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Colon (large intestine)

Macroscopic anatomy

Several distinct parts make up the continuous tube of the colon (also called the large intestine) (Adopted from http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php). Each part contributes to the movement of the undigested food materials, the formation of stools and its regulation. The parts include (Adopted from http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php) (Figure 2.1).

- **Ileocecal Valve**: It is a mucus membrane fold that acts as a gateway for materials to pass from the small intestine into the colon (Adopted from http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php).

- **Cecum**: It is a reservoir to receive the materials entering the colon where muscles advance the materials (now called fecal matter) upwards (Adopted from http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php).

- **Vermiform appendix**: It is a twisted coiled tube attached to the cecum (Adopted from http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php).

- **Ascending colon**: The right side of the colon where most of the water absorption occurs as the fecal matter moves upward (Adopted from http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php).
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- **Hepatic flexure**: A bend in the colonic tube connecting the ascending colon to the transverse colon (Adopted from [http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php](http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php)).

- **Transverse colon**: It is the lateral section of the colonic tube where stools begin to take consistency and form (Adopted from [http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php](http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php)).

- **Splenic flexure**: It is a bend in the colonic tube connecting the transverse colon to the descending colon (Adopted from [http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php](http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php)).

- **Descending colon**: It is the left side of the colon where stools, now more solid in form, move downward. (Adopted from [http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php](http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php)).

- **Sigmoid colon**: "S" shaped curve in the colon which angles to the right, then curves down and inward and then curves slightly upward (Adopted from [http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php](http://fruiteze.com/Product_Information/Colon_Anatomy_Regularity.php)).

  Stools may be stored here for a short period of time.

  The waste matter that is left after going through the colon is called feces or stool and it goes into the rectum (the final 6 inches of the digestive tract) where it is stored until it passes out of the body through the anus (Adopted from [http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancerwhat-is-colorectal-cancer](http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancerwhat-is-colorectal-cancer)).

  The length of the human colon is of the order of 100-150 cm (Tanaka, 2009), while that of rat is of 15-20 cm ([http://books.google.co.in/books](http://books.google.co.in/books)).

  Macroscopically, the colon has numerous distinct morphological features as compared to the small intestine (Figure 2.2) (Adopted from [https://www.kenhub.com/en/library/anatomy/the-colon](https://www.kenhub.com/en/library/anatomy/the-colon)). The semilunar folds arise in the inner surface through the muscle contractions and as these are simply caused functionally they are therefore movable (Adopted from [https://www.kenhub.com/en/library/anatomy/the-colon](https://www.kenhub.com/en/library/anatomy/the-colon)).

  Pouches are formed by these semilunar folds on the external surface which are termed as haustra, while the longitudinal musculature of colon is concentrated in three strong ribbon-like strips called taeniae coli (Adopted from [https://www.kenhub.com/en/library/anatomy/the-colon](https://www.kenhub.com/en/library/anatomy/the-colon)). Furthermore, the middle part of the colon called the mesocolon is attached to the mesocolic taenia and the greater omentum to the omental taenia.
whereas the free taenia is unbound and fully visible (Figure 2.3). An additional characteristic feature of colon is the small sacculations filled with fat which is formed by the serosa called appendices epiploicae (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon).
Microscopic anatomy

The colon has the characteristic histological structure of an epithelium which comprises of mucosa, submucosa, muscularis and serosa/adventitia (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon) (Figure 2.4). The mucosa is lined by simple columnar enterocytes (lamina epithelialis), covered by a layer of mucus thus helping the fecal transport (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon). The mucosa does not contain villi although it has numerous crypts of Lieberkuhn containing several goblet cells as well as enteroendocrine cells. Furthermore, the connective tissue layer (lamina propria) is filled with macrophages, plasma cells and other immune cells (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon). On the other hand, submucosa comprises of blood vessels, lymph nodes and predominantly fat tissues. The inner circular musculature of the muscularis mucosa is strongly prominent, however, the outer longitudinal musculature is practically only found in the taeniae (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon).

Function

The chief function of the colon is the temporary storage and transport of the feces (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon). It also daily absorbs about 1 liter of water which leads to a thickening of the stool. Further, it absorbs sodium, potassium and chloride but can also secrete potassium into the lumen itself. The physiological intestinal flora is rich in anaerobic bacteria which live in a symbiotic relationship with the human body (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon). These bacteria fulfill various crucial functions such as decomposing...
the indigestible food ingredients (e.g. cellulose), vitamin K generation, promoting the intestinal peristalsis and supporting the immune system (Adopted from https://www.kenhub.com/en/library/anatomy/the-colon).

