CHAPTER II

REVIEW OF RELATED LITERATURE AND RESEARCH

Anatomy of large intestine

Colon and rectum are parts of digestive tract. The digestive tract removes and processes nutrients from foods and helps pass waste material out of the body. The digestive tract is made up of the esophagus, stomach, small, and large intestines. The first 6 feet of the large intestine (Figure 1) are called large bowel or colon which composes of cecum, ascending, transverse, descending, and sigmoid colon. The last 6 inches are rectum and anal canal. The anal canal ends at anus by opening outside of the body.

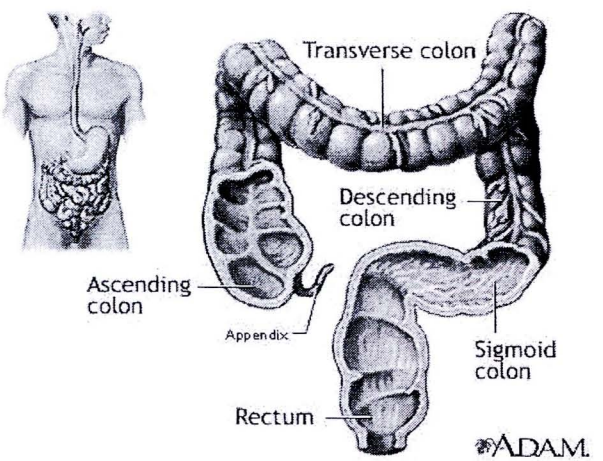


Figure 1 Anatomy of large intestine

Source: http://healthguide.howstuffworks.com/large-intestine-picture.htm

The absorptive epithelium of the large intestine contains many of epithelial cells (Figure 2). Stem cells self-renew to regenerate the epithelium after injury while progenitor cells arrest their cell cycle and differentiate when they reach the tip of the crypt (Clevers, 2006). All the cells within the crypt are derived from the stem cells. One of the mitotic stem cells remains as a stem cell at the bottom of crypt and another

cell is gradually pushed up to the luminal surface of the crypt as an epithelial cell. The cells that reached the uppermost part of the crypts undergo apoptosis and peel off without replication or differentiation (Bach, et al., 2000). Therefore, any mutations in these cells did notimpact on the normal turnover of mucosa. The cells with damaged DNA do not cause apoptosis and reach the uppermost part in the crypt, and continue proliferation which developed to cancer.

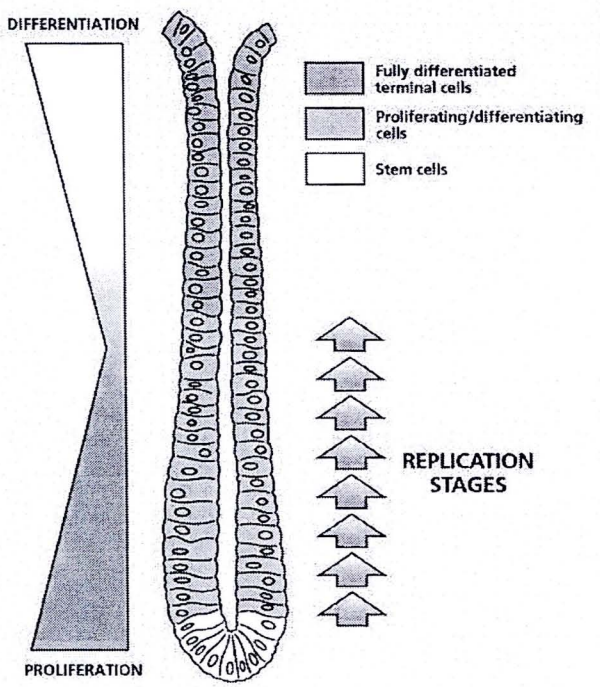


Figure 2 Absorptive epithelium of large intestine

Source: http://ehp.niehs.nih.gov/docs/2002/110-7/niehsnews.html

General information of colorectal cancer

Colorectal cancer is a disease of cells in colon or rectum become abnormal and divides without control. Colorectal cancer is one of most commonly diagnosed cancer in the world (Ferlay, et al., 2010). The incidence rate of colorectal cancer in Thailand is lower than other countries in 1998 - 2000. However, in Thailand colorectal cancer is the third in frequency of malepeople after the liver and lung cancer, and the fifth after cancer of the cervix, breast, liver and lung for female as shown in Figure 3 (Khuhaprema and Srivatanakul, 2008). This can reflect that colorectal cancer is a major cause of morbidity and mortality in Thai people.

