CHAPTER II

REVIEWS OF RELATED LITERATURE AND RESEARCH

Colon cancer

1. Incidence

Colon cancer is one of the most common cancers in both men and women. The incidence in men is higher than those for women. Although colon cancer exhibits universal distribution, there is higher incidence in developed and/or industrialized countries such as Australia, New Zealand, Northern Europe and North America, but it is now increasing in middle and low income countries, including Thailand [1, 13, 14]. About 70% of patients with colon cancer are over 65 years of age and in the age group of 45-54 years, colon cancer incidence is approximately 20%. The colon cancer is rare under the age of 45 years [14].

The incidence of colon cancer in Thailand is low when compared with other countries. It is the third in frequency in males after the cancers of liver and lung, and the fifth after the cancers of cervix, breast, liver and lung for female. The highest incidence rate of colon cancer is in Bangkok and the lowest incidence rate is found in Nakhon Phanom [2, 19]. This rate was expected to rapidly increase in the next decade probably due to the acquisition of Western lifestyle diet such as high levels of fat and protein, especially red meat and low dietary fiber meals [3, 5]

2. Risk factors

Nowadays, it is well recognized that a complex interaction between genetic factors and environment factors, especially diet, is involved in the etiology of colon cancer. Data from epidemiological studies found that most of colon cancer occurs sporadically and 5% of cases are inherited [15, 16]. According to human and animal studies, the risk of colon cancer can be divided into three factors, dietary factors, non-dietary factors and genetic factors [3, 15].

Dietary factors: Diet is widely believed to be associated with colon cancer development and is a modifiable risk factor. Epidemiological studies suggested that 70-90% of colon cancer influence by diet. Therefore, there has been a great interest in better understanding is to investigate which dietary factors may be associated with

higher or lower colon cancer risk. Among dietary properties, Western diet stands out for being rich in fats, animal proteins and calories, as well as poor in fibers [6]. Increased consumption of dietary fat and protein, especially animal fat and red meat, has been shown as potential risk for colon cancer [17, 18]. The saturated fats in red meat may influence on colon cancer risk by increasing the production of secondary bile acids that can promote colon carcinogenesis [19] as well as heme iron in red meat can damage the colonic mucosa and stimulate epithelial cell proliferation in animal model [20]. The components of processed red meat, n-nitroso compounds and the products while cooking red meat at high temperatures also contains heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) that has the potential mutagenic and carcinogenic effect [21].

Obesity and high level of alcohol consumption have been implicated as potential risk factors [22-25]. In part, the relation between obesity and colon cancer risk may be explained by alterations in the metabolism of endogenous hormones such as insulin and sex steroid, which can destroy the balance between cell proliferation and cell apoptosis [23]. Excessive alcohol consumption is one cause of colon cancer. The association between alcohol consumption and the risk of colon cancer remains controversial because ethanol is not carcinogenic. However, its first metabolite, namely acetaldehyde, has recently been shown to be a local carcinogen in human studies [26]. In addition, low intake of fiber, fruits and vegetables were associated with colon cancer development [7, 27, 28]. The potential anti-carcinogenic effect of fruits and vegetables can be attributed either to their high content of fiber or to the presence of various micronutrients such as several anti-oxidant vitamins (vitamin C, D and E), calcium, selenium and folate.

Non-Dietary factors: From an environmental and behavioral perspective, risk factors also include non dietary factors such as smoking, low physical activity and sedentary lifestyle. Smoking increases risk of colon cancer since it is associated with the development of adenomatous polyps [29]. Low physical activity and sedentary lifestyle has consistently been shown to be associated with the increased risk of colon cancer [28, 29].

Genetic factors: Epidemiological studies have suggested that 5% of colon cancer cases are inherited. Hereditary colon cancer is defined by an inherited

predisposition to diseases and has been divided into two types. The first is familial adenomatous polyposis (FAP) which is associated with mutation or loss of adenomatous polyposis coli (APC) gene. FAP is a mutation at one of the alleles of APC gene (chromosome 5q). Defects in this gene are thought to result in the potential for tumor initiation in colon cancer. The second is hereditary non-polyposis colorectal cancer (HNPCC) which is associated with mutation in DNA mismatch repair genes [15]. HNPCC recently has been associated with mutations in any five genes involved in DNA mismatch repair such as *hMLH1*, *hMSH2*, *hPMS1*, *hPMS2* and *hMSH6* [30].