Cell turnover

Subsequent evidences demonstrated that the absorptive epithelium of the large intestine is surrounded by a large number of cryptal cells (Reya and Clevers, 2005). Differentiated cells (enterocytes, enteroendocrine cells and goblet cells) occupy most of the crypts (Figure 2.5) and the remaining part is made up of stem cells as well as the proliferative progenitor compartment (Reya and Clevers, 2005). The stem cells are present near the bottom of the crypts and give rise to the progenitor cells which are capable of differentiating towards all the epithelial lineages. 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). These cells make the colonic epithelium the most rapidly self-renewing tissue in the adult animals (Clevers, 2006).

Figure 2.5: (a) Figure showing the colonic mucosa and (b) aberrant crypt fission in a FAP patient. (a) progenitor cells and stem cells are present at the crypts ‘A’ zone. Transit-amplifying cells are present at the ‘B’ zone of the crypt. ‘C’ zone contains mature and terminally differentiated cells. (b) Presence of aberrant crypt fission in a monocryptal dysplasia (Adopted from Tanaka, 2009)
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Epithelial renewal occurs in the crypts through a coordinated series of events such as the proliferation, differentiation and migration toward the large intestinal lumen (Van de et al, 2002). Therefore, in this way, a great number of cells formed by the crypt compartment is counterbalance by apoptosis at the tip of the crypt in a process that requires about 2–3 days. Proliferating crypt precursors and differentiated cryptal cells form a continuous sheet of cells in perpetual upward motion, however, stem cells at the crypt bottom escape this flow (Potten, 1998).

In abnormal states, the mucosa is capable of repairing mucosal integrity including the reversible ulcer-associated cell lineage, metaplasia (irreversible constitutive change of phenotype), dysplasia (abnormal differentiated phenotype) and neoplasia (abnormal cell count and abnormal differentiated phenotype) (Bykorez and Yu, 1984; Bach et al, 2000). All the cells within the crypt are originated from the stem cells where one of the mitotic stem cells remains as a stem cell at the bottom of crypt while another one is gradually pushed up to the luminal surface of the crypt as an epithelial cell. The cells that reached the uppermost part execute apoptosis and peel off without replicating or differentiating (Bykorez and Yu, 1984; Bach et al, 2000). Therefore, any mutations in these cells have essentially no influence on the normal turnover of mucosa. On the other hand, the cells with damaged DNA (mutated genes) in the stem cells do not cause apoptosis and therefore they reach the uppermost part in the crypt and continue proliferating. This pre-cancerous change termed as aberrant crypt foci (ACF), is now being widely used as one of the biomarkers of colon carcinogenesis in chemopreventive experiments (Pretlow et al, 1991; Pretlow et al, 1992; Bird, 1995).

Definition of colon cancer and its World scenario

Colon cancer is the cancer that forms in the tissues of the colon. Most colon cancers are adenocarcinomas (cancers that begin in cells that make and release mucus and other fluids). Colon cancer is the fourth most common cancer in men while, the third most common cancer in women worldwide (Parkin et al, 2005). Considerable variations in the distribution of colon cancer have been observed internationally (Parkin, 2004; Center et al, 2009a; Center et al, 2009 b). There are several risk factors for colon cancer such as obesity, a diet low in fruits and vegetables, physical inactivity and smoking (Giovannucci, 2002; Giovannucci and Wu, 2006; Botteri et al, 2008). It was a disease once primarily identified
in longstanding developed nations whose populations typically reveal these aspects (Popkin, 2004). On the other hand, in recent years, high colon cancer rates have been reported in newly developed countries around the globe in which the risk was once low (Popkin 1994; Parkin et al, 2005). Colon cancer is more prevalent in countries like North America, Argentina, Australia, New Zealand and parts of Europe, Japan and Israel, and for this reason, is usually considered as a western lifestyle disease (Center et al, 2009a; Center et al, 2009b). Although the incidence and mortality rates are higher in countries with a western lifestyle, the global incidence is rising and the majority of the world’s cases of colon cancer occur outside of the countries in which traditional western lifestyle features are dominant (Center et al, 2009a; Center et al, 2009b).

Worldwide, estimates showed that each year nearly 1 million new cases of colorectal cancer occur and nearly 500,000 deaths result from the disease (Parkin et al, 2005). Estimates show that in 2007, there were more than 150,000 new cases of colorectal cancer and over 50,000 deaths occurred from this disease in the United States, making it the second most common cause of death from cancer (Parkin et al, 2005).