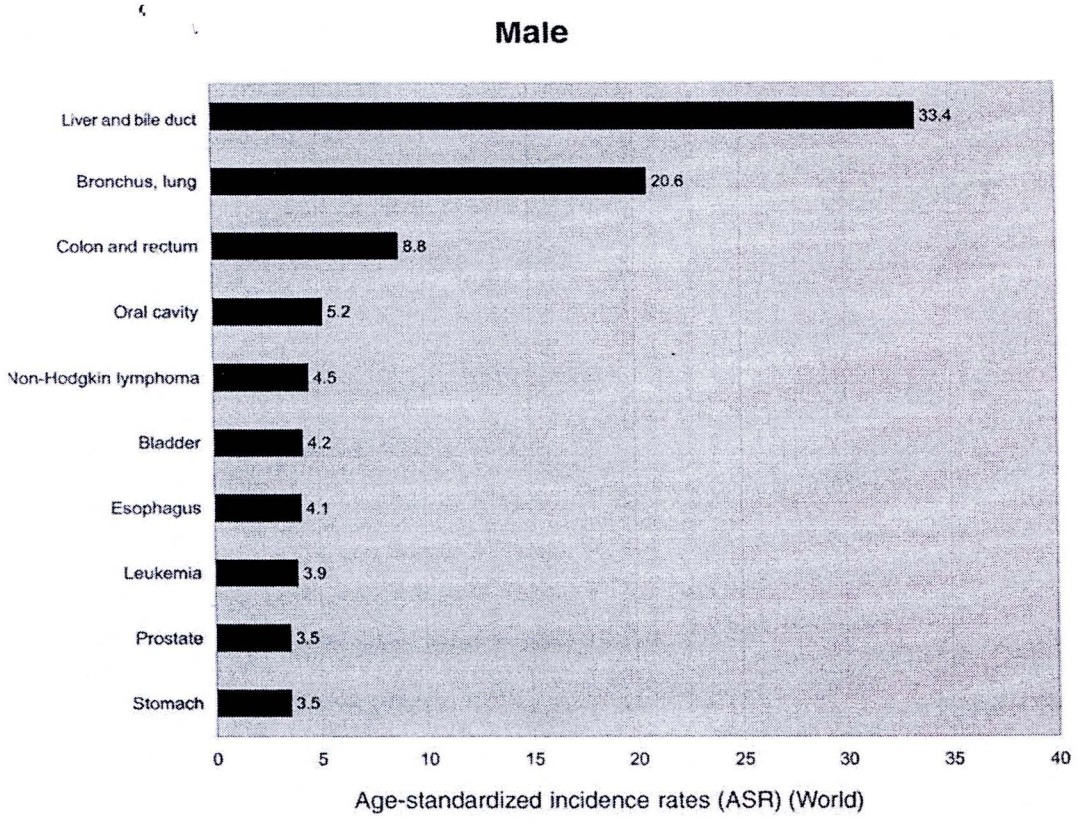


Figure 3 Incidence of colorectal cancer in 1998-2000 Thailand

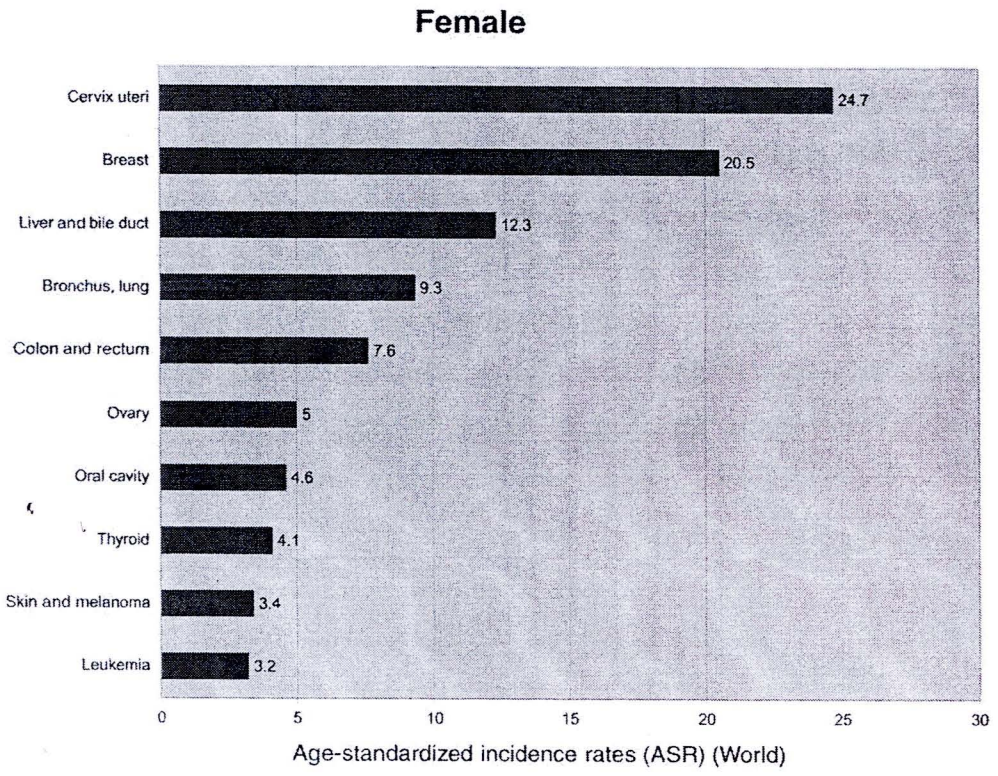


Figure 3 (Cont.)

Source: Khuhaprema and Srivatanakul, 2008

Risk factors of colorectal cancer

The exact causes of colorectal cancer are not clear, but risk appears to be associated with genetic or family inheritance such as Familial Adenomatous Polyposis (FAP) which causes by mutation in the tumor suppressor gene, Hereditary Non-Polyposis Colorectal Cancer (HNPCC) which associate with mutations in DNA mismatch repair genes, high fat intake cause increase bile acids to damage DNA, family history of colorectal cancer and polyps, and long duration of chronic ulcerative colitis, a chronic inflammation of the large intestine.

1. Genetic or familial inheritance

Hereditary colon cancer syndromes are disorders affected family members have inherited cancer-causing genetic defect from one or both of the parents. These forms of colorectal cancer represent a smaller percentage of all colorectal cancer cases. However, family history of colorectal cancer is an important risk factor. There are two basic forms recognized as having a genetic basis:

1.1 Familial adenomatous polyposis (FAP)

FAP is a genetic disorder that is characterized by the development of many hundreds to thousands of polyps form mainly in the epithelium of the large intestine. It equally occurs in both sexes, and accounts for less than 1% of colorectal cancer cases. People who have inherited the hereditary colorectal cancer syndrome genes are at risk of developing large number of colon polyps, usually at young ages, and very high risk of developing colon cancer early in life (Half, et al., 2009).

The classical FAP is resulting from a mutation in the adenomatous polyposis coli (*APC*) gene.*APC* is a tumor suppressor gene that located on the long arm of chromosome 5 in band q21 (5q21) (Groden, et al., 1991). The *APC* protein has an important role in cell proliferation, signal transduction, and transcriptional activation which prevent the uncontrolled growth of cells. Mutation in *APC* gene leads to loss of ß-catenin regulation, altered cell migration and chromosome instability lead to abnormal cell proliferation and develop to cancer.

1.2 Hereditary non-polyposis colon cancer (HNPCC) or Lynch syndrome (LS)

HNPCC is caused by inherited germline mutations in mismatch repair (MMR) genes lead to microsatellite instability (Lynch, et al., 2009). It is represents 1-7% of all colorectal cancer cases and characterized by an early age of onset, frequent occurrence of multiple lesions, and a striking association with tumors of other organs, such as endometrium, urinary tract, ovary, stomach, and small intestine (Stigliano, et al., 2008).

Mismatch repair (MMR) genes particularly in MSH2 and MLH1 genes which were found on chromosome arms 2p and 3p, respectively (Soreide, et al., 2006). MMR genes have several functions relating to genetic stabilization, such as correcting errors in DNA synthesis. Mutation in these genes impair the function of

MMR proteins lead to inactivation of genes and thus to a defect in DNA replication or repair.

2. Dietary intake

Diet is important in the etiology of colorectal cancer. Previous studies implicated that red meat and high fat diet as factors in colon carcinogenesis (Sesink, et al., 1999; Geter, et al., 2004).

High fat intake results in a significantly higher excretion of fecal secondary bile acids, mainly deoxycholic acid (DCA) and lithocholic acid (LCA) (Reddy, et al., 1980b; Bianchini, et al., 1989). Previously study reported that high fecal secondary bile acids associated with high incidence of colorectal cancer as demonstrated in rats by its ability to increase proliferation of colonic crypt cells (Seraj, et al., 1997) and significantly increase tumor size and number in animal models (Reddy, et al., 1980a; Wasan, et al., 1997). Furthermore, epidemiologic studies have also found that DCA concentration was high in patients with adenomas (Bayerdorffer, et al., 1995). However, the bile acids did not produce any tumors by themselves (Reddy and Watanabe, 1979). It is suggested that secondary bile acids may play a role in the promotion of colorectal carcinogenesis. The mechanism of bile acids that induced colon carcinogenesis is still unclear. Several studies have shown that bile acids may induce DNA damage, cell proliferation and activate some genes, including COX-2 which leads to alteration in cell growth and carcinogenesis (Booth, et al., 1997; Pool-Zobel and Leucht, 1997; Glinghammar, et al., 2002).

Red meat (beef, pork or lamp) consumption is associated with an increased risk of colorectal cancer in the promotion step of the carcinogenesis, but the mechanism is not clear. Several studies reported that heme, highly found in red meat, causes cytotoxic effects in the colonic lumen which may effect the proliferation of the colonic epithelium. Histological examination in rats fed heme diet without carcinogen induction, caused increase colonic epithelium injuryresulted in a compensatory of colonic epithelial cells by significantly increased proliferation more than the control rats, and inhibited the colonic mucosal apoptosis (de Vogel, et al., 2008). The hyperproliferation and reduction of apoptosis in colonic epithelium are considered early risk markers of colorectal cancer (Lipkin, 1988). Moreover, the chronic inflammation caused by the continuous heme intake might have promoted

the ACF growth (Rhodes and Campbell, 2002). According to this hypothesis, Pierre and his colleagues demonstrated that heme promotes preneoplastic lesions in the colon by strikingly increased the size and number of aberrant crypts in DMH-induced colorectal carcinogenesis rats (Pierre, et al., 2003).

3. Chronic inflammation of the large intestine

Chronic inflammation of gastrointestinal mucosa, including ulcerativecolitis (UC) and Crohn's colitis (CC), is an increased risk for development of colorectal cancer. Darren and his colleagues shown that oral administration of dextran sulfate sodium (DSS) to mice induced moderate severity of colorectal inflammation, mucosal ulceration and 65% of the mice developed colorectal tumors with tumor multiplicity (Seril, et al., 2007). Furthermore, DSS-induced chronic UC in rodents have clinical and histological of chronic UC similar to human UC (Okayasu, et al., 1990). However, the molecular mechanisms linking inflammation and colorectal cancer carcinogenesis are incompletely understood. A possible hypothesis is many malignancies that arise from areas of infection and inflammation. Persistent infections within the host induce chronic inflammation (Figure 4). Leukocytes and other phagocytic cells induce DNA damage in proliferating cells, through their generation of reactive oxygen (ROS) and nitrogen species (RNS) that are normally produced by these cells to fight infection (Maeda and Akaike, 1998).