From epidemiological study, dietary factors can influence all stages of colon carcinogenesis from crypt cell proliferation to aberrant crypt and adenoma formation, and finally to malignant changes [31]. Thus, diet is definitely important for colon cancer development. The exact mechanism of how the diet causes colon carcinogenesis is not yet completely clear [29, 30]. Thus, dietary interventions have received much attention as one of the approaches to prevent this type of cancer [4, 5, 31].

3. Colon cancer and diet

Several experimental studies have proposed that the protective effects of diets rich in fruits and vegetables against colon carcinogenesis are thought to be due to their high content of anti-oxidant vitamins and fibers [6-8]. Vitamin D and calcium intakes may also prevent colon cancer [32]. Moreover, many types of dietary proteins have been found to reduce the development of colon cancer in animals [9, 11, 31-35].

Glucosinolate breakdown products from cruciferous plants

Consumption of brassica vegetables is associated with a reduced risk of cancer of alimentary tract including colon cancer. Brassica vegetable such as cabbage, broccoli and cauliflower are members of a Cruciferae family containing high level of glucosinolate. The hydrolysis product of glucosinolate, namely isothiocyanates has been suggested to have anti-carcinogenic properties [33]. Smith et. al. have reported that a semi-synthetic diet containing glucosinolate sinigrin (high levels in mustard and brussels sprouts) could induce the colonic crypt cells to undergo apoptosis in DMH-treated rats. This apoptotic effect might be due to allyl isothiocyanate (AITC) released from glucosinolate sinigrin in colonic lumen [34].

Phenolic substances

Polyphenolic compounds are abundant throughout the plant kingdom and are found in a wide variety of human foods. The flavonoids are the best defined group of polyphenols in the human diet, presenting in fruits and vegetables as glycosides such as quercetin, myricetin and kaemferol. They have been shown to interact with the cellular signal pathways such as proliferation, differentiation and apoptosis of various tumors cells [35]. Gee et al. fed quercetin to rats and observed a suppression of crypt cell proliferation [36]. They also showed a statistically significant reduction in the frequency of crypt cell mitosis in small intestine and in the distal colon [37].

Vitamins and minerals

There are several epidemiological studies reporting the association between risk for colon cancer and vitamins or minerals. Vitamin C, D, E and calcium have been suggested to have chemopreventive effect against the development of colon cancer [38]. Vitamin C and E are well known antioxidants. They were reported to inhibit the formation of carcinogenic nitrosamine, by blocking the reaction between secondary amines and nitrite under acidic condition in the stomach [39]. Vitamin D is a micronutrient that is essential to prevent many diseases including colon cancer. Vitamin D regulates calcium absorption and is essential for the maintenance of systemic calcium homeostasis. Epidemiological data have suggested that increased intake of vitamin D and calcium might have some protective effect on colon cancer by binding with bile and fatty acids and induction of cellular differentiation [40]. Vitamin D and calcium may have roles in the control of cell proliferation beyond those associated with the maintenance of blood-calcium concentrations [32].

Several epidemiological studies have provided support for the beneficial effect of calcium on colon cancer. Calcium might have effect on colonic epithelium by binding with fatty and bile acids in the colonic lumen, then forming insoluble soaps that are safe to colonic mucosa [41]. Studies in animal model have shown that high calcium intake cause a reduction of colonic epithelial hyperproliferation and the formation of tumors in rats. This effect was attributed by calcium ions binding to excess bile acids and free fatty acids in colonic lumen or by directly inhibiting the proliferation of colonic epithelial cells [42, 43].

A micronutrient, selenium, which is a key component of various enzymes, also shows their anti-carcinogeneic effects via several mechanisms such as inhibition of growth of malignant colonic epithelial cells, induction of apoptosis in transformed cells, regulation of immune response and DNA repair [44-46]. High levels of serum selenium and folate have been reported to reduce colon cancer risk [47].

