATURE REVIEW

Liver Cancer or Hepatocellular Carcinoma

Liver cancer or hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide in terms of numbers of cases (626,000 or 5.7% of new cancer cases) but because of the very poor prognosis, the number of deaths is almost the same (598,000). It is therefore the third most common cause of death from cancer. Survival rates are very low, only 3% to 5% in cancer registries for the United States and developing countries (Parkin *et al.*, 2005). Presently much is known about the development and causes of HCC. It is nearly always developed in the setting of chronic hepatitis or cirrhosis, the conditions which many hepatocytes are killed, and many inflammatory cells in the liver and connective tissue is deposited (Thorgeirsson and Grisham, 2002).

Cancer has been the leading cause of death in Thailand with the age-adjusted mortality rate from the two cancer registries in Khon Kaen being 89.7 per 100,000 in males, 67.2 per 100,000 in females and in Chiang Mai being 133.3 in males, 121.0 in females. HCC is the most common cancer in men [age-standardized incidence rate (ASR) = $37.4/10^5$] and the third most common in women (ASR =$16.3/10^5$). HCC, which is associated with hepatitis B virus, is a major problem in all regions of Thailand, with the exception of Khon Kaen and the Northeast. Liver fluke [*Opisthorchis viverrini* (OV)], related to cholangiocarcinoma, account for 80% of all liver cancer in Khon Kaen, which has the highest incident rate of liver cancer in the world ($97.4/10^5$ in male and $39.0/10^5$ in females) (Figure 1) (Vatanasapt *et al.*, 2002).

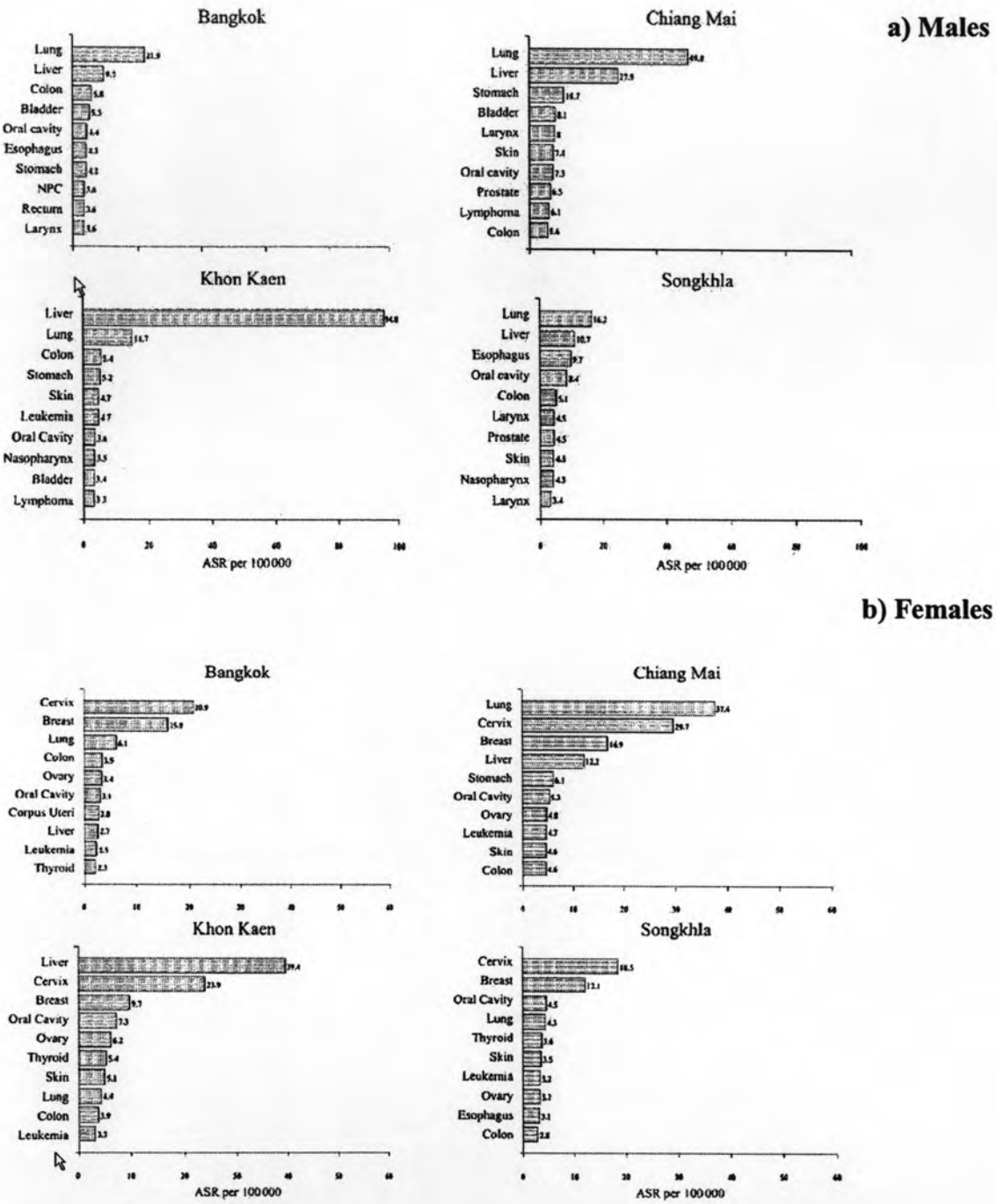


Figure 1 Leading cancer in four registries in (a) males and (b) females in Thailand (Vatanasapt et al., 2002).