Nitric oxide (NO) and peroxynitrite, the product of the reaction of NO with the superoxide anion may also play a role in the enhancement of colon cancer risk in UC patients (Nguyen, et al., 1992; Wink, et al., 1998; Seril, et al., 2007). NO and peroxynitrite, the product of the reaction of NO with the superoxide anion, are mutagenic activities *in vitro*, inhibits the functions of DNA repair enzymes (Jaiswal, et al., 2001) and stimulate new blood vessel growth via increase vascular permeability and endothelial cell proliferation (Wink, et al., 1998; Seril, et al., 2007).

Hence, repeated tissue damage and regeneration of tissue, in the presence of highly RNS released from inflammatory cells, interacts with DNA in proliferating epithelium resulting in permanent genomic alterations such as point mutations, deletions, and rearrangements (Yamanishi, et al., 2002).

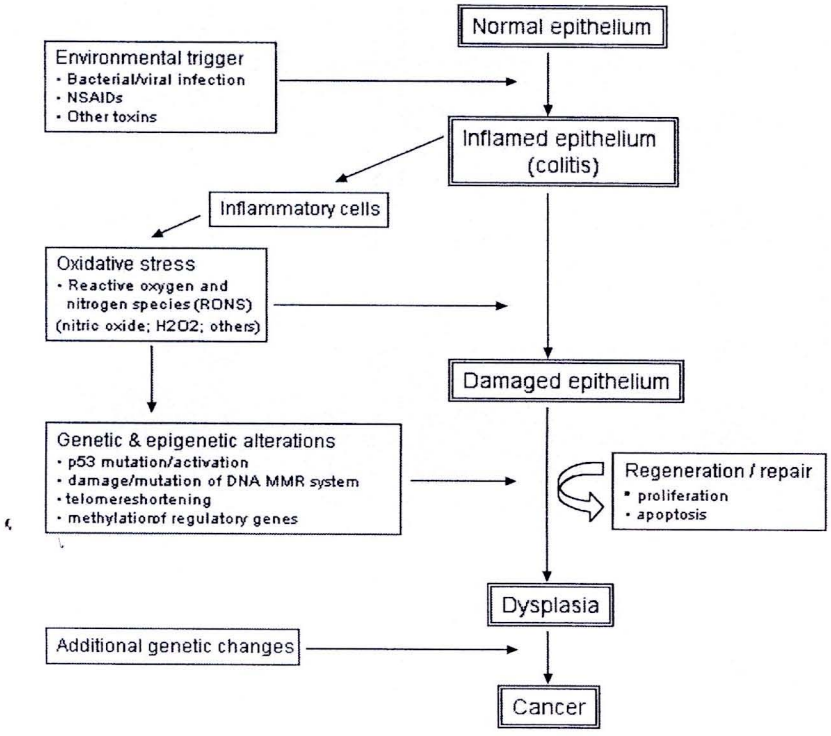


Figure 4 Diagram showing chronic inflammation associate with development of cancer

Source: Itzkowitz and Yio, 2004, p.G 10

Mechanism of colorectal carcinogenesis

At the cellular level, the development of colorectal cancer develop through multistep process involving mutation and selection for cells with progressively increasing capacity for proliferation, survival, invasion, and metastasis. The carcinogenesis process consists of tumor initiation, tumor promotion, and tumor progression.

Tumor initiation, the first step in the process (Figure 5), is thought to be the result of a genetic alteration leading to abnormal proliferation of a single cell. The second step is tumor promotion, the tumor promoter increase proliferation of this cell leads to the outgrowth of a population of clonally derived tumor cells and called ACF, pre-cancerous change, and now being widely used as one of the biomarkers of colon carcinogenesis in chemopreventive experiments (Pretlow, et al., 1991; Bird, 1995).

Tumor progression continues as additional mutations occur within cells of the tumor population. Further, some of these mutations confer a selective advantage to the cell, such as more rapid growth will become dominant within the tumor population and then continuously become more rapid-growing and increasingly malignant (Hausman, 2007; Oliveira, et al., 2007).

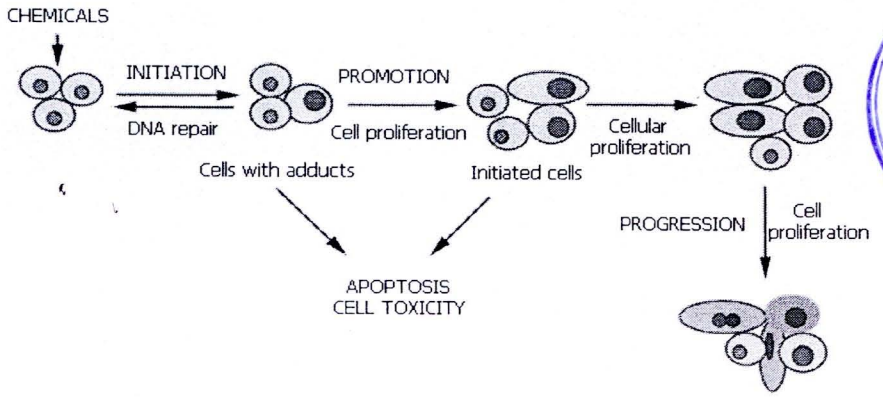


Figure 5 The multistep process of human carcinogenesis

Source: Oliveira, et al., 2007, p.597

APC gene mutation is typically occurring at the first stage of human colorectal carcinogenesis process (Figure 6). The *APC* protein that translated from the *APC* gene is main factor in the Wnt signal pathway and regulates cell proliferation by binding and degrading ß-catenin that promotes cell proliferation (Ilyas, 2005).The mutant *APC* protein cannot bind and degrade ß-catenin protein and, as a result, ß-catenin protein translocates to the nucleus and binds to the T-cell factor or lymphocyte enhancer factor transcription factor, which targets *c-myc*, *cyclin D1* and *c-jun* genes and promotes cell proliferation (Figure 7) (Fodde, et al., 2001).