Dietary fibers

The role of dietary fiber in prevention of colon cancer has been extensively investigated. Although it has been suggested that dietary fibers can protect against colon cancer, there is still controversial about their efficacy [5, 48]. The protective role of dietary fiber is partially associated with butyrate produced in the gastrointestinal tract by anaerobic fermentation of fiber and resistant starch. Butyrate, an energy source for colonocytes, can promote or inhibit cell proliferation as well as apoptosis, and plays important role in the maintenance of colonic health. It also has anti-neoplastic in the *in vitro* study and has implicated role in the protective effect of fibers in the *in vivo* study [28, 49]. Wheat bran and cellulose inhibit colon tumorigenesis in the animal following exposure to chemical carcinogens whereas others fibers do not have consistent effect [50, 51]. Takahashi et al. have examined the inhibitory effect of micro-fibril in mice and suggested that high butyrate content in micro-fibril wheat bran might play a role to prevent colon cancer development [52]. Evidence of possible association between colon cancer and dietary fiber has been shown by several studies. There are alteration of intestinal transit time and raising of the concentration of luminal contents allowing a shorter time contact of the colonic mucosa with carcinogenic agents, generation of bacterial fermentation products especially short chain fatty acids leading to alteration of luminal pH [53]. Protective factors from vegetables and fiber have physical effects such as forming high stool volume, shortened stool transit time and they also affect the releasing of certain beneficial fermentation products by gut microflora [54].

Dietary proteins

Soy protein: Consumption of soybeans and soy-based products including soymilk, tofu and soy flour has been shown to prevent colon cancer in animal models [55]. Soy protein diet can reduce the incidence of colon tumors from 50% to 12% in rats [56]. Soy protein diets also reduced the incidence of the large size ACF in AOM-

treated rats by decreasing the activation of pro-carcinogen to carcinogen and regulating of genes involved in signal transduction pathways [57]. A study by Kahlon and Woodruff has demonstrated that bile acid binding with soy protein might improve gastrointestinal health and reduce the risk of colon cancer [58].

Buckwheat: Consumption of buckwheat protein suppresses the development of colon cancer. Rats fed with buckwheat have 47% reduction in the incidence of colonic adenocarcinoma that is associated with reduced cell proliferation and protein expression of c-*myc* and c-*fos* relating to cell proliferation [59]. Additionally, consumption of buckwheat protein reduces the incidence of bloody stool, possibly relating to tumor development. The high contents of arginine and glycine in buckwheat diet may be responsible for its preventive effect [59].

Whey protein: A study by Belobrajdic et al. has demonstrated that rats fed with whey protein have fewer numbers of ACF than those of rats fed meat diet. This study has suggested that the components of whey protein such as cysteine, lactose and conjugated linoleic acid are responsible for its anti-cancer effect [60]. Consumption of whey protein also reduces colon cancer development in AOM-treated rats [60]. The possible mechanism is thought to be involved in the increase in tissue glutathione concentration, leading to increased detoxification of the free radicals produced by metabolism of carcinogenic compound [61]. In addition, consumption of whey protein can reduce the level of C-peptide concentration in serum associated with reduced tumor occurrence in the colon [62].

Silk protein: Silk sericin has been reported to reduce the incidence and number of colon tumors in DMH-treated mice and rats [9, 10]. Sericin reduces cell proliferation in rat colonic mucosa. Decreased c-*myc* and c-*fos* protein expression may, at least in part, be responsible for the protective effect of sericin against colon tumorigenesis. In addition, the anti-oxidant properties of sericin, which suppress the level of 8-hydroxydeoxyguanosine (8-OHdG), 4-hydroxynonenal (4-HNE) and inducible nitric oxide synthase (iNOS) protein expression in rat colon, might be involved in reduction of colonic epithelial cell proliferation [9]. Anti-oxidant activity of undigested sericin in colon contents has been thought to play a role in reduction of colonic oxidative stress and tumorigenesis [10].

4. Colon cancer development

Colon cancer development is a multi-stage process consisting of initiation, promotion and progression phases. Colon carcinogenesis is a long-term process starting from changing of normal epithelial cells to aberrant crypt, progression to adenoma stages to form carcinomas and then to metastasis (Figure 1) [63, 64]. The occurrence of colon cancer is mainly associated with the incidence of aberrant crypt foci (ACF) formation [65].