The molecular model of hepatocacinogenesis is summarized in Figure 2. Initial hepatocellular alterations that precede the appearance of HCC include foci of phenotypically altered hepatocytes and, subsequently, dysplastic hepatocytes that form foci and nodules 3–5. Some agents can trigger HCC, and account for much of the marked variation in its incidence, have been identified and their impacts quantified. The various causes of HCC are perhaps better understood than those of any other major cancer in humans. Furthermore, the main causative agents—hepatitis B virus (HBV), hepatitis C virus (HCV) and aflatoxin B1 (AFB) which together are responsible for about 80% of all HCC in humans, leave 'molecular marks' on hepatocytes that enable the causes of individual HCC to be determined accurately in many instances. The molecular interactions between hepatocytes and the specific etiologic agents of HCC, and how these interactions disrupt hepatocellular genes and gene products leading to the development of HCC, are being elucidated (Thorgeirsson and Grisham, 2002).

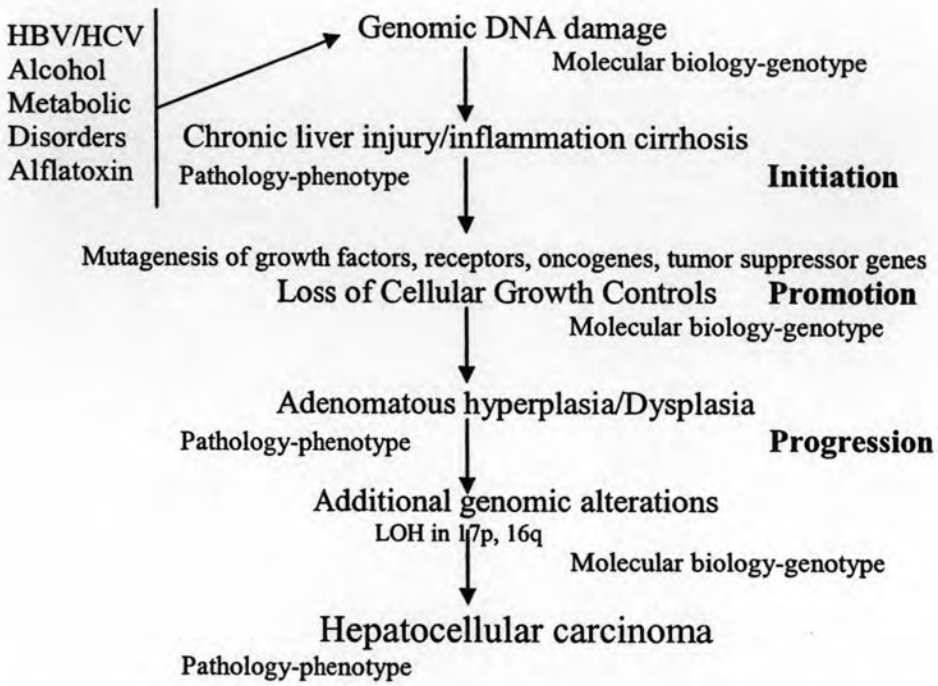


Figure 2 Molecular model for hepatocarcinogenesis (Nita et al., 2002).

Oxidative Stress-Induced Cancer

Oxygen-free radicals, more generally known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS) are well recognized for playing a dual role as both deleterious and beneficial species. The cumulative production of ROS/RNS through either endogenous or exogenous insults is termed oxidative stress and is common for many types of cancer cell that are linked with altered redox regulation of cellular signaling pathways. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions (8-OH-G) have been noted in various tumors, strongly implicating such damage in the etiology of cancer. It appears that the DNA damage is predominantly linked with the initiation process (Halliwell, 2007).

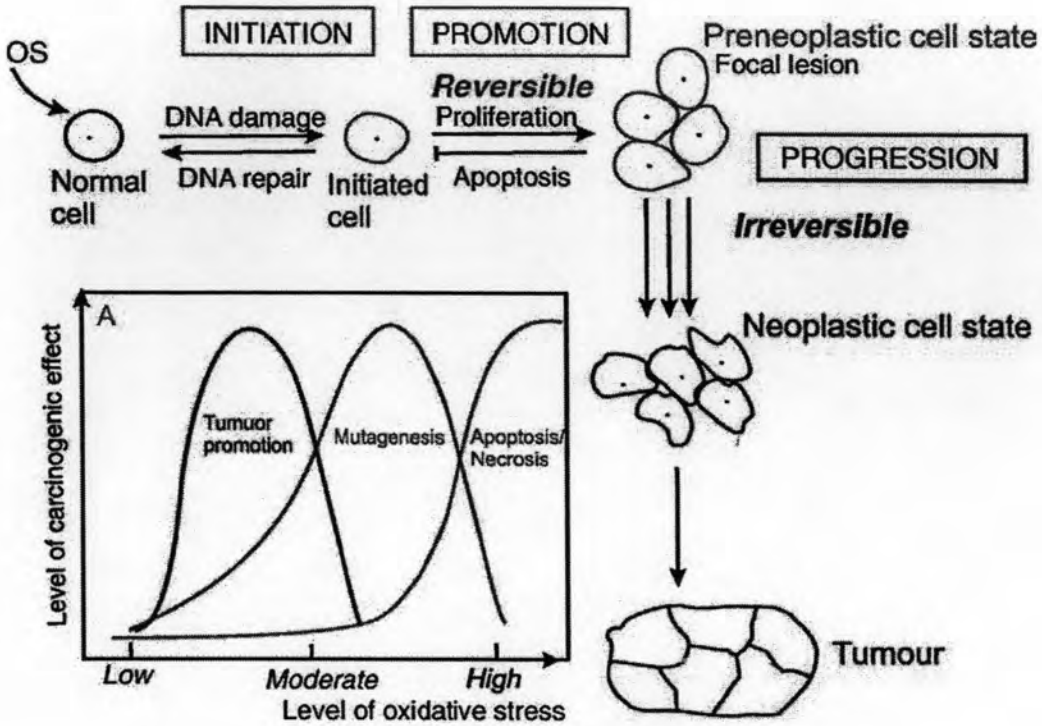


Figure 3 Three stages model of carcinogenesis and the level of carcinogenic effect vs. level of free radicals at various stages of carcinogenic process (Valko et al., 2006).