Incidence of colon cancer

Worldwide incidence rates of colon cancer vary significantly with rates per 100,000 among males in the time period of 1998 – 2002, reported to range from 4.1 in India (Karunagappally, Tamil Nadu) to 59.1 in the Czech Republic (Center et al, 2009a) (Figure 2.6). Among females, these rates ranged from 3.6 in India (Karunagappally, Tamil Nadu) to 39.5 in New Zealand. The majority of registries with the highest incidence rates of colon cancer were found in Europe, North America, and Oceania (Center et al, 2009a). On the other hand, the lowest rates were observed from registries in Asia, Africa and South America (Center et al, 2009a).
Notably, colon cancer rates (1998–2002) among males in the Czech Republic, Japan and Slovakia have exceeded the peak incidence rates observed in longstanding, developed nations such as New Zealand, Australia and the United States, which previously reported the highest colon cancer incidence worldwide (Center et al, 2009b). Although, the data regarding risk factors for colon cancer are limited in various parts of the world, increase in colon cancer rates in newly developed or economically transitioning countries such as the Czech Republic and Slovakia and some others in Eastern Europe are most likely the result of the increased prevalence of obesity associated with “westernization,” including the consumption of high calorie dense food and lack of physical exercise (Popkin, 2004; Chrzanowska et al, 2007; Knai et al, 2007; Baillie K, 2008). Moreover, raised smoking prevalence as indicated by lung cancer mortality rates in the Czech Republic and Slovakia as compared to the longstanding, economically developed countries such as the United States (Center et al, 2009b) perhaps may play a significant role in the growing colon cancer rates reported in these countries.

Although the majority of the highest colon cancer incidence among males were observed in Europe, North America and Oceania, selected registries in Asia also recorded
high rates in Japan, Singapore and Israel (Center et al., 2009b) and are most likely due to the environmental or lifestyle factors. In Japan, a developed country with one of the strongest economies worldwide, the high incidence of colon cancer, particularly among males, is most likely due to modifications in dietary intake (Kono, 2004).

Among females, the highest colon cancer incidence rates were observed in New Zealand, Australia (Tasmania) and Israel (Figure 2.6). New Zealand and Australia, in addition to many other long standing developed nations such as Europe and North America, have historically had high incidence rates of colon cancer that are most likely the result of behaviors associated with urbanization. However, colon cancer incidence rates in recent years among females have declined in New Zealand and stabilized in Australia (Tasmania), but have continued to increase in Israel (Center et al., 2009b).

Figure 2.7: The registries with the lowest age-standardized colorectal cancer incidence rates by sex, 1998-2002. Source: Cancer incidence in five continents. Curado et al, 2007.

High colon cancer rates among females were also observed in the Asian registries of Japan and Singapore, although rates for females are considerably lower than those for males in these and other registries. Reasons for the lower rates observed among females (Figure
compared with males may be related to the differences in risk behaviors associated with colon cancer, such as smoking (Mackay and Amos, 2003) and the differing effect of obesity in men and women (Frezza et al, 2006). Among both males and females, the lowest rates of colon cancer incidences were observed for registries in India (Nagpur, Poona and Karunagappally), Oman, Egypt (Gharbiah), Algeria (Setif) and Pakistan (South Karachi) (Figure 2.7). Low colorectal cancer incidence in these economically developing regions of the world may reflect a lower prevalence of known risk factors (Center et al, 2009b).

Colon cancer mortality

Mortality trend analyses for selected countries have revealed that it has been decreased in both males and females in 13 out of the 29 countries (Center et al, 2009b). This decrease was mainly confined to longstanding, economically developed nations such as the United States, Australia, New Zealand and the majority of Western Europe, including Austria, France, Germany, Ireland and the United Kingdom (Figure 2.8). However, colon cancer mortality trends have also decreased in some Asian and Eastern European countries in which incidence rates are among the highest worldwide (Center et al, 2009b). In Japan, death rates decreased by 0.9% per year from 1996 - 2005 in men and by 5% per year from 1992 - 2005 in women. Similarly, in the Czech Republic, in which death rates were the second highest in 2005 among both males and females, it decreased by 1% per year from 1994 through 2005 in men and by 1.2% per year from 1988 through 2005 in women. In addition to these 13 countries in which decreasing colon cancer mortality rates were noted among males and females, there are 4 additional countries which recorded decreasing mortality among females only, including Latvia, Slovakia, South Africa and Spain. The decreasing rates may reflect improvements in colon cancer treatments which increased the survival (Chia et al, 2001; Ribes et al, 2009) or possibly earlier detection due to opportunistic screening or symptom recognition.
Colon cancer risk and screening

Stryker et al found a relative risk of colon cancer development of approximately 1% per year for adenomas >1 cm (Stryker et al, 1987). In addition to size, important determinants of colon cancer risk include adenoma number (O’Brien et al, 1990) and clinical features such as histological architecture type. For example, tubular histology is associated with the lowest lifetime risk (5% overall), while villous lesions are associated with the highest (up to 50%), and tubulovillous lesions with an intermediate lifetime risk 15-20% (Tobi, 1999), respectively.