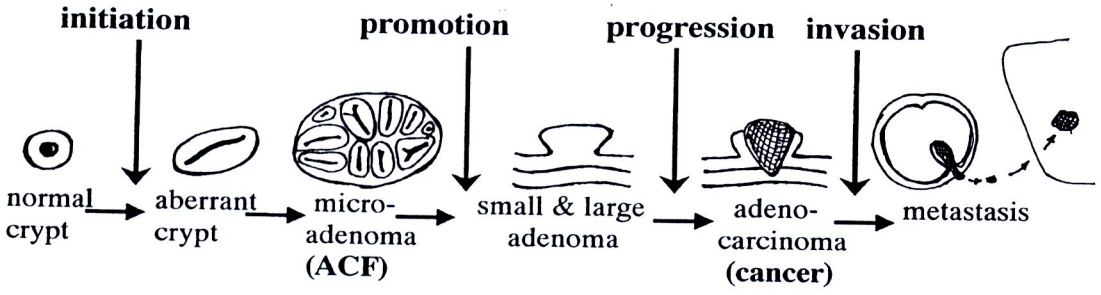

Figure 1 Process of colon carcinogenesis [64]

ACF formation is an earliest morphologic alteration in colonic mucosa that occurs during the development of colon cancer. ACF analysis is widely used as the endpoint to assess the ability of chemopreventive agents to reduce colon cancer development. ACF are generally appeared within two weeks after carcinogen administration in animal models. Initially they appear as single crypts with dilated irregular luminal opening and thicker epithelial lining. As the time progresses many foci of aberrant crypts contain more than one crypt in a focus, so called crypt multiplicity [66, 67]. ACF development can be detected under a microscope by methylene blue staining. The stained ACF are darker and larger than the surrounding normal crypts. ACF have not been reported as spontaneous lesions in rodents, but they are inducible in rodents by certain carcinogens that specifically induce colon cancer development.

5. Biomarkers of colon carcinogenesis

DMH-induced colon cancer is a multi-step process with morphological and histological features sharing many similarities with human sporadic colon cancer [68]. The transformation of adenoma to carcinoma is characterized by histological changes that begin with aberrant crypts. These early lesions have the potential to progress to advanced adenomas and then transform into adenocarcinomas [69].

ACF, the early lesions in the development of colon carcinogenesis that can be identified on the surface of colon under low-magnification stereomicroscope after methylene blue staining [66]. At present, ACF have been used as an endpoint to evaluate the effectiveness of chemopreventive agents, natural, pharmaceutical, and or dietary compounds to prevent colon cancer process [70]. When using ACF as biomarkers, it is important to consider that ACF are heterogeneous lesions. The total number of ACF may be considered to be a valid biomarker only at an early stage of colon carcinogenesis. While in long term study, ACF with crypt multiplicities (more than 4 crypts) are considered a more specific biomarker than total number of ACF [71].

ACF with dysplastic features (dysplatic crypts) may be more directly associated with tumorigenesis than ACF [72]. Dysplatic ACF have been identified by various investigators using different approaches. It can be identified based on the surface morphology of ACF with thicker epithelial lining, compressed luminal openings and mildly enlarged crypts, which are not elevated from surrounding epithelium [73].

Identification of mucin depleted foci (MDF) is based on an absent production of mucous, which is a common feature of severe dysplastic crypts. MDF can be stained by high-iron diamine alcian blue (HID-AB) of mucosal surface of unsectioned colon. In contrast to ACF, MDF are composed of dysplasic crypts [74]. Although, MDF have been demonstrated as a potential biomarker for evaluation of the chemopreventive effects of any agents, there are only a few using this marker.

The mutation of β-catenin gene or accumulation of β-catenin are the first step in rat colon carcinogenesis [75]. Identification of β-catenin accumulated crypts (BCAC) can be performed based on an immunohistochemical method in sectioned colon. BCAC are intraepithelial lesions that accumulate β-catenin protein in the

cytoplasm and nucleus. However, determination of BCAC is relatively costly and time consuming than other biomarkers.