Carcinogenesis is a complex multi-sequence process leading a cell from a healthy to a precancerous state and finally to an early stage of cancer (Greenwald, 2002). A multi-stage process of cancer development is characterized by cumulative action of multiple events occurring in a single cell and can be described by three stages: initiation, promotion and progression. ROS can act in all these stages of carcinogenesis. The three stages model of carcinogenesis is shown in Figure 3 (Valko *et al.*, 2006).

The step of initiation involves a non-lethal mutation in DNA that produces an altered cell followed by at least one round of DNA synthesis to fix the damage (e.g. 8-OH-G) produced during the initiation. If dividing cells are damaged for whatever reason, they are able to interrupt temporarily their cell cycle at stage G1, S, or G2 ("checkpoints"), repair the damage, and resume division. Oxidative DNA damage can occur via action of ROS, e.g. hydroxyl radicals, formed through the Fenton-type mechanism, along with other species. The process of initiation further proceeds through oxidative stress-induced Ca (II) can changes leading to increase in intracellular free calcium as a result of its release from intracellular Ca (II) stores and through the influx of extracellular Ca (II). The promotion stage is characterized by the clonally expansion of initiated cells by the induction of cell proliferation and/or inhibition of programmed cell death (apoptosis). This process results in the formation of an identifiable focal lesion. This stage dose-dependently requires the continuous presence of the tumor promotion stimulus and therefore it is a reversible process. Many tumor promoters have a strong inhibiting effect on cellular antioxidant defense systems such as SOD, catalase, glutathione, etc. While a high level of oxidative stress is cytotoxic to the cell and halts proliferation by inducing apoptosis or even necrosis, a low level of oxidative stress can in fact stimulate the cell division in the promotion stage and thus stimulate the promotion of tumor growth. This implies that production of ROS during this stage of carcinogenesis is the main line of ROS-related tumor promotion. Progression is the third and final stage of the carcinogenic process. This stage involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state. This stage is irreversible and is characterized by accumulation of additional genetic damage, leading to the transition of the cell from benign to

malignant. This stage is characterized by genetic instability and disruption of chromosome integrity (Valko *et al.*, 2006).

Considerable laboratory evidence from chemical, cell culture, and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer. However, information from recent clinical trials is less clear. In recent years, large-scale, randomized clinical trials reached inconsistent conclusions.

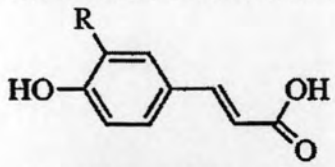
Polyphenol Compounds

Fruits and vegetables are excellent sources of fiber, vitamins, and minerals, but they also contain components like polyphenols, terpenes, alkaloids, and phenolics that may provide substantial health benefits beyond basic nutrition. Research over the last decade has shown that several micronutrients in fruits and vegetables reduce cancer. Phenolic compounds comprise one of the largest and most ubiquitous groups of plant metabolites. They are formed to protect the plant from photosynthetic stress, reactive oxygen species, wounds, and herbivores. Phenolic compounds are an important part of the human diet. The most commonly occurring ones in foods are flavonoids and phenolic acids. Current interest stems from the observations that dietary polyphenolic compounds have antioxidative, anti-inflammatory, and anticarcinogenic activities. Some representative structures are shown in Figure 4. Many of these compounds are usually glycosylated by sugars such as glucose, rhamnose, galactose, and arabinose (Yang *et al.*, 2001).

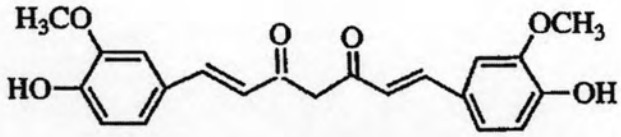
Plant polyphenols are well recognized for their antioxidative activities. They scavenge free radicals, thus breaking the free radical chain reaction of lipid peroxidation. The main structural features for these activities are exemplified by the ortho-dihydroxy structure in the B-ring, 2,3 double bond in conjunction with a 4-keto

function, and hydroxyl groups at positions 3 and 5 in flavonols, the di and trihydroxyphenol structures in catechins, and the side chain double bonds in conjugation with the ortho-dihydroxyphenol structure in caffeic acid. Polyphenols also quench reactive oxygen and nitrogen species generated in biological systems. Another antioxidative mechanism is the chelating of metals such as iron and copper ions, which prevent their participation in Fenton-type reactions and the generation of highly reactive hydroxyl radicals (Robards *et al.*, 1999; Yang *et al.*, 2001; Williams *et al.*, 2004).

Phenolic acids and derivatives



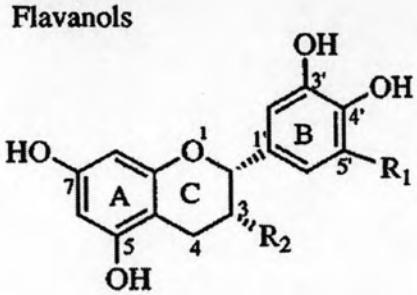
R = OH Caffeic acid
R = OCH_3 Ferulic acid



Curcumin

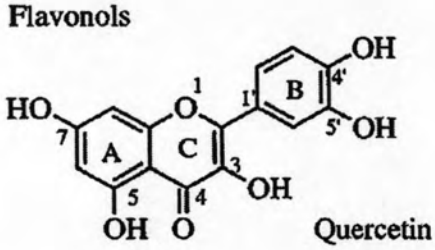
Flavonoids

Flavanols



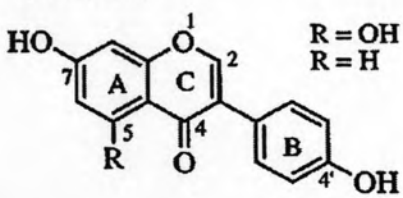
	R1	R2
EGCG	OH	gallate
ECG	H	gallate
EGC	OH	OH
EC	H	OH

Flavonols



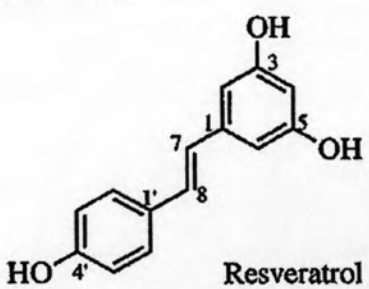
Quercetin

Isoflavones



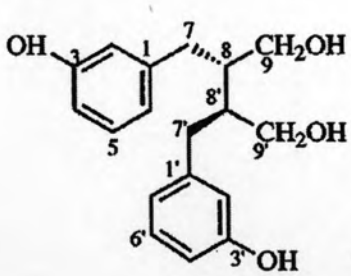
R = OH Genistein
R = H Daidzein

Stilbenes



Resveratrol

Lignans



Enterodiol

Figure 4 Representative structures of selected classes of dietary polyphenols (Yang et al., 2001).