Screening studies have indicated that colon cancer is typically diagnosed 10-15 years after adenoma detection (Kozuka et al, 1975). This lag provides a convincing rationale and opportunity for screening and intervention, as colon cancer represents a significant public health burden worldwide (Kozuka et al, 1975). Adenomas are very prevalent lesions, nearly half of men and 30% of women develop adenoma by the age of 50 years (Villavicencio and Rex, 2000). In addition to environment and lifestyle factors (dietary fat, calcium and lack of exercise and so forth), multiple inherited and acquired genetic factors contribute to the colon cancer risk (Potter, 1999).
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The presence or absence of colon cancer screening programs is an important consideration when evaluating the colon cancer burden worldwide, because screening may increase colon cancer incidence in the short term through the increased detection of prevalent cases (Bonneux et al, 1995) and reduce the incidence in the long term through the removal of precancerous polyps (Winawer et al, 1993; Citarda et al, 2001). Hence, over the time, screening can lower colon cancer mortality by reducing the incidence or by detecting tumors at earlier stages, which then have better prognosis (Walsh and Terdiman, 2003; Hewitson et al, 2008; Baxter et al, 2009). A population-based colon cancer screening program based on colonoscopy is more resource-intensive, less feasible and is less practical in low-resource countries (Center et al, 2009a). Although less sensitive than structural examinations, the fecal occult blood test (FOBT), which is inexpensive and easy to perform, is a more feasible colon cancer screening option in many areas of the world (Center et al, 2009a).

In the United States, current screening recommendations for the detection of adenomatous polyps and colon cancer in adults with an average risk (those aged 50 years and older) include either annual stool testing with a high-sensitivity guaiac- or immunochemical-based test, periodic stool DNA testing, flexible sigmoidoscopy, colonoscopy, double-contrast barium enema, and computed tomographic colonography (Levin et al, 2008).

Colon cancer prevalence in India

In India the incidence of colon cancer is low which varies from 3.7 to 0.7/100,000 among men and 3 to 0.4/100,000 among women in eight population regions (Mohandas and Desai, 1999).

Rural incidence rate in India is approximately half to that of urban rate, however, significant increase in the incidence of colon cancer has been reported for both men and women over the last two decades (Mohandas and Desai, 1999). The notable trends have been the high incidence of these cancers in the urban population and amongst young Indians. This is due to the rise in increasing migration of rural population to the cities, increase in life expectancy and change in lifestyle (Mohandas and Desai, 1999).
Molecular basis of colon cancer progression

Several studies revealed that the colon cancer development is a multi-step process which takes place during the accumulation of several discrete genetic events (Fearon and Vogelstein, 1990). It develops in an orderly histopathologically detectable manner from normal mucosa through the stages of hyperproliferative epithelium, early adenomas, late adenomas, carcinoma-in-situ, frank cancer and metastasis as shown in Figure 2.9.

![Figure 2.9: The progression of mutations that commonly lead to colon cancer. The fatal metastasis is the last of six serial changes that the epithelial cells lining the rectum undergo. One of these changes is brought about by mutation of a proto-oncogene, and three of them involve mutations that inactivate tumor-suppressor genes (Adopted from www.txtwriter.com).](image)

Each genetic event offers a selective growth advantage for the cell. Additionally, it has been demonstrated that each physical stage of colon tumorigenesis is due to a specific genetic event. Information on genetic mechanisms involved in colon carcinogenesis has been largely derived from studying the genetic alterations in familial cancer syndromes such as familial adenomatous polyposis (FAP), hereditary non-polyposis colon cancer syndromes (HNPCC), and murine models such as the Min mouse and knockout mouse models (APC, COX-2, p53). FAP syndrome was found to arise from a mutation in adenomatous polyposis coli (APC), a tumor suppressor gene which resulted in a dominant negative effect (Groden et
al, 1991; Nishisho et al, 1991; Spirio et al, 1993) and the loss of second copy may further present an advantage to the cancer development.