6. Colon cancer models

In vitro models

The *in vitro* model of colon cancer can be performed in both primary colonic cell culture and colon carcinoma cell lines. Both models can be used to investigate the cellular mechanisms underlying tumor development, progression and are useful for screening or identification of chemopreventive agents from a large number of compounds. Primary cell cultures contain the same combination of cell populations that are presenting in the tissue or *in vivo* model, but they are generally not sufficient for high-throughput screening of many compounds. Because of a short lifespan of primary colon cells, colon cancer cell lines have been widely used instead. Cell lines can grow rapidly in standard media and can be easily transfected or their genes of interest can be knockdown. Nowadays, there are several cell lines established from colon carcinomas such as SW480, HT-29 and Caco-2 cells. However, the drawback of cell lines is a loss of tissue characteristics due to passaging. Thus, more than one type of cell lines are suggested when investigating certain effects of any compounds [76].

In vivo models

The genotoxic chemical 1,2-dimethylhydrazine (DMH) or its metabolite azoxymethane (AOM), and methoxymethane (MAM) have been widely used to induce colon carcinogenesis in mice and rats. DMH is one of agents most frequently used to induce the initiation and promotion stages of colon carcinogenesis in rodents. These surface abnormalities often appear in the distal colon within 2 weeks of carcinogen treatment [77]. Number of ACF in colon varies with time, concentration and frequency of carcinogen exposure and experimental design [66]. The chemical-induced colon cancer in animals covers the developmental process of normal epithelial cells into malignant carcinomas and shares many of the histopathological characteristics of human colon cancer. However, the drawback of this model would be time consuming and high cost.

7. Colon cancer and chemoprevention

Colon cancer development consists of three distinct phases: initiation, promotion and progression. The initiation phase is a rapid, irreversible event that occurs when normal cells are exposed to a chemical carcinogen. The promotion phase consists of expansion of mutated cells to form actively proliferating cells. The last phase progression, is an irreversible process in which new clones with increased proliferative capacity, invasion and metastasis are developed [78]. During the carcinogenesis process, the initiation and progression phases are irreversible and relatively transient events, thus the promotion of carcinogenesis may provide the best targets for cancer prevention [79].

Chemoprevention involves modulation the process of carcinogenesis in order to slow the progression, reverse, or inhibit carcinogenesis, thereby lowering the risk of cancer development [80]. Chemopreventive effect of any agents can be demonstrated by testing their abilities to suppress cancer cell proliferation, induce cell apoptosis, suppress the expression of anti-apoptotic proteins, induce cell cycle arrest and inhibit growth factor signaling pathway. Many studies are focused on the use of agents that eliminate pre-malignant cells by inducing them to undergo apoptosis, regulation of apoptotic proteins and inducing cell cycle arrest. Therefore, apoptotic and cell cycle processes are the major targets to eliminate the transformed cells for cancer therapies.

Cell proliferation

The structural and functional properties of the colon are not the same throughout its entire colonic length. The proliferating stem cells in the proximal colon are located in the mid-crypt and migrate up toward the luminal surface, whereas in the distal colon, the stem cells are located in the crypt base and these cells migrate up toward the luminal surface [81]. In normal colonic mucosa, the predominant area of cell proliferation is localized to the lower one third of the crypts, cells then migrate from the base of the crypt up towards the luminal surface, where they are slough off [82].

Cell apoptosis pathway

Apoptosis appears to be an important mechanism in deletion of tumor cells rather than inhibition cell proliferation [83]. Apoptotic cells exhibit several morphological features and biochemical modifications such as cytoplasmic shrinkage,

plasma membrane blebbing, nuclear condensation, and the later fragmentation of both cytoplasm and nucleus into apoptotic bodies after that these bodies are phagocytosed by macrophages [84]. Loss of plasma membrane is one of the earliest features of apoptotic cells. The membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment [85].

The Bcl-2 is the protein families that regulate cell apoptosis either to induce or inhibit apoptotic process. Bax is pro-apoptotic whereas Bcl-2 is anti-apoptotic protein. Both proteins are expressed in mitochondrial membrane and modulate apoptosis by permeablization of the inner and/or outer membrane, leading to release of cytochrome c. Cytochrome c directly activates Apaf-1 and induces the formation of apoptosome. The apoptosome mediates the activation of the initiator caspases, caspase-9, which subsequently activates the effector caspases, caspase-3, -6 and -7, which are responsible for cells to undergo apoptosis [86].