Numerous studies have suggested that polyphenolic compounds are corresponding protective effect against chronic and degenerative disease (Katiyar and Muktar, 1997; Yang *et al.*, 2001; Kris-Etherton *et al.*, 2002). Several specific plant phenolic compounds and plant extracts exhibiting antioxidant activity were reported to inhibit mutagenesis and carcinogenesis. Possible mechanisms for chemopreventive activity of phenolic acids (Aggarwal and Shishodia, 2006) which is can be summarized in Figure 5.

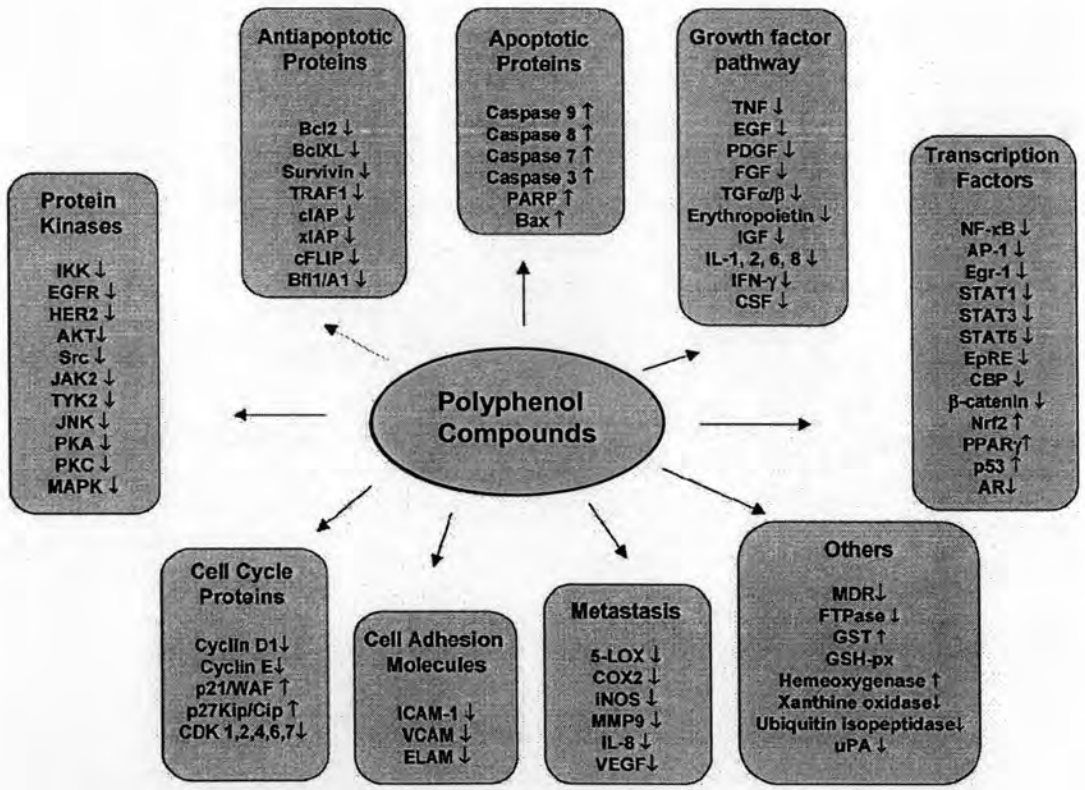


Figure 5 Molecular targets of dietary agents.

(Adapted from Aggarwal and Shishodia, 2006: 1403).

The polyphenol compounds are believed to suppress the inflammatory processes that lead to transformation, hyperproliferation, and initiation of carcinogenesis. Their inhibitory influences may ultimately suppress the final steps of carcinogenesis as well, namely angiogenesis and metastasis. Tumorigenesis is a multistep process that can be activated by any of various environmental carcinogens. These carcinogens are known to modulate the transcription factors (e.g., NF-kB, AP-1, STAT3), anti-apoptotic proteins (e.g., Akt, Bcl-2, Bcl-XL), proapoptotic proteins (e.g., caspases, PARP), protein kinases (e.g., IKK, EGFR, HER2, JNK, MAPK), cell cycle proteins (e.g., cyclins, cyclin-dependent kinases), cell adhesion molecules, COX-2, and growth factor signaling pathways. They also inhibit oxygen radical-forming enzymes or enzymes that contribute to DNA synthesis or act as ATP mimics and inhibit protein kinases that contribute to proliferating signal transduction. They may be prevent tumor development by inducing tumor cell apoptosis by inhibiting DNA topoisomease II and p53 down regulation or causing mitochondrial toxicity, which initiates mitochondrial apoptosis. The active gradients identified in plants and molecular targets modulated may be the basis for how these dietary agents not only prevent but also treat cancer and other diseases. (Galati and OBrien, 2004; Aggarwal and Shishodia, 2006; Nichenametla and Turuscio, 2006).