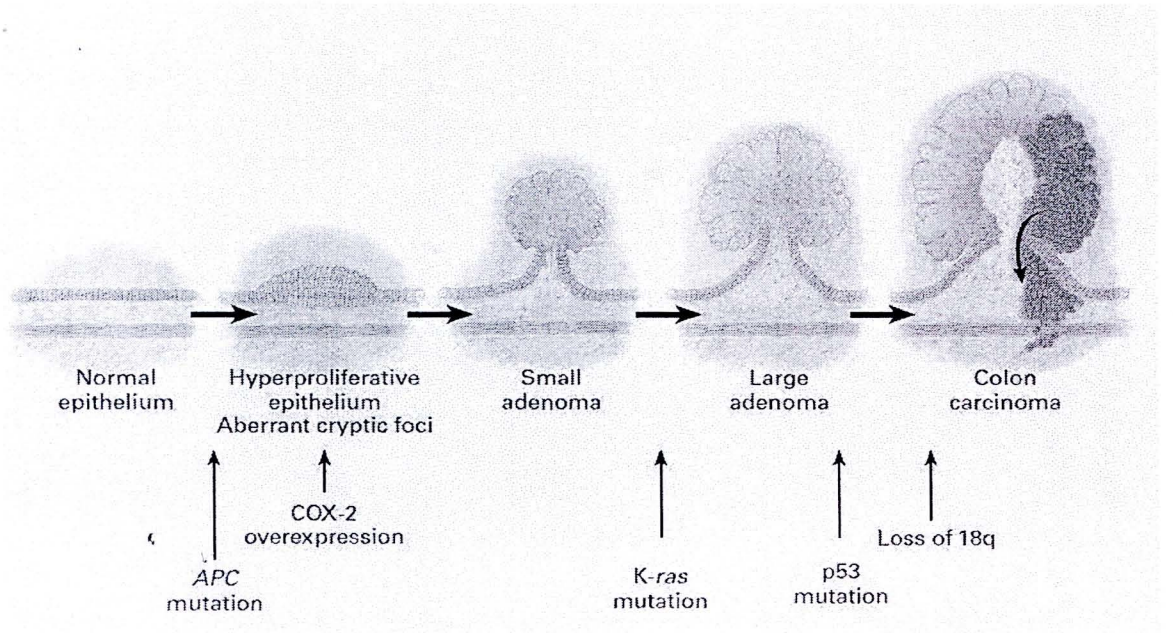


Figure 6 Genetic mutations in human colon cancer

Source: Pasi and Robert, 2000

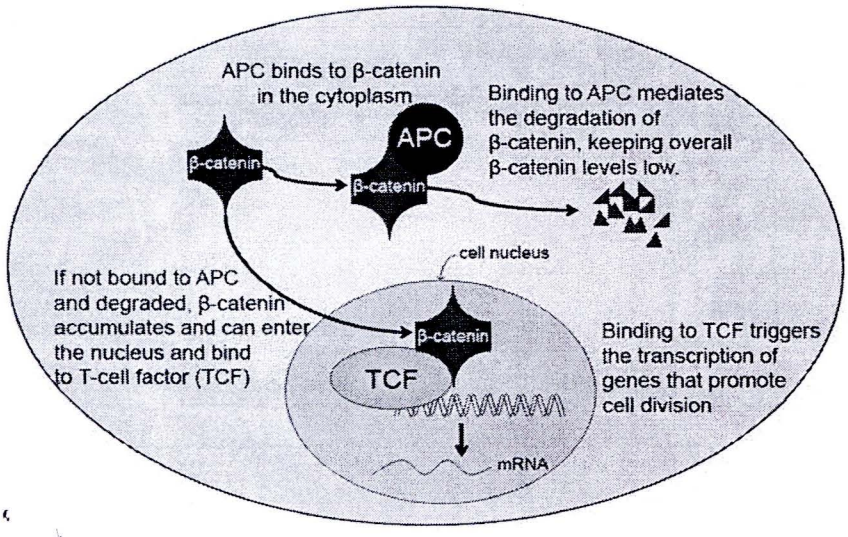


Figure 7 Diagram showing one of APC's cellular functions

Source: http://www.sciencecases.org/colon_cancer/case4.asp

During the progression of the adenoma, such as increases in adenoma size, degree of dysplasia, and degree of villous histology, induction of the K-*ras* mutation occur after the mutation of *APC* gene (Itzkowitz and Yio, 2004). K-*ras* mutations were identified in ACFand normal mucosa adjacent to tumor of colorectal patients (Pretlow, et al., 1991; Zhu, et al., 1997). The functional of the *ras* proteins are involved in the transduction of external stimuli, most likely induced by growth factors or factors involved in cell differentiation (Bos, 1989). Therefore, mutation of *ras* proteins stimulates cell division and proliferation in the absence of a growth factor and facilitates the development of cancer cells.

Loss of *p*53 gene function occurs late and is believed to be the defining event that drives the adenoma to carcinoma. The *p*53 protein is encoded by the *p*53 gene regulates the cell cycle, and protect the normal cells from proceeding to replicate damaged DNA. The *p*53 gene function as a tumor suppressor is involved in preventing cancer (Fazeli, et al., 1997). Therefore, mutation of *p*53 gene cause stimulates cell proliferation, transformation and differentiation of cancer cells (Fazeli, et al., 1997).

Aberrant crypt foci (ACF)

ACF has been described as single or clusters of abnormally large crypts of the colon mucosal surface after stained with methylene blue. They have been generally accepted as precancerous lesions both in rodents and human colorectal cancer (McLellan, et al., 1991; Cheng and Lai, 2003), morphologically distinguishable from normal crypts, induced in colons of several animal models, remarkable similarity between human and rodents, rapidly detectable (Shpitz, et al., 1998; Bouzourene, et al., 1999; Raju, 2008), and proved to be a reliable biomarker in short-term screening assay for colon carcinogenesis in laboratory rodents (Velmurugan, et al., 2008). They represent lesions that can also be characterized by genetic and biochemical alterations (Pretlow, et al., 1992; Cheng and Lai, 2003). Furthermore, ACF are easily induced by colon-specific carcinogens, such as azoxymethane (AOM) and DMH in rodents and can be used to learn more about the process of colon carcinogenesis.

Several studies described the characteristic of ACF (Figure 8) by the following criteria: the size, luminal opening of the cryptand thickened epithelial are larger than normal surrounding crypts (Cheng and Lai, 2003; Yamada and Mori, 2003).

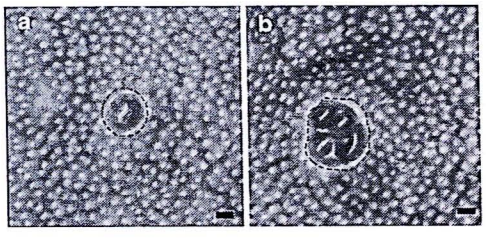


Figure 8 ACF in methylene blue stained-colon whole mount. ACF (circumscribed) are easily distinguished from surrounding normal colonic crypt, bars = 100 µm.

Source: modified from http://www.springerimages.com/Images/Biomedicine/1-10.1007_s12575-010-9032-x-1

The mechanism of ACF increase in size seems to be a process of crypt division, which begins at the base of the crypt and proceeds upwards until two crypts are generated. Thus, the number of crypts per ACF, also termed "crypt multiplicity", would be an important parameter for evaluating ACF progression. It has been demonstrated that the number of ACF with 4 or more crypts (Figure 8b) significantly increased in advanced stages of carcinogenesis (Pretlow, et al., 1991; McGinley, et al., 2010).

Cyclooxygenase and arachidonic metabolism

The cyclooxygenase (COX), also known as prostaglandin H_2 synthases (PGH_2s), are the enzyme for the prostaglandin production. COX changes arachidonic acid into prostaglandin H_2 (PGH_2) that is further metabolized to a variety of prostaglandins, such as prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostacyclin and thromboxane A_2 (Figure 9) by different prostaglandin synthases (Kawai, et al., 2002). The COX enzyme consists of COX-1 and COX-2. Both COX enzymes are located intracellular on the luminal surface of the endoplasmic reticulum and in the nuclear envelope. However, function and pattern of expression of COX-1 and COX-2 differ substantially. COX-1 enzyme is constitutively expressed in most tissues and functions as a housekeeping enzyme by the production of prostacyclins, prostaglandins and thromboxane (Dubois, et al., 1998). These products are essential for protection of gastric mucosa by inhibiting acid secretion and stimulating bicarbonate and mucus secretion (Ashburn and Rubingh, 1999). COX-2 enzyme, which catalyzes the production of PGE_2 (Eberhart, et al., 1994), is only constitutively expressed in brain, testis and renal parenchyma, nearly absent in other tissues under normal conditions, but rather is induced by numerous stimuli including mitogen, pro-inflammatory cytokines and tumor promoters, leading to increased accumulation of prostanoids in neoplastic and inflamed tissues (Eisinger, et al., 2007). Many studies reported that PGE_2 levels are increased in scenarios of inflammation, human colon cancer in comparison with adjacent normal tissue (Rigas, et al., 1993; Kargman, et al., 1995).

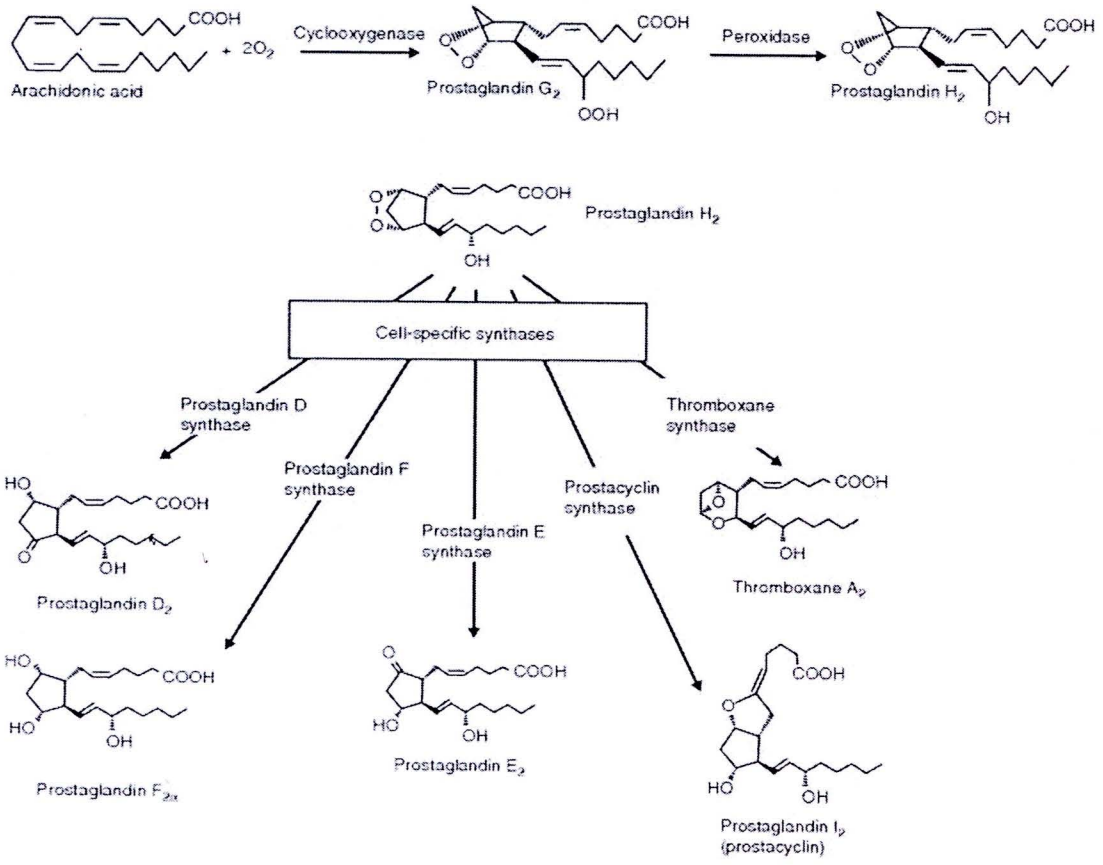


Figure 9 Production of prostaglandins by COXs and the cell-specific synthases that are involved in the conversion of prostaglandin H_2 to the five principalprostaglandins.

Source: Chandrasekharanand Simmons, 2004

1. COX-2 expression in inflammation and cancer

PGE_2 and COX-2 have been increased in inflammatory tissue. Olivoand his colleagues demonstrated that PGE_2 and COX-2 levels significantly increase after injected with venom-induced edema formation (Olivo Rdo, et al., 2007). Many studies reported that breast cancer cell lines and human breast cancer tissues expressed COX-2 with no detectable expression in normal breast tissue (Half, et al., 2002; Basu, et al., 2005). On the other hand, COX-2 up-regulation has also been frequently reported in other cancer types, particularly in the prostate (Yoshimura, et al., 2000), skin (Denkert, et al., 2001), and lung (Schroeder, et al., 2007).

2. **COX-2 expression in colorectal cancer**

COX-2 is directly involved in colorectal tumor development. Many studies demonstrated an elevated expression of COX-2 mRNA and protein in colon carcinoma cell lines (Yamazaki, et al., 2002; Janssen, et al., 2006), Min mouse adenoma (Williams, et al., 1996) and polyp formation of adenomatous polyposis coli (APC)-mutated mice (Sunayama, et al., 2002), whereas COX-1 expression was present at equal or reduced levels compared to normal tissue.

3. Role of COX-2 expression in carcinogenesis

Previous report showed that knock out of COX-2 gene was associated with a lower incidence of intestinal polyps in adenomatous polyposis coli mutant mice, a murine model of familial adenomatous polyposis (Oshima, et al., 1996). These result provided that COX-2 is involved in colorectal carcinogenesis. The relationship of COX-2 and carcinogenesis may involve several pathways as described below:

3.1 Activation of carcinogens

The COX-2 enzyme has both oxygenase and peroxidase functions. The peroxidase activity catalyzes the conversion of pro-carcinogens to carcinogens in extrahepatic tissues such as the upper digestive tract which initiate tumor formation (Altorki, 2004). Xenobiotics (natural non-human organic compounds) can be co-oxidized into mutagens by the peroxidase activity of COX. In the liver, these types of oxidative reactions are catalyzed principally by cytochrome P-450s, thus preventing the formation of mutagens. In contrast, the colon has low concentrations of P-450s, leading to co-oxidation of significant amounts of xenobiotics to mutagens by the peroxidase activity of COX (Eling, et al., 1990).

3.2 Inhibition of apoptosis

The evolution of tumor size depends on the balance between cell proliferation and cell death. In pre-malignant and malignant lesion, decreased apoptosis, or programmed cell death, has been largely observed. Various experimental studies have shown a positive correlation between the expression of COX-2 and inhibition of apoptosis. Intestinal epithelial cells of rats engineered to over-express COX-2, were showed to have increased amounts of Bcl-2 anti-apoptotic protein. Nevertheless, treatment with the a COX-2 inhibitor, sulindacsulphide was able to induced apoptosis (Tsujii and DuBois, 1995).

Additionally, increased apoptosis in human colon carcinoma cell lines have been observed after exposure to various NSAID, a selective COX-2 inhibitor (Sakoguchi-Okada, et al., 2007). Furthermore, celecoxib, a selective COX-2 inhibitor, and in combination with cisplatin caused human osteosarcoma cells apoptosis related to significantly decreased protein levels of phosphatidylinositol-3-kinase (PI3K)/Akt and bcl-2 (Liu, et al., 2008), which up-regulates apoptosis resistant factors.

3.3 Promotion of angiogenesis

The growth of solid tumors and the formation of metastases depend on the generation of new blood vessels, also known as tumor angiogenesis. Previousstudies shown that COX-2 stimulates endothelial cell migration and promotes angiogenesis both *in vitro* and *in vivo* studies (Tsujii, et al., 1998; Jones, et al., 1999) by directly stimulate the production of angiogenic factors, vascular epithelial growth factor (VEGF). VEGF is highly expressed in colonic adenomas than normal tissues and closely correlated with prognosis in colorectal cancer patients (Ishigami, et al., 1998; Wong, et al., 1999). Furthermore, vascular density of xenograft tumor model with COX-2 knockout mice was decreased 30% than tumors from wild-type mice (Sunayama, et al., 2002).

Angiogenesis inhibition by COX-2 inhibitor was demonstrated in several experiments. JTE-522 inhibited the VEGF expression of macrophage in the submucosa of large adenomas, resulting in a decrease in vascular area (Sunayama, et al., 2002). Human colorectal cancer liver metastases treated with Rofecoxib had decrease microvessel density (MVD) for 29% compared with placebo-treated tissues (Fenwick, et al., 2003).

3.4 Invasion and metastasis

Invasion and metastasis is a complex series of steps in which cancer cells leave the original tumor site and migrate to other parts of the body via the bloodstream or the lymphatic system. Tumor cells must penetrate basement membranes and the extracellular matrix (ECM) by collagenases, such as matrix metalloproteinase (MMP-2 and MMP-9). These MMPs are degrading the collagen IV component of the basement membrane in ECM, and the malignant cells can invade to organs and enter the bloodstream. Many studies reported that over-expression of

COX-2 involved in tumor cell invasion and metastasis. Li and his colleagues investigated the relationship of COX-2 and tumor invasive ability by transfected COX-2 expression vector to human transitional cell carcinoma (TCC). The results showed that COX-2 significantly stimulated inversion of cancer cells and the motility of the cancer cells was inhibited by a selective COX-2 inhibitor indomethacin (Li, et al., 2002).

Colorectal cancer treatments

There are different types for colorectal treatment, such as standard surgical treatment and standard chemotherapy.

1. **Standard chemotherapy drug, 5 -Fluorouracil (5-FU)**

5-FU is widely used as a chemotherapeutic drug for treatment various cancersincluding colorectal cancer. Previous studies have reported that 5-FU treatment increase the accumulation of colon cancer cells in S - phase, and induce apoptosis (Oka, et al., 1997; Wiebke, et al., 2003). 5-FU is an analogue of uraciland rapidly enters the cell using the same facilitated transport mechanism asuracil. 5-FU is converted intracellularly to several active metabolites mainly, fluorodeoxyuridine monophosphate (FdUMP) and fluorouridine triphosphate (FUTP), within the cell (Figure 10). FdUMP inhibits irreversibly the enzyme thymidylate synthase (TS), preventing DNA synthesis. FUTP incorporated into RNA, interfering with protein synthesis (Longley, et al., 2003).

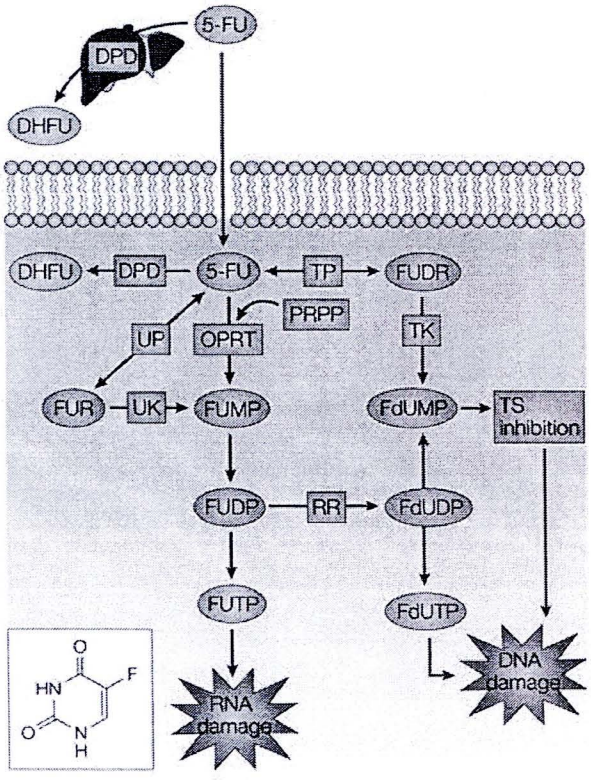


Figure 10 Metabolism of 5-fluorouracil

Source: Longley, et al., 2008, p. 331

Although 5-FU has successful effect to treatment of various cancers; however, the 5-FU single treatment in advanced colorectal is limited response rate. Long-term treatment with 5-FU often develops drug resistant. The main factor in resistant to 5-FU is over- expression of TS (Chu, et al., 1993; Parr, et al., 1998), which is essential for DNA synthesis. Johnston and his colleagues demonstrated that patients with responsive disease had low level of TS protein and TS gene, whereas highly level in patients who did not response to 5-FU, indicated that both TS protein and TS gene expression associated with response to 5-FU chemotherapy (Johnston, et al., 1995). Moreover, reduced activities of 5-FU metabolizing enzyme, orotatephosphoribosyltransferase (OPRT), orotatephosphoribosyltransferase (OPRT), dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP), and uridinephosphorylase (UP) (Figure 10), has been associated with the sensitivity to 5-FU in colorectal cancer treatment (Isshi, et al., 2002; Inokuchi, et al., 2004). In addition, long-term exposed to 5-FU treatment showed a low level of the pro-

apoptotic Bax protein and high levels of anti-apoptotic Bcl-2 and Bcl-xL protein which correlated with a higher resistance of cancer cells to 5-FU treatment (Violette, et al., 2002). However, 5-FU has remained the main agent for treatment of both early and advanced stages of colorectal cancer. Therefore, strategies that explore to modulate the anticancer activity of 5-FU including decreasing 5-FU resistance should be further development.

2. **Cyclooxygenase-2 inhibitor**

Many studies showed that COX-2 is a major target for prevention and treatment of colorectal. Therefore, agents that are non-toxic and can inhibit COX-2 activity might be useful for the inhibition of colorectal carcinogenesis.

2.1 Non-Steriodal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are commonly used medications for reduce pain and inflammation by blocking COX-2 (Figure 11). Many preclinical studies have suggested that NSAID or COX-2 inhibitors have anticancer effects in animal models of colon, urinary bladder, skin, and breast cancer (Kawamori, et al., 1998; Pentland, et al., 1999; Grubbs, et al., 2000; Harris, et al., 2000). Previous study demonstrated that regular use COX-2 inhibitor decrease risks of colorectal and adenomatous polyps 20-50% (Kawamori et al., 1998) or reduces the number of colorectal polyps in patients with FAP by suppression of PGE_2 levels which produce by the COX-2 enzyme (Steinbach, et al., 2000).

However, NSAIDs have important adverse effects. Many of NSAIDs such as aspirin and ibuprofen are general nonselective COX inhibitors. This lack of selectivity cause inhibit constitutively COX-1, (Traversa, et al., 1995). Moreover, some NSAIDs have also been associated with an increase risk of serious cardiovascular events, such as myocardial infarction (Chou, et al., 2006), inactivation of thromboxane in platelets, results in impair aggregating and increase of bleeding time (Leese et al., 2000), while the selective COX-2 inhibitor makes less toxic for gastrointestinal tract and cardiovascular system.

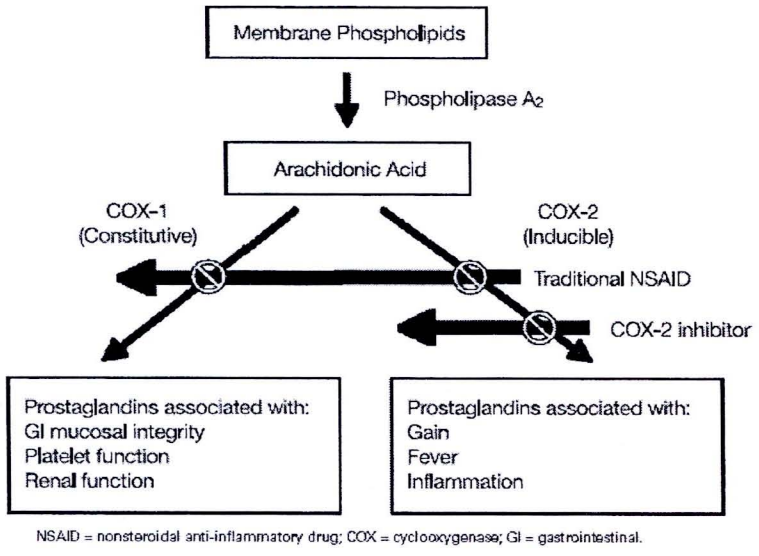


Figure 11 Pharmacology of NSAIDs and selective COX-2 inhibitors on prostaglandinsynthesis

Source: https://secure.pharmacytimes.com/lessons/200607-01.asp

2.2 Curcumin

CUR extracted from the root of *Curcuma longa* L., commonly called turmeric, a member of ginger family (Figure 12). Turmeric has been used as a spice and food-coloring agent for centuries in Southeast Asia and available commercially at a low cost. Fractions of turmeric known as curcuminoids (CUR, demethoxycurcumin, and bisdemethoxycurcumin) (Figure 13) are considered the active compounds and possess a yellowish orange color.

Figure 12 Characteristics of *Curcuma Longa* L.

Source: http://www.itmonline.org/arts/turmeri3.htm
http://health-food-nutrition.blogspot.com/2010_03_14_archive.html

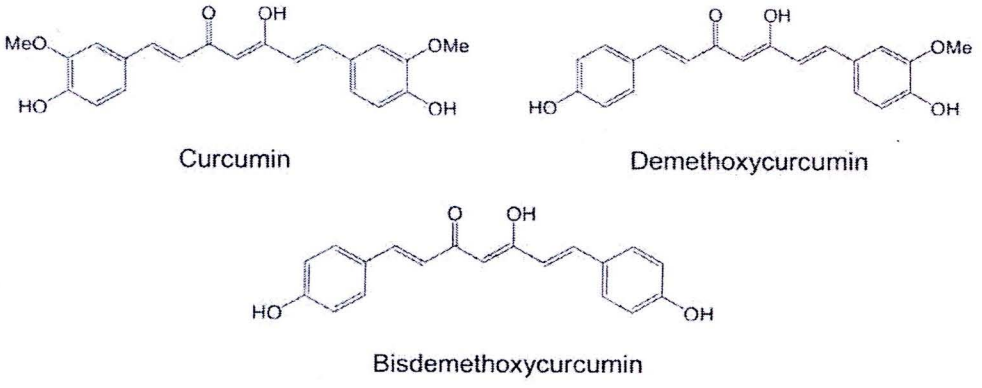


Figure 13 Structures of Curcumin, Demethoxycurcumin and Bisdemethoxycurcumin

Source: Laurie, et al., 2008, p. 1595

The anticancer properties of CUR

The previous data showed that CUR can act as a preventive effect against cancers and serve in cancer therapy as a drug. For example, administration CUR in the diet of mice during the initiation and post-initiation stages significantly inhibited colon tumor incidence and increased apoptosis in the colon tumors (Huang,

et al., 1994; Kawamori, et al., 1999). Dietary CUR also significantly suppressed the colon tumor volume (57%) which compared to the control diet (Rao, et al., 1995).

The anticancer properties of CUR have shown in culture cells and animal studies. CUR play important role in induction of apoptosis, specific inhibition of COX-2 expression, inhibits the initiation of carcinogenesis by inhibiting the cytochrome P-450 enzyme activity, increasing the levels of glutathione-S-transferase, arrest of cancer cells in S, G_2 / M cell cycle phase, and inhibits the growth of DNA mismatch.

1. Induction of apoptosis

Previous studies found that CURinhibits the growth of HT-29 human colon cancer cells by induction of apoptosis in a dose-and time-dependent manner (Jaiswal, et al., 2002; Moos, et al., 2004). Song and his colleagues reported that CUR could up-regulate the serine phosphorylation level of *p*53 tumor suppressor protein and the Bax pro-apoptotic protein, while down-regulate the levels of Bcl-2 anti-apoptotic protein. Moreover, CUR could also down-regulate the expression of pro-caspase-3 and procaspase-9 in a time-dependent manner (Song, et al., 2005).

2. Specific inhibition of COX-2 expression

Inhibition of prostaglandin biosynthesis by inhibition of COX-2 expression is an important mechanism that contributes to the chemopreventive activity of CUR. Goel and his colleagues reported that CUR markedly inhibit of COX-2 mRNA and protein expression in colon cancer cell lines without effected on COX-1 expression (Goel, et al., 2001). Moreover, CUR directly suppress the expression of COX-2 in several gastrointestinal cell lines (SK-GT-4, SCC450, IEC-18 and HCA-7) inducted by chenodeoxycholate (CD) or phorbol ester (PMA) (Zhang, et al., 1999). In addition, curcumin has ability to inhibit the production of PGE_2 induced by PMA in human colonic epithelial cells (Ireson, et al., 2001).

3. Interfere cell cycle

Cellular proliferation of normal cells is process by a cell grows, replicates its DNA and then divides to give two daughter cells. The cell division is dividing into four sequential phases as show in Figure 14. Movement through each phase of the cell cycle and transition from one phase to the next is regulated at a number of positions within the cell cycle known as checkpoints. Cells that have

temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase but not found in cancer cells.

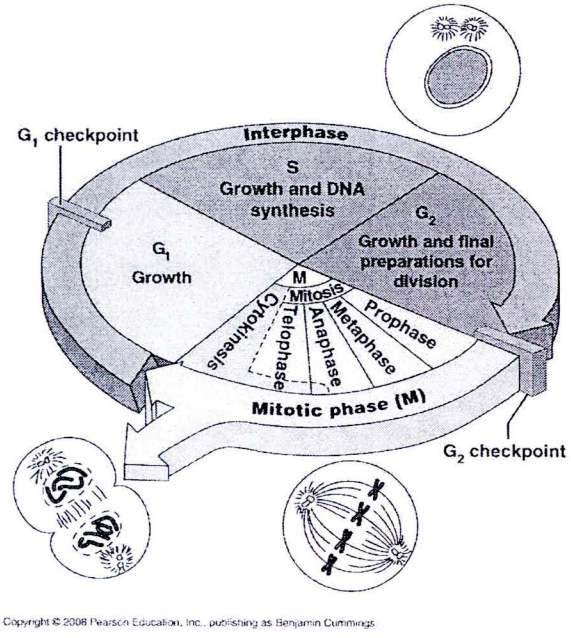


Figure 14 Cell cycle regulation

Source: http://stevebambas.com/AP%20220%20Cells.htm

Many studies reported that CURinhibits growth or proliferation of human colon cancer cells by interfere cell cycle. Hanif and his colleagues demonstrated that CUR accumulated many colon cancer cells (Lovo, HT-29 and HCT-15) in S and G_2/M phase which prevent cells from entering the next cycle (Hanif, et al., 1997). CUR down-regulated the expression of many genes involve in cell cycle control, such as BUB1B (mitotic checkpoint gene), STK6, STK12, and cyclin G_1 ($CCNG_1$). Additionally, they found that percentage of cells in G1 phase was decrease and percentage of cells in G2/M phase and S phase were increase when compared to cells that were not exposed to CUR. Moreover, Polo-like kinase (PLK) gene, involve in spindle formation and chromosome segregation during mitosis, highly express in colorectal cancer than in normal colon tissues was down-regulated after exposed to CUR(Van Erk, et al., 2004). The down-regulation of PLK resulted in an increase in

the percentage of cells in the G_2/M phase (Spankuch-Schmitt, et al., 2002). Depletion of PLK also found in HeLa cells which resulted in G_2/M phase arrest and apoptosis (Spankuch-Schmitt et al., 2002).

Cyclin D1 plays a key role in G_1/ S phase cell cycle progression during proliferation. Over-expression of Cyclin D1 has been found in a variety of human tumors, including colorectal cancer (Salomon, et al., 1995; Kenny, et al., 1999). Previous studies reported that CUR inhibited the expression of cyclin D1 in gastric, and colon cancer cells (Moragoda et al., 2001; Chen and Xu, 2005) resulted in inhibit the growth and proliferation of cancer cells.

4. Increase levels of glutathione-S-transferase (GTS)

Glutathione (GSH) is a tripeptide that functions as an important intracellular radical scavenger. It protects cells against reactive oxygen species (ROS) as well as against many toxins, mutagens, and drugs. GSTs, enzymes of phase II, belong to a family of enzymes that catalyze the conjugation of GSH to a wide variety of chemical toxins (Syng-Ai, et al., 2004). Induction of GST is regarded as a potential mechanism of blockade of the early stages of carcinogenesis.

It is known that dietary CUR can induce activity of antioxidant or phase-II enzymes in livers of rats treated with DMH or in liver and kidney of mice. Diet containing 2% CUR increased hepatic GST in rodent models of chemically induced carcinogenesis (Sharma, et al., 2001) and also increased in human breast cancer (MDAMB) and hepatocellular carcinoma cell line ($HepG_2$) (Syng-Ai, et al., 2004). Moreover, CUR also increase the level of phase-II enzyme gene (e.g. AKR1C1, NQO2 and EPHX1) expression in colonic cells (Van Erk, et al., 2004). Induction of phase-II genes can be a mechanism to protect against development of cancer. Therefore, CUR play important role in blockade of the early stages of carcinogenesis.

5. Suppress activation of nuclear factor-kappa beta (NF-κB)

NF-κB is a transcription factor which regulated genes involve in carcinogenesis. Under resting condition, NF- κB is retain in cytoplasm by binding to inhibitory IκB proteins, which blocks the nuclear localization of NF-κB (Figure 15) (Baldwin, 1996). On activation by free radicals, inflammatory stimuli, cytokines and carcinogens, IκB undergoes phosphorylation and degradation leading to nuclear translocation and modulate transcription of many genes involved in anti-apoptosis

(e.g. *bcl-2* and *bcl-xl*), proliferation (COX-2 and cyclin D1) and metastasis (MMP-9) (Shishodia, et al., 2005), which promoting the carcinogenesis. Many cancer cell types, including colon cancer express constitutively active NF-κB(Mukhopadhyay, et al., 2001) and also exhibit in tissue sample obtained from cancer patients (Garg and Aggarwal, 2002). Therefore, inhibit the expression of NF-κB could prevent cancer growth and metastasis.

CUR selectively inhibited NF-κB of melanoma cells, but not inhibited NF-κB in normal melanocytes (Marin, et al., 2007). CUR inhibits the NF-κB and IKK of mantle cell lymphoma leading to suppression of NF-κB-regulated gene products that resulted in the suppression of proliferation, cell cycle arrest and induction of apoptosis (Shishodia, et al., 2005). Additionally, CUR inhibited TNFα and IL-1ß could induce the degradation of IκBα and decreased NF-κBand p65 in cytosol (Moon, et al., 2006).

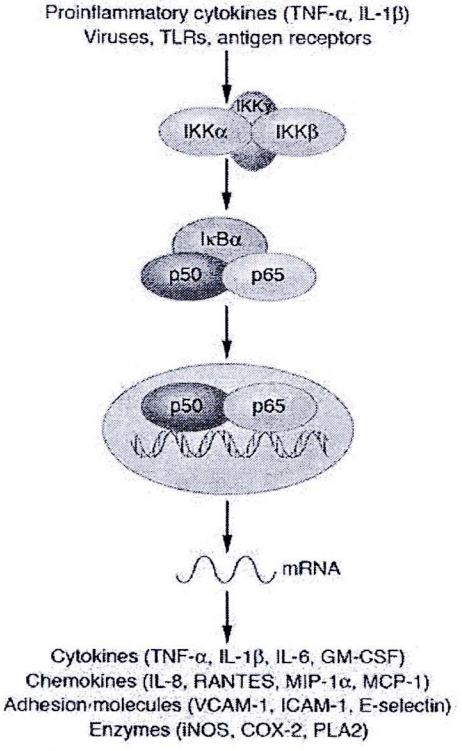


Figure 15 IKK/NF-κB signaling pathways

Source: Luo, et al., 2005

6. Inhibit angiogenesis

Angiogenesis is the formation of capillaries derived from a pre-existing vasculature. This process is central to a wide range of debilitating human pathologies, including solid tumor growth, arthritis, and corneal ulceration. In the initial stages of capillary formation, microvascular endothelial cells of preexisting blood vessels locally degrade the underlying basal lamina and invade into the stroma of the tissue to be vascularized. Previous experimental evidence has shown that this process requires a wide array of degradative enzymes (Mignatti and Rifkin, 1996).

Matrix metalloproteinases (MMPs) are suggested to play a critical role in extracellular matrix degradation and remodeling during inflammation and wound healing processes (Swarnakar, et al., 2005). Gelatinase B (MMP-9) is important member of the MMP in the angiogenesis. These enzymes may contribute to growth of new capillaries in several ways including activation of growth factors that stimulate endothelial cell migration and tube formation and dissolution of endothelial basement membranes at the sprouting capillary tips (Figure 16). CUR inhibits the expression of MMP-9 both *in vitro* and *in vivo* studies and also inhibits invasion and metastasis. Kunnumakkara and co-worker showed that CUR inhibits the expression of MMP-9 in orthotopically implanted pancreatic tumors (Kunnumakkara, et al., 2007)and ovarian tumors in nude mice (Lin, et al., 2007).

VEGF plays an essential role in endothelial proliferation and angiogenesis during embryonic development as well as periods of increased physiological demand,such as menstrual cycle, pregnancy and wound healing (Carmeliet, et al., 1996; Gerber, et al., 1999).VEGF plays a key role in cancer biology and contributes to tumor new blood supply to the increased demand for delivery of nutrients and oxygen (Shweiki, et al., 1992; Ferrara, 1999). CUR inhibited VEGF - induced transmigration, and decreased the number of endothelial tubes(Binion, et al., 2008). These results suggested that CUR is a potent inhibitor of angiogenesis in cancer progression.

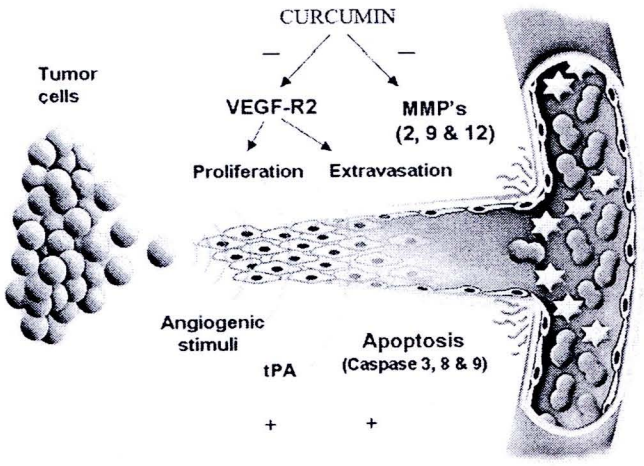


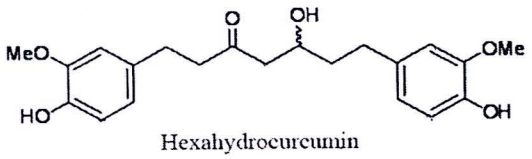
Figure 16 CUR inhibit angiogenesis of tumor

Source: Modified from http://www.angioworld.com/DominiqueGarrel.html

Although CUR is a very important agent in preventing and treatment colon cancer, its disadvantages include poor solubility and poor absorption in the gastrointestinal tract. Previous report have indicated that after oral administration of CUR, about 60% of the dose was absorbed and 38% remained in the large intestine of rats (Ravindranath and Chandrasekhara, 1980) and it rapidly decomposed in human blood (Wang, et al., 1997).

7. Hexahydrocurcumin

HHC (Figure 17) is one of major metabolites of CUR (Ireson, et al., 2002). Previous studies revealed that this compound exhibits stronger antioxidant activity than CUR. Further, HHC greater inhibited lipid peroxidation and free radical induced red blood cell hemolysis than CUR (Somparn, et al., 2007). Moreover, this compound inhibits the biosynthesis of PGE_2 in LPS-stimulated macrophages (Shao, et al., 2003). PGE_2 is a major product of COX-2 enzymes implicated in colorectal carcinogenesis and has been shownto stimulate the growth of human colorectal carcinoma cells. In addition, HHC decreases the PGE_2 levels of phorbol ester-induced PGE_2 production in human colonic epithelial cells (HCECs) but weakly inhibits COX-2 protein (Ireson, et al., 2001).



Hexahydrocurcumin

Figure 17 Structure of HHC

Source: Changtam, et al., 2010