Bcl-2 (B cell lymphoma-2) proto-oncogene is located on chromosome 18q21. It was initially identified in follicular B cell lymphoma with chromosome translocation (t14:18) [87]. Bcl-2, known as an inhibitor of apoptosis and prolongs the cell life, encodes 25 kDa protein that localizes to the nuclear envelope, mitochondrial membrane and endoplasmic reticulum [88]. The ability of Bcl-2 to inhibit apoptosis depends on the intracellular balance among other members of Bcl-2 family such as Bax and Bad [89]. Expression of Bcl-2 has been studied in a number of human tumors, including colon cancer. Bcl-2 immunohistochemical expression has been evaluated as a prognostic factor for clinical use. In colonic crypts, Bcl-2 protein expression may contribute to the relative resistance of colonic epithelial cells to apoptosis [90, 91].

Colon cancer is believed to result from a series of genetic alterations that destroy normal mechanisms controlling the cell growth. Apoptosis appears to be an important mechanism in deletion of cancer cells rather than inhibition of cell proliferation. The bcl-2 proto-oncogene is a known inhibitor of apoptosis and may therefore allows an accumulation of genetic alterations that become propagated by cell division and potentially contribute to neoplastic development. In normal mucosa, Bcl-2 staining is restricted to basal epithelial cells, whereas abnormally the Bcl-2 protein expression is found in parabasal and superficial mucosal layers [92]. In the

hyperplastic colorectal epithelial, Bcl-2 expression is restricted to the basal epithelial cells as found in the normal condition. The alteration of Bcl-2 protein expression has been found in adenomas and carcinomas [90]. One possible explanation is that loss of p53 could lead to deregulate expression of Bcl-2 protein [93]. Watson *et. al* have demonstrated that Bcl-2 is expressed in a high rate in adenomas but it is often lost during progression to carcinoma, suggesting that Bcl-2 protein expression is an early event in adenomas formation and occurs before change in p53 [94].

Bax is an important regulator of Bcl-2 mediated apoptosis, as the overall regulation of apoptotic depends on ratio of Bax to Bcl-2. Thus, tumors with elevated Bcl-2 expression may also have high Bax expressors. Loss of Bax expression may be seen in tumors with decreased Bcl-2, which may then lead to metastasis [89]. Bax, a dominant repressor of Bcl-2 forms, heterodimers with Bcl-2 and accelerates the rates of cell death [89].

Cell cycle pathway

The cell cycle is a complex process, in which the cells received different growth controlling signals that are integrated and processed at various points known as checkpoints [95]. Blockade of the cell cycle is regarded as an effective strategy in the development of cancer therapies [96]. Treatment with flavonoid apigenin inhibit the proliferation of SW480, HT-29 and Caco-2 cells by inducing G2/M cell cycle arrest [97]. Joe et al. have reported that polyphenolic compound resveratrol induces growth inhibition, S phase arrest and apoptosis in SW480 cells [98]. Deregulated cell cycle progression driven by activation of growth-stimulating oncogenes is one of the primary characteristics of cancer cells. Cell cycle progression is tightly controlled by the regulation of expression and activity of cyclin/clin-dependent kinase (CDK) complexes. A key regulator of the G2/M transition of cell cycle is a complex of cell division cycle 2 (Cdc2) and cyclin B [99].

Lipid peroxidation

Lipid peroxidation is one of the most investigated consequences of reactive oxygen species (ROS) actions on membrane structure and function. It is involved in formation of lipid radicals and rearrangement of unsaturated lipid that subsequently give a variety of degraded products such as malondialdehyde (MDA) [100]. MDA is a mutagenic and genotoxic agent that is associated with the development of cancer.

8. Immune system and colon cancer

The role of immune response for controlling colon cancer progression has been investigated for a long time. The potential advantage of immunotherapy over radiotherapy and chemotherapy is that it selectively targets cancer cells [101]. Nowadays immune-base therapeutic approaches are successful in renal cancer and melanoma, although they have not been successful in colon cancer. The role of the host response to colon cancer might influence survival and outcomes in colon cancer [102]. Immune response to colon cancer is a complex process involving both innate and adaptive immunity [103].

The immune response can be divided into innate and adaptive components. The innate response composes of macrophages, natural killer (NK) cells, dendritic (DC) cells, neutrophils, eosinophils and mast cells. The adaptive response has two specialized classes of cells: T cells which are responsible for the cellular response and B cells which are responsible for the humoral response [104].