Cell Cycle and Tumor Development

Cell cycle control is a major regulatory mechanism of cell growth. In response to extracellular proliferate signals, a cell enters the cycle from the resting state (G0) and becomes committed to division once it passes the restriction point (R) late in the first gap phase (G1), the point at which it prepares for DNA replication. Beyond this

point, the cell-cycle programmed becomes autonomous and its fidelity is interrogated at various stages. DNA-damage checkpoints operate throughout the cycle, especially before (G1–S transition), during and after the DNA-synthesis phase (S), where the cell enters the second gap phase (G2) and prepares for mitosis (M). During mitosis, centrosome separation, chromosome condensation and formation of the mitotic spindle are instrumental prerequisites in separating the sister chromatids and in dispatching them to the nascent daughter cells. These processes are also subject to checkpoints before the cell-cycle programmed can be resumed. Depending on the circumstances, activation of a checkpoint enables extra time for repairing the detected lesion or prevents progression to cell division altogether, either by imposing a cycle arrest or by induction of apoptosis. The cell cycle check point may function to ensure the cell have time for DNA repair. Several proteins are known to regulate the timing of the events in the cell cycle. The loss of this regulation is a hall mark of cancer. The cell cycle and key point processes as shown in Figure 6 (Fischer, Glover, and Lane, 2004).

The major control acting like switches of the cell cycle is cyclins and serine/threonine kinase known as cyclin-dependent kinases (Cdks). This process is regulated by the coordinated action of Cdks in association with their specific regulatory cyclin proteins which successively act together in G1 to initiate S phase and in G2 to initiate mitosis. The ability of Cdks to phosphorylate specific substrates is regulated by phosphorylation and dephosphorylation of the catalytic subunit and by association with cyclins also. To prevent abnormal proliferation, cyclin-Cdks complexes are precisely regulated by binding of negative regulatory subunits called as cyclin-dependent kinases inhibitor (Ckis). There are two families of Ckis have been

characterized. The first class of inhibitors includes the INK4a proteins (include $p15^{Ink4b}$, $p16^{Ink4a}$, $p18^{Ink4c}$, and $p20^{Ink4d}$) that bind only cdk4-cdk6 kinases and not to cyclins and are therefore specific for early G1 phase. The second family of inhibitors is composed of Clip/Kip proteins (include $p21^{Cip1/Waf1}$ $p27^{Kip1}$, and $p57^{Kip2}$) that inhibit all cyclin-CDK complexes and are not specific for particular phase (Hartwell and Kastan, 1994; Coffman and Studzinski, 1999).

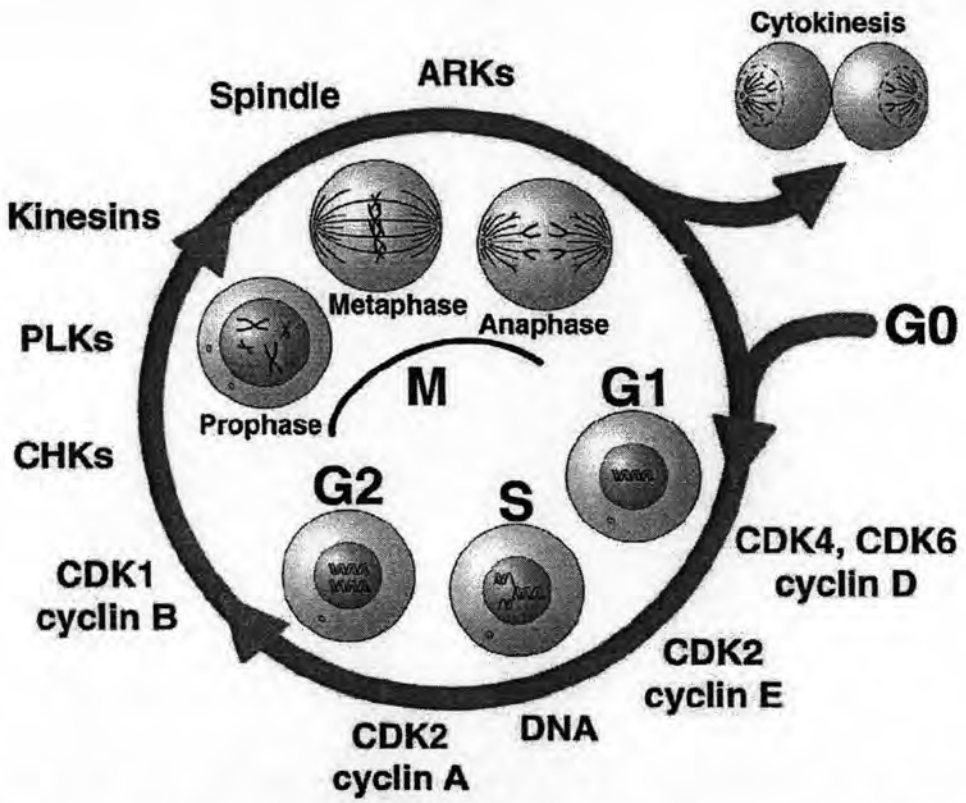


Figure 6 The mammalian cell cycle. The key processes of DNA replication, chromosome segregation and cell division are indicated diagrammatically, and the phases of the cycle are labeled. Key cell-cycle components that currently serve as targets in drug discovery and development are shown. Abbreviations: ARK, aurora kinase; CDK, cyclin-dependent kinase; CHK, checkpoint kinase; PLK, polo-like kinase. The cell-cycle phases are as follows: G0, quiescent phase; G1, first gap phase; S, DNA-synthesis phase; G2, second gap phase; M, mitosis (Fischer, Glover, and Lane, 2004).

The abrogation of cell cycle regulatory proteins is a common mechanism in tumor development. HCC patients have loss of cell cycle regulation in HCC is frequently found, and can be involved in the hepatic carcinogenesis, since it could lead to an unordered growth of the hepatocyte (Nita *et al.*, 2002).