The functions of the normal wild-type APC gene may involve cell signaling and adhesion and thus its inactivation may lead to uncontrolled colonic epithelial proliferation. APC may control apoptotic processes, and introduction of wild-type APC into a cell line with mutant APC can restore apoptosis (Jen et al, 1994). APC binds β-catenin and β-catenin in turn binds E-cadherin which is required to form intraepithelial adherens junctions (Krishnan et al, 2006). In rodent models, APC mutations (APC min, APC716) result in intestinal polyposis that progress to invasive transformed neoplasms. Chemical carcinogens can generate adenomas that facilitate the invasive colon cancer growth. Data from the National Polyp Study support the concept that adenomas progress to frank malignancy. Moreover, resection of adenomas results in a 70–90% reduction in the incidence of colon cancer in comparison to the reference group (O’Brien et al, 1990; Winawer et al, 1993).

Apart from mutations of APC, other abnormalities of the replication-signaling pathway have been described in various studies which include the deletion in colorectal cancer (DCC) gene and mutations of the p53 tumor suppressor gene (Krishnan et al, 2000). Although the molecular changes underlying neoplastic transformation of the normal colonic epithelium can occur in any order, deletions of DCC gene and mutations of p53 are probably late events.

Heritable colon cancer
Familial colon cancer

There are different types of inherited colon cancer conditions, including familial adenomatous polyposis (FAP) and Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC) (Adopted from http://www.med.upenn.edu/gastro/ColonCancerSyndromes.shtml).

FAP accounts for approximately 1% of all colon cancers. FAP predispose people to develop 100’s to 1,000’s of colon polyps, which are benign growths (Adopted from http://www.med.upenn.edu/gastro/ColonCancerSyndromes.shtml). Although benign, polyps have the ability to become cancer and therefore, people with FAP are at increased risk to develop colon cancer and at a much younger age than the general population (20’s, 30’s,
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and 40's). There is also a milder version of this condition, called attenuated FAP, where individuals tend to develop fewer than 100 polyps, often at later ages than with the classic form of FAP (Adopted from http://www.med.upenn.edu/gastro/ColonCancer Syndromes.shtml).

The link among a stability gene aberration and colon cancer is revealed by HNPCC/Lynch syndrome. Lynch syndrome accounts for approximately 3-5% of all colon cancers. Instability of short tandem repeats or microsatellite genes (MSI) is a characteristic of the tumors from these patients (Haydon and Jass, 2002). In most HNPCC colon cancers, MSI has been shown to result from mutations in the DNA mismatch repair genes (hMSH2, hMLH1, hPMS1, hPMS2 and hMSH6) (Kinzler and Vogelstein, 1996; Peltonaki and Vasen, 1997). Most microsatellite sequences in the genome are located within the non-coding or intronic sequences and mutations in these introns are believed to be silent and insignificant. However, some genes may contain MSI within their coding regions which have been identified as receptors for growth factors, such as transforming growth factor-β receptor II, insulin-like growth factor-II receptor, regulators for cell cycle as well as those of apoptosis (Tanaka et al, 2009). Therefore, transformation to malignancy occurs when these target genes are mutated.

Non-heritable colon cancer

In non-heritable colon cancer, at least seven independent genetic events are needed over decades and in correct order to develop colon cancer (Vogelstein et al, 1988). This process begins with a normal colonic epithelial cell developing an APC mutation, migrating to top of the colonic crypts, expanding, and then forming an early adenoma (Smith et al, 1993; Miyashiro et al, 1995). Accumulation of K-ras mutation then promotes intermediate adenoma formation followed by the transition to a late adenoma after mutations on chromosome 18q21 (candidate genes DCC, DPC4, JV18) occur. Mutations in the p53 genes then transform the premalignant lesion to invasive carcinoma, and other additional genetic hits that lead to metastasis (Kinzler and Vogelstein, 1996).
Relevance of rodent model to human colon cancer

The ability to reliably induce colon tumors in animals has imparted the opportunity to study diverse aspects of the carcinogenic process. These models offer suitable information on the tumor initiation, promotion and progression, including details on the cellular transformation and the subsequent events leading to the development of neoplastic lesions. The established models can be used for chemoprevention studies as well. These animal models are chemically induced (Reddy, 1998; Rosenberg et al, 2009) or genetically modified (Boivin et al, 2003). Oncogenesis studies using these models have revealed the role of genetic and environmental factors and other influences on the various aspects of cancer. Moreover, animal colon cancer models have also been used to evaluate immunological, chemical and surgical therapy regimens.

The study of experimental colon carcinogenesis in rodents had a remarkably long history, dating back almost 80 years (Krebs, 1928). However, the most commonly used model for sporadic colon cancer takes advantage of the organotropism of the colon carcinogens, 1, 2-dimethylhydrazine (DMH) and azoxymethane (AOM). DMH, a metabolic precursor of methylazoxymethanol (MAM), was used in several earlier studies to induce tumors in rats (Rosenberg et al, 2009). Repetitive treatment with this methylating agent was reported to produce colonic tumors in rodents that exhibit many of the pathological features associated with the human disease (Rosenberg et al, 2009). Thus, DMH has provided cancer researchers with a reproducible experimental system for studying ‘sporadic’ (nonfamilial) forms of colon cancers (LaMont and O'Gorman, 1978).

There are a number of benefits to study the pathogenesis of carcinogen-induced colon cancer in rodent models, which includes great reproducibility i.e., they can be readily tested on animals with different genetic backgrounds and the pathogenesis recapitulating human colon cancer, at least at the early stages. Historically, the majority of colon carcinogenesis studies have been carried out in rats (Weisburger et al, 1977; Madara et al, 1983). However, the high frequency of tumors generated within the distal colon of mice, as well as the histogenesis of multiple adenomas along with subsequent growth of adenocarcinomas, also confirm the significance of this species for studying the pathogenesis of colon cancer (Chang, 1978; Nambiar et al, 2003). However, on the whole, the role of rodent models to
Recapitulate the adenoma–carcinoma sequence which is found in human colon cancer has been used broadly to study the cancer chemopreventive agents as well as the dietary factors.

**Colonic carcinogens**

Colonic carcinogens are the chemical agents that have been utilized for the induction of colon tumors in animal model (Tanaka, 2009). They comprises of direct and indirect-acting agents. The direct-acting carcinogens are compounds that do not require biological catalysis, such as the action of enzymes to form the final reactive species that can modify the cellular macromolecules (Tanaka, 2009). These agents spontaneously break down in an aqueous environment to form electrophilic species that react with the nucleophilic centers on the DNA molecules (Tanaka, 2009).

On the other hand, indirect-acting carcinogens require the enzymatic action to be converted into the ultimate electrophilic species (Tanaka, 2009). Several researchers deal mainly with the experiments involving dimethylhydrazine and its metabolites such as AOM.

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**Figure 2.10:** Metabolism of DMH involves multiple transcriptionally regulated xenobiotic metabolizing enzymes (Adopted from: Rosenberg et al, 2009).
and MAM acetate in the colon tumor model in rodents. Most of the experiments involving chemical colon cancer induction have been performed with these carcinogens using rats or mice (Tanaka, 2009).

**Mechanisms of action of DMH**

The mode of action and metabolism of colon carcinogens such as AOM and DMH were examined by Fiala, 1977 (Figure 2.10). The primary step in the activation of these carcinogens comprises a series of oxidative steps that occur in the liver (Fiala and Stathopoulos, 1984). These compounds then subsequently enter the intestine through the bile or the blood stream and before entering the bile, they are conjugated in the liver with glucuronic acid and/or sulfate or glutathione (Fiala et al, 1977).

The conjugates entering the intestine can then be converted into free compounds by hydrolytic bacterial enzymes. The compounds formed in this sequence can then be activated to the ultimate electrophilic carcinogens by the action of colonic tissue enzymes or perhaps by the further action of the bacteria as well as the colonic tissue-activating systems. The bacteria are also capable of deactivating the proximal carcinogens thus providing protection against tumor induction (Wheeler et al, 1975). In this sequence, the initial activation occurs by the action of hepatic oxidative enzymes leading to the formation of MAM. Initially, it was considered that MAM spontaneously broke down to a methyl carbonium ion which then alkylated the DNA. An alternate pathway exists (Grab and Zedeck, 1977) in which the alcohol functional group on MAM is further oxidized by extrahepatic tissue dehydrogenases to the aldehyde and acid, which then spontaneously form the carbonium ion. It was found that both the mechanisms may occur simultaneously, making MAM both a direct and an indirect-acting carcinogen. The MAM molecule is small and hence its transport from the liver to the intestine primarily occurs via the circulatory system (Fiala, 1977). However, a small percentage of MAM may be conjugated and excreted in the bile itself. Evidence for this also appears from the observation that animals receiving metabolites of DMH may develop small intestinal tumors just distal to the point of entrance of the bile duct into the intestine (Ward et al, 1973).
Inflammation as a predisposing factor for colon carcinogenesis

The relationship between inflammation and cancer was first suggested by Rudolph Virchow in 1863, when he demonstrated the presence of leucocytes in the neoplastic tissues. Inflammation, a host defense mechanism, is an immediate response of the body to tissue injury caused by microbial infection and other noxious stimuli. Acute inflammation is characterized by vasodilation, leakage of the vasculature, and infiltration of leukocytes into the site of infection to destroy invading pathogens and is followed by a rapid resolution phase and repair of the damaged tissue. Thus, acute inflammation plays a beneficial role against infection and injury. However, inadequate resolution of inflammation and uncontrolled inflammatory reactions can evoke a state of chronic inflammation which is a common etiologic factor for various human ailments including cancer (Balkwill et al, 2005; Aggarwal et al, 2006).

Since the 19th century proposition of an inflammation–cancer connection, the persuasive role of inflammation in carcinogenesis has become more and more evident in recent times (Balkwill and Mantovani, 2001). There are several mechanisms by which inflammation contributes to carcinogenesis which includes: (i) induction of chromosomal instability, (ii) alterations in epigenetic events and subsequent inappropriate gene expression, (iii) enhancement of cell proliferation, (iv) evasion from apoptosis, (v) stimulation of intratumoral neo-vascularization, (vi) invasion through tumor-associated basement membrane, and (vii) stirring up the metastatic movement (Hussain and Harris, 2007; Kundu and Surh, 2008; Porta et al, 2009; Colotta et al, 2009).

Central to these altered biochemical processes is the elevated expression, overproduction, or abnormal activation of diverse mediators of inflammation. Such proinflammatory mediators include, but are not limited to cytokines, chemokines, cyclooxygenase-2 (COX-2), prostaglandins (PGs), inducible nitric oxide synthase (iNOS), nitric oxide (NO) and advanced glycation end products (Figure 2.11).

During chronic inflammation (Figure 2.11), a wide array of intracellular signaling pathways comprising cell surface receptors, cytoplasmic and nuclear kinases, adaptors and scaffold proteins and transcription factors, are often dysregulated, thereby leading to an abnormal expression of proinflammatory genes involved in malignant transformation. In general, inflammation-driven activation of various protein kinases that include Janus-
activated kinase (JAK), Akt (protein kinase B), and mitogen-activated protein (MAP) kinases that inappropriately transmit growth signals, allowing cells to acquire a malignant phenotype.

Studies from an animal model revealed that a sequence of histopathological events from chronic gastritis to gastric carcinogenesis occurs (Cordon-Cardo and Prives, 1999), for example, the risk of colon cancer was 10-fold greater if linked with inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease (Seril et al, 2003; Itzkowitz and Yio, 2004). Additionally, the role of colitis by various anti-inflammatory agents suppressed the colon cancer incidence (Moody et al, 1996; Eaden et al, 2001). Further, in the gastrointestinal tract, gastric Helicobacter pylori infection is the leading cause of adenocarcinoma and mouse-associated lymphoid tissue lymphoma (Coussens and Werb, 2002; MacArthur et al, 2004). However, a detailed mechanism applicable to the colon cancer is still unknown.

Relationship among the inflammatory cells and tumor progression

Subsequent evidences showed that the neoplastic cells are capable of attracting
several different cell types into the tumor microenvironment through the secretion of extracellular proteases and pro-angiogenic factors, along with the cytokines (Macarthur et al, 2004). Several pro-inflammatory cytokines such as interleukins-10 (IL-10) are secreted by tumor cells as well as macrophages, and among other effects, it inhibits cytotoxic T cells and consequently assists in suppressing the immune response against the tumor (Coussens and Werb, 2002). Furthermore, chemokines, which comprise the largest family of cytokines, characterized by their ability to induce migration and activation of leukocytes to the specific sites including tumor stroma are the C-C chemokine and monocyte chemotactic protein (MCP)-1, which has been shown to be a major determinant of monocyte/macrophage infiltration in tumors. Besides this, tumor epithelial areas have also been found to express MCP-1, whereas additional chemokines such as macrophage inflammatory protein-1β (MIP-1β) has been detected in the stroma that regulate the infiltration of other inflammatory cells including T cells. Other functions of the chemokines include the activation of cells to release proteolytic enzymes, serving the digestion of extracellular matrix and providing a path for further inflammatory cell migration, tumor growth and metastasis (Macarthur et al, 2004).

Arachidonate metabolism and role of cyclooxygenases (COX) in colon cancer

Arachidonic acid is a 20 carbon unsaturated fatty acid distributed throughout the lipid bilayer of the cell and is usually esterified at the SN-2 position of the phospholipids (Williams et al, 1999). Phospholipase enzymes cleave membrane bound arachidonate, thus making it available for conversion to bioactive lipids (Williams et al, 1999). Once liberated, the arachidonic acid can be metabolized through one of three major pathways: (1) the COX pathway, (2) the lipoxygenase pathway, or (3) the cytochrome P-450 monooxygenase pathway (Williams et al, 1999) (Figure 2.12).
The COX pathway, which is the focus of this study, is the most extensively studied of the major pathways in the inflammation. COX or prostaglandin G/H synthase catalyzes the conversion of arachidonic acid to prostaglandin H$_2$ (PGH$_2$), the immediate substrate for a number of cell specific PG and thromboxane synthases. This occurs via a two-step process, in which the first step introduces two molecules of oxygen to arachidonate, forming the bicyclic peroxide intermediate, PGG$_2$. The second step occurs in a distinct reactive site located on the other side of the molecule, and requires the diffusion of PGG$_2$ to this site. Here peroxidation results in the reduction of PGG$_2$ to the freely diffusible PGH$_2$ (Figure 2.12). Though the COX enzymes are membrane bound, they do not contain transmembrane domains, rather, they possess four amphipathic helices juxtaposed such that they form a localized region of hydrophobicity. The hydrophobic region serves to anchor the lower portion of the enzyme in the membrane. The COX active site is located in an area of hydrophobicity near the amphipathic helices. Access to this site occurs via a channel buried in the lipid bilayer. Both substrate and inhibitors use this channel to reach the active site.
Discovery of COX enzymes and their expression

COX-1 was first purified from bovine vesicular glands in 1976 (Miyamoto et al, 1976). COX-1 is found to be constitutively expressed in many tissues including kidney, lung, stomach, duodenum, jejunum, ileum, colon and cecum of rat, dog, Rhesus monkey and human (Kargman et al, 1996). COX-1 activity is believed to be responsible for producing cytoprotective prostaglandins, such as the prostacyclin and PGE$_2$, which are thought to be critical to maintain integrity of gastric mucosa (Miller, 1983; Soll et al, 1991; Allison et al, 1992). However, in 1989, Simmons et al. identified a novel COX cDNA, of which the corresponding mRNA was induced by v-src transformation of chicken embryo fibroblasts (Simmons et al, 1989).

Further, in 1991, Kujubu et al. independently isolated a cDNA encoding this isoform by differential screening of 3T3 fibroblasts treated with phorbol ester (Kujubu et al, 1991). This second isoform, now known as COX-2, shares significant sequence homology and catalytic activity with COX-1. However, its expression pattern is markedly different. Most tissues, with the exception of the placenta, the macula densa of the kidney and brain, do not constitutively express COX-2 (Harris et al, 1994; Hirst et al, 1995). However, a variety of extracellular and intracellular stimuli will rapidly induce COX-2. These stimuli include lipopolysaccharide (LPS) (Fu et al, 1990; Lee et al, 1992; O'Sullivan et al, 1992), forskolin (Kujubu and Herschman, 1992), interleukin-1 (IL-1), tumor necrosis factor (TNF) (Coyne et al, 1992; Jones et al, 1993; Geng et al, 1995), epidermal growth factor (EGF) (Hamasaki et al, 1993), transforming growth factor alpha (TGF$\alpha$) (DuBois et al, 1994), interferon-\gamma (Riese et al, 1994), retinoic acid, platelet activating factor (PAF) (Bazan et al, 1994), endothelin (Kester et al, 1994) and arachidonic acid.

Finally, COX-3 has been identified as a splice variant of COX-1, and it is present mainly in brain and spinal cord (Sarkar et al, 2007; Kis et al, 2003). Currently, the role of COX-3 is not known. Some pieces of evidence suggest a possible role of it in pain sensitivity, based on studies focused on the mechanism of action of acetaminophen (paracetamol), which recently evoked as a selective inhibitor of COX-3 (Chandrasekharan et al, 2002). However, this hypothesis is debated because other findings argue that acetaminophen targets at the same time to COX-2 (Hinz et al, 2008).
**Structural differences of COX enzymes**

The human gene for COX-1 is located on chromosome 9 \((\text{Kam and See, 2000})\). It is a membrane bound haemoglycoprotein having a molecular weight of 71 kDa and is found in the endoplasmic reticulum (ER) of PG producing cells \((\text{Kam and See, 2000})\). It has 3 folding units: a membrane binding domain, an early growth factor (EGF) like domain, and an enzymatic (catalytic