Macrophages represent the first line of the innate system defense capable of killing tumor and presenting tumor antigens to T cells [105]. Macrophages can be transformed into 2 different phenotypes; tumoricidal (cytotoxic) and tumor-promoting, depending on the presence of cytokines [106]. Natural killer cells (NK) are one of the cell populations in the innate immunity that plays an important role in the immune response against cancer cells. NK cells can induce tumor cell apoptosis via cytokine production and cytotoxic responses. There are limited reports on the role of NK cells in colon cancer. Increased numbers of CD57-positive cells which include NK cells and certain $CD8^+$ and $CD4^+$ T cells are found in colon cancer patients [107]. Colon cancer patients without metastasis have a significantly increased percentage of NK cells in peripheral blood whereas patients with metastatic colon cancer have a significant decrease in the number of NK cells when compared to control patients [108]. The number of dendritic cells decreased in colorectal cancer primaries compared with normal colonic mucosa. These cells are rare in metastatic tumors [104].

T cells are divided into two subsets with distinct functions. The $CD8^+$ cytotoxic T lymphocytes (CTLs) are key effector cells to eliminate both viral infected cells and potentially play a role in the elimination of tumor cells. $CD4^+$ T helper cells function primarily to regulate other cells of the immune system by secreting cytokines

[104]. $CD4^+$ T cells are also required for the generation and maintenance of $CD8^+$ T cells [109]. The activation of NK cells to kill cancer cells is enhanced by cytokines secreted by T cells. $CD4^+$ T cells secrete cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ) that activate $CD8^+$ cytotoxic T cells and macrophages. Macrophages also participate in killing cancer cells by producing tumor necrosis factor (TNF). $CD8^+$ T cells can get rid of cancer cells through induction of cell death by perforin/granzymes mediated pathway [110]. In the sporadic colon cancer, the balance between immunosurveillance through the function of $CD4^+$ and $CD8^+$ T cells as well as NK cells and tumor-promoting inflammation conveyed by innate immune cells as well as T and B cells are associated with stages of carcinogenesis [111]. Studies in mice have shown that activation of $CD4^+$ and $CD8^+$T cells leads to anti-tumor activities, whereas $CD4^+CD25^+$ Treg cells may suppress anti-tumor responses by other T cell subsets because removal of these immunosuppressive $CD25^+CD4^+$T cells can result in effective immune response to tumors both *in vivo* and *in vitro* [112, 113]. Thus, understanding the mechanisms underlying immunity to tumor would help to devise effective immunotherapy for chemoprevention in cancer.

Sericin protein

1. Structure and biochemical properties

Sericin is a natural silk protein which is removed from silk cocoons in a process of deguming. It is a glue protein secreted from the middle part of silk gland of silkworm, *Bombyx mori*. Sericin contributes 20-30% of total cocoon weight, and functions to envelop the fibroin, the fibril protein of cocoon (Figure 2) [72-74]. Its molecular weight is ranging from 20 to 400 kDa [70, 71]. Sericin has high content of serine (33.3-39.0%), glycine (14.1-16.0%) and aspartic acid/asparagines (11.3-15.7%) [114]. Sericin consists of polar side chain made of hydroxyl, carboxyl and amino groups that enable easy cross-linking, copolymerization and blending with other polymers to form improved biodegradable materials [115]. Sixty-three % of sericin appear in random coil, 35% in β-sheet, and no α-helical form. The major molecular conformation of easily soluble sericin is random coil, whereas β-sheet structure is more difficult to dissolve. [116].