In cases of DNA damage induced by carcinogens or irradiation, p53 is activated and induces the transcription of genes such as $p21^{Cip1/Waf1}$, which allows cell cycle arrest or apoptosis to occur. Little p21 mRNA or protein is detected in normal liver tissue, but its expression is abruptly up-regulated in response to hepatic injuries. Wakagama *et al.* (2002) suggest that p21 $^{Cip1/Waf1}$ expression in hepatocytes is a useful marker in predicting cellular stress on hepatocytes and thus in predicting the development of HCC in HCV-associated chronic liver diseases. The down-regulation of p21 $^{Cip1/Waf1}$ may be due to impaired function of p53. Notably, the $p27^{Kip1}$ expression level is also significantly decreased in biologically aggressive HCC. $p27^{Kip1}$ is regulated at post-translational levels by the ubiquitin-proteosome pathway rather than by mutations or gene methylation. Jing *et al.* (2005) indicated that the p53 mutation and decreased $p27^{Kip1}$ expression might be involved in the imbalance of proliferation and apoptosis in HCC.

Cell cycle-related proteins may be good targets for the design of diagnostic marker sets that can define various stages of HCC development (Chetty, 2003; Wong *et al.*, 2006).

Apoptosis and It Potential Pharmacological Target of Caspases

Apoptosis or programmed cell death is a major control mechanism by which cell die if DNA damage is not repaired. Apoptosis is also important in controlling cell

number and proliferation as part of normal development. Some types of cancers such as leukemia, lymphoma, hepatoma, lung cancer, pancreatic cancer are characterized by defects in apoptosis leading to immoral clones of cells. Cancer chemotherapy utilized apoptosis to eliminate tumor cells. The initial definition of apoptosis was morphological: dying cells exhibit a characteristic pattern of changes, including cytoplasmic shrinkage, active membrane blebbing, chromatin condensation, and, typically, fragmentation into membrane-enclosed vesicles. This readily visible transformation is accompanied by a number of biochemical changes. Changes at the cell surface include the externalization of phosphatidylserine and other alterations that promote recognition by phagocytes. Intracellular changes include the degradation of the chromosomal DNA into high-molecular-weight and oligonucleosomal fragments, as well as cleavage of a specific subset of cellular polypeptides (Watson, Cai, and Jones, 2000).

There are two mechanisms of apoptosis. The first, referred to as the extrinsic or cytoplasmic pathway, is triggered through the Fas death receptor, a member of the tumor necrosis factor (TNF) receptor family. The second pathway is the intrinsic or mitochondrial pathway that when stimulated leads to the release of cytochrome *c* from the mitochondria and inactivation of the death signal. Both pathways converge to a final common pathway involving the activation of a cascade of proteases called **caspases** that cleave regulatory and structural molecules, culminating in the death of cell (Figure 7). Caspases, being the key effector molecules in apoptosis, are potential targets for pharmacological modulation of cell death. Caspases inhibitors have therapeutic potential for cancer therapy (Ghobrial, Witzig, and Adjei, 2006).

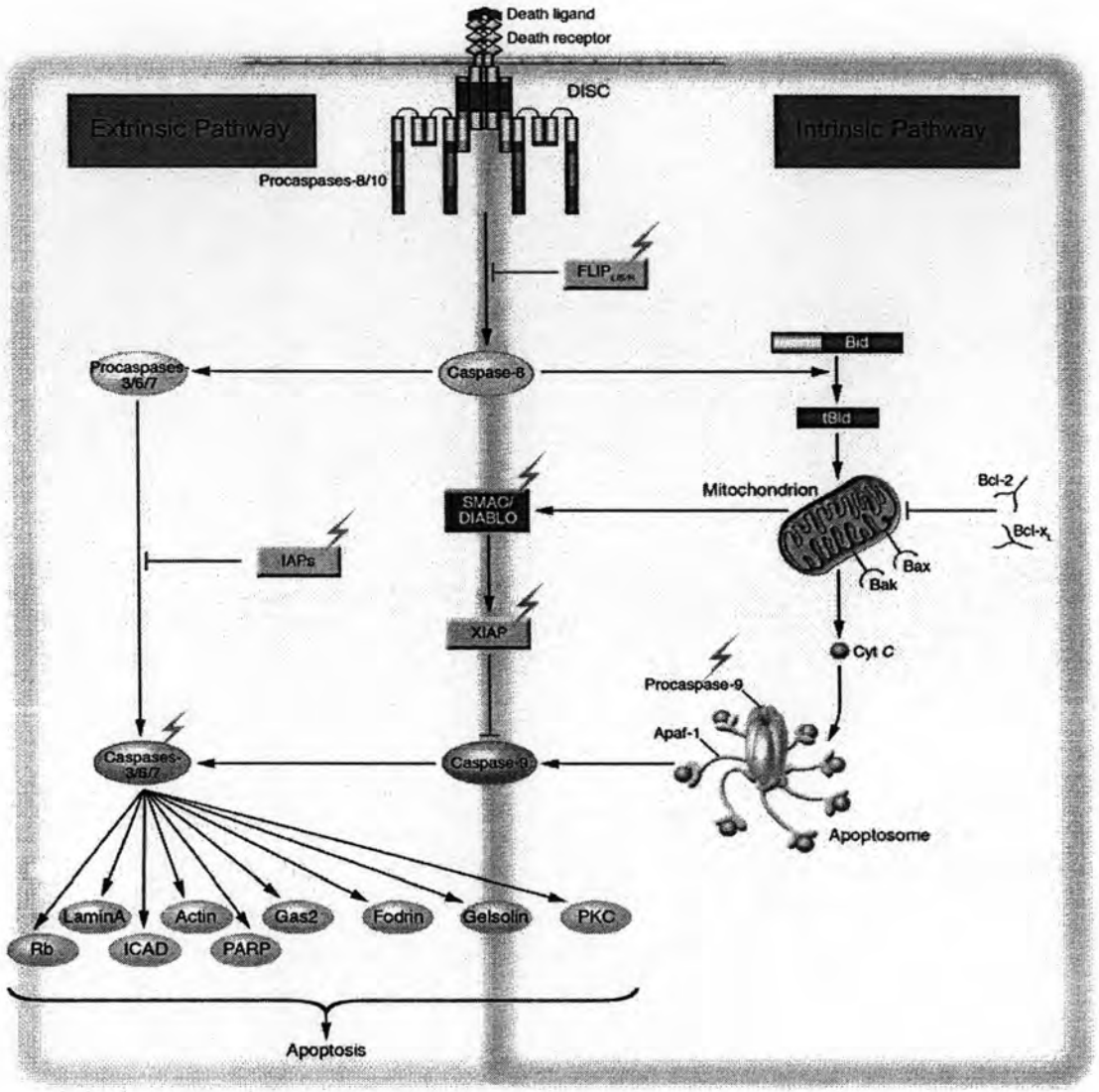


Figure 7 Caspase signaling and its modulation. In the extrinsic pathway, DISC formation leads to caspase-8 activation. Two signaling pathways downstream from the receptor were established. Extrinsic pathway, caspase-8 directly cleaves caspase-3, which starts the death cascade. For intrinsic pathway, an additional amplification loop is required, which involves tBid-mediated cytochrome *c* release from mitochondria followed by apoptosome formation. Initiation of the intrinsic pathway results in mitochondria-mediated apoptosome formation, followed by caspase-9 and -3 activation, leading to destruction of the cell. Caspase action can be modulated on several levels. Activation of caspases at the DISC is inhibited by c-FLIP proteins; activation of effectors caspases is inhibited by IAPs. (Lavrik, Golks, and Khammer, 2005).

Topoisomerases as Biomarkers Screening for Chemopreventive Agents

Topoisomerases are enzymes that change the topology (or conformation) of a segment of DNA by a complex catalytic cycle which involves DNA strand cleavage, strand passage and religation. These enzymes are essential to the cell because of the nature of the structure of the double helix which, although normally stored highly complexes as chromatin, can impose topological problems (supercoiling) when it is unwound and processed (Cummings J and Smyth JF, 1993). At least five different topoisomerases have been reported to be present in higher eukaryotes, namely topoisomerases I, and topoisomerases IIIα and IIIβ, which are type I, and topoisomerases IIα and IIβ, two isozymes belongs to the type II family (Cortés *et al*, 2003).

Type I enzymes, which do not require ATP, act by forming a transient single-strand break (ssb) through which the other DNA strand passes to achieve relaxation. While type II, usually ATP-dependent is able to do so with the double strand that make up duplex DNA creating a DNA-linked protein gate though which another intact duplex passes. Both type I and type II enzymes are proficient in relaxing supercoiled DNA, while only topoisomerases II can decatenate intertwined DNA molecules. Whereas the biological functions of topoisomerases III and IIβ are poorly understood, many investigations have dealt with the roles of both topoisomereases I and IIα (Cortés *et al*, 2003).

DNA Topoisomerase	Type	Structure	M.W. (kD)	DNA cleavage	Gene localization (Human chromosome)	Function
I	IB	Monomer	100	ssb	20	Replication, transcription, recombination
IIIα	IA	2 isoforms (alternative splicing)	110	ssb	17	Recombination, rDNA metabolism
IIIβ	IA	3 isoforms (alternative splicing)	96	ssb	22	Recombination
IIα	II	Homodimer	170	dsb	17	Chromosome condensation and segregation. Replication
IIβ	II	Homodimer	180	dsb	3	Not well defined

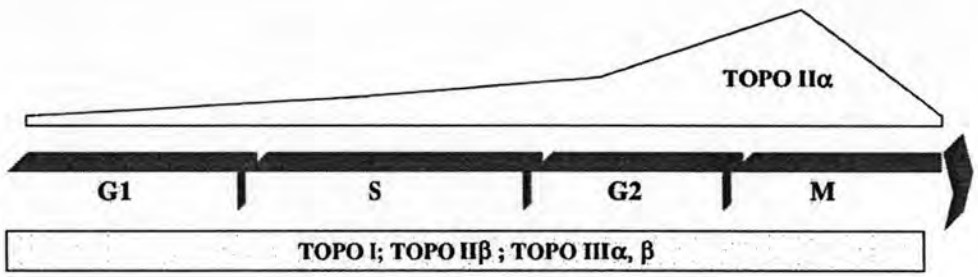


Figure 8 Human DNA topoisomerases and their expression throughout the cell cycle. Human type 1A topoisomerases (topoisomerases IIIα and IIIβ) are homologous to bacterial type I topoisomerases (bacterial topoisomerases I and III) and, like these bacterial enzymes, have activity toward negatively supercoils DNA, but not positively supercoiled DNA substrate. Topoisomerases IIα and IIβ are isozymes. As to their mode of DNA cleavage, type I enzymes act by forming a transient single-strand break (ssb) in DNA to achieve relaxation of the supercoiled molecule before resealing, while type II topoisomerases form double-strand breaks (dsb) to facilitate unknotting or decatenation of entangled DNA molecules. Levels of topoisomerases IIα mRNA increase several-fold (normally over 10 times) in late S and G_2/M, while other topoisomerases are expressed constitutively in a cycle-dependent fashion.

Topoisomerases are required during DNA replication, transcription (mainly topoisomerase I) and homologous recombination and a specific and unique role for topoisomerase II in segregation of daughter chromatids after DNA replication as well as in chromatin condensation and anaphase segregation during mitosis has been purposed. The amount and stability of topoisomerases I and topoisomerases IIβ show no significant fluctuations though the cell cycle while topoisomerases IIα proteins levels vary as a function of the proliferate stage (higher in cancer cells that in normal cells) and cell cycle position (Figure 8). This particular has made topoisomerases II the primary cellular target for a number of widely used antineoplastic drugs considerably more lethal to cells that contain high levels of topoisomerases II and which are undergoing high rates of DNA replication. Levels of topoisomerases IIα mRNA peak in late S and G2/Mseveral-fold (normally over 10 times) over the amount in G1 cells. These high levels are consistent with the idea that topoisomerases IIα is required mainly during the final stages of DNA replication to facilitate chromosome untangling condensation and mitotic segregation (Cortés *et al*, 2003; Larsen, Escargueil, and Skladanowski, 2003). Inhibition of topoisomerase II enzymes has been considered as an important biomarker in screening chemopreventive and therapeutic properties of potential anticancer agents and the mode of action of many anti-cancer drugs has been based on this inhibitory capacity (Heck and Earnshaw, 1986; Hande, 1998; Walker and Nitiss, 2002). Overexpressed topoisomerase II proteins were detected in 60% of HCC patients (Yuwen *et al.*, 1997; Watanuki *et al.*, 2002; Yeh *et al.*, 2007)

Morus alba. L

An experimental studies have been shown that mulberry leaves have some chemopreventive and these studies have been reviewed extensively in recent year. Kim *et al.* (2000) have reported that *M. alba* leaves contain two flavonoids that induce the differentiation of the leukemic cells. These two flavonoids are quercetin-3-*O*-*β*-D-glucopyranosyl-(1->6)-*β*-D-glucopyranoside and quercetin-3,7-di-*O*-*β*-D-glucopyranoside. The latter has also been shown to induce differentiate on the HL-60 cell line to express CD66b and CD14 antigens and this toward mature granulocytes and monocyted. This flavonoid, therefore can induce differentiation and inhibit the *in vitro* proliferation of human myeloid leukemic cell.

Oh *et al.* (2002) have shown that compounds isolated from dried twigs of *M. alba* including 5,7-dihydroxycoumarin 7-methy ether, 2 two prenylflavones (cudraflavone B and cudraflavone C) and oxyresveratol exhibit superoxide scavenging effects and exhibits a DPPH free radical scavenging effect. Cudraflavone B and oxyresveratol have also been shown to have hepatoprotective effects on tarcrine-induced cytotoxicity in human liver-derived Hep G2 cells (Oh *et al*, 2002).

Nam *et al.* (2002) have shown tha Cortex Mori (CM, commonly known as 'Sangbaipi' is water extract from the root bark of *M. alba*) against tumor cells and its mechanism. CM found to exhibit cytotoxic activity on K-562, B380 human leukemia cells and B16 mouse melanoma cells. By using DNA fragmentation, Poly (ADP-ribose) polymerase cleavage and nuclear condensation assay, it was found that those cells exposed to CM underwent apoptosis. A protein-binding test using Biacore and a microtubule assembly-disassembly provide evidence showed that CM bound to the tubulins resulted in a marked inhibition of the assembly. Overall, the water extract of

CM appear to induce exerted cytoxicity against tumor cells by inducing apoptosis through inhibition of microtubule assembly.

The inhibition of prostaglandin biosynthesis and nitric oxide production has been considered as a potential anti-inflammatory and cancer chemopreventive agents. Hong *et al.* (2002) have evaluated 170 methanol extracts of natural products for the inhibition of prostaglandin E_2 production for cyclooxygenase-2 inhibitors (COX-2 inhibitors) and nitric oxide formation for nitric oxide syntase (iNOS inhibitors) in lipopolysaccharide (LPS)-induced mouse macrophage RAW264.7 cells. They have found that methanol extract from twigs of *M. alba* is as potential inhibitors of iNOS activity (88 % inhibition at the test concentration of 10 μg/ml) but fruits and leaves extracts are weak inhibitors (1.3 and 57.9 % of inhibition respectively). For inhibition of COX-2 activity, fruits, leaves and twigs are weak inhibitors (0, 11.4 and 2.2 % of inhibition, respectively). These active extracts mediated iNOS inhibitory are warranted for further elucidation of active principle for development of new cancer chemopreventive or anti-inflammatory agents.

Lorenz *et al.* (2003) have investigated antioxidative and antinitrosative capacity of oxyresveratol which is present in high amounts in mulberry wood, in comparison to resveratol. Both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are elements of the apoptotic cascade that cause oxidative damage to proteins, DNA and other biological targets. They found that oxyresveratol was more effective scavenger for 2,2-diphenyl-1-picryl-hydrazyl (DPPH) used as a general free radical model, compare to resveratol. When primary glial cell culture were loaded with ROS/RNS-sensitive fluorochrome 2,7-dichlorodihydrofluorecein, the lowest rise in the fluorescence signal after H_2O_2 exposure was seen when the cell were pretreated

with oxyresveratol. Accordingly, cultures of the murine microglial cell line N9 and primary mixed glial cultures were used to test the drug effects of NO production upon expression of the inducible isoform of nitric oxide synthase (iNOS). The results showed both oxyresveratol and resveratol diminished NO levels, resveratol more effectively than oxyresveratol. Resveratol down-regulated the expression of iNOS protein, but both did not alter iNOS activity. Furthermore, oxyresveratol displayed a generally lower cytotoxicity than resveratol. The radical and ROS scavenging properties, as well as the lower cytotoxicity towards microglial and the known good water solubility suggesting oxyresveratol as a potential protectant against ROS/RNS.

Mizushina *et al.* (2003) have tested 1,4 –dideoxy-1,4-imino-D-ribitol (DRB) which was isolated from root of *M. alba* and found to strongly inhibit the activities of eukaryotic DNA polymerases and had almost no effect on the prokaryotic DNA polymerases. Their kinetic studies showed that inhibition of both DNA polymerase α and β by DRB was competitive with respect to dNTP substrate. It would be scheme to examine the biological activity including antitumor effects of DRB.

These have been extensive research into the some possible mechanisms of cancer prevention by mulberry tree. The widespread consumption and availability of herbal teas throughout the world creates the possibility of exploiting their properties as chemopreventive agents. Thus, it has become necessary to define the actual magnitude of their health benefits, and elucidate the potential mechanism of action of tea and its constituents, particularly those of polyphenols. Several assays have been developed to evaluate the ability of compounds to modulate biochemical events presumed to be mechanistically linked to carcinogenesis.