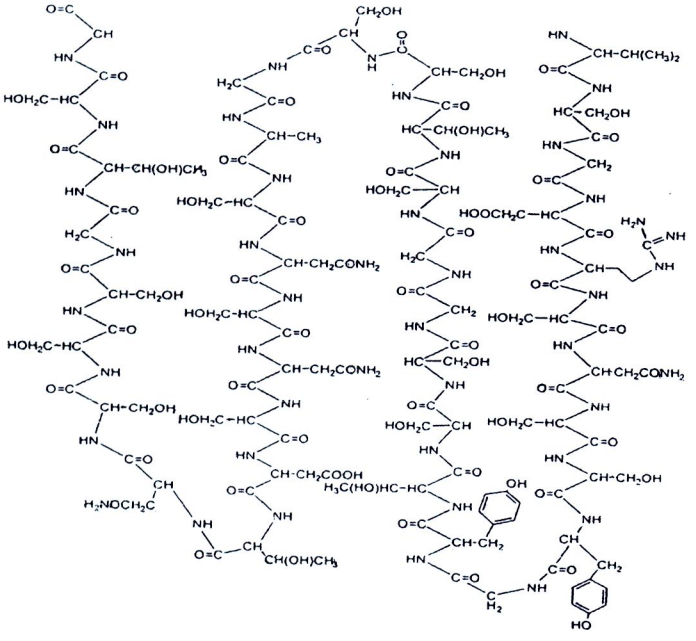

Figure 2 Structure of sericin (Nantong Dongchang Chemical Industrial)

2. Application of sericin

Sericin has more hydrophilic properties due to the presence of several hydroxyl groups when compared to fibroin [117], as well as good compatibility and biodegradation [118]. This property makes it a biocompatible and biodegradable material. When sericin is dissolved in a polar solvent, hydrolyzed in acid or alkaline solutions, or degraded by proteases. The size of the sericin molecules depends on several factors such as temperature, pH, and processing time of extraction. Lower molecular weight sericin peptides (≤ 20 kDa) are used in cosmetics, medical and pharmaceutical applications. High molecular weight sericin peptides (≥ 20 kDa) are mostly used as medical biomaterial and biodegradable material [119].

Cosmetics

Sericin helps to enhance the elasticity of skin such as anti-wrinkle and anti-aging effects when used as lotion and cream [119]. Increase in the intrinsic moisturization of skin by sericin may be attributed to restoration of the amino acids and its occlusive effect. Therefore, sericin would become an important moisturizing ingredient in moisturizing formulations [120]. Sericin has also been found to suppress lipid peroxidation and tyrosinase inhibition activity [121].

Biodegradable materials

Sericin has been applied to develop biodegradable materials, functional membrane materials and functional biomaterials and fabrics [119]. Polymer films, foams and fibers containing sericin (0.01-50% w/w) have excellent moisture-absorbing and desorbing properties [122].

Biomedical applications and dietary food

Sericin has been used as a dietary fiber with anti-oxidative activity [123]; anti-coagulant [124] and chemoprevention [15, 16, 75]. Sericin has protective effect against UVB-induced acute damage and tumor promotion in mouse skin [82] and inhibits UVB-induced apoptosis in human skin keratinocytes [125]. Sericin promotes the wound healing process without causing inflammation [126]. The study of macrophage activation response to silk protein has shown that sericin when attached to fibers induces inflammatory response [127]. Sericin in the presence of lipopolysaccharide induces inflammatory response by releasing tumor necrosis factor (TNF) and is associated with native silk fiber-induced immune response [128, 129]. Diet containing sericin is reported to suppress the incidence and number of colon tumors by DMH due to reduction of oxidative stress and cell proliferation [12, 13]. Sericin showed protective effects on alcohol-mediated liver damage in mice by increasing first-pass metabolism in the stomach and ethanol elimination [130]. Consumption of dietary sericin suppressed atropine-induced constipation in rats due to its low digestibility and high water–holding capacity [131]. Also, sericin consumption in rats is reported to elevate intestinal absorption of zinc, iron, magnesium and calcium [132].

Bioconjugates

Bioconjugation with natural or synthetic polymers provides methods of delivering drugs [133]. Several investigators have exploited sericin as a natural polymer bioconjugation with therapeutic protein, enzymes and polysaccharides such as insulin. Insulin is a therapeutic protein used to lower the levels of blood glucose in diabetes. The conjugation of sericin and insulin (SS-Ins) has a good bioavailability, longer half life in the blood and no immunogenicity and antigenicity [134].

Cell attachment and proliferation

Sericin has been used as additive in serum-free medium. Terada et al. have reported that when sericin is added to the cell culture of mammalian cell lines and hybridomas, there is acceleration in the proliferation of all cells. Sericin with low molecular weight (5-100 kDa) accelerates cell proliferation better than that of sericin having higher molecular weight (50-200 kDa). This study has suggested that sericin could be used as a supplement for cell culture [135]. Sericin also helps to protect insect cells from acute serum deprivation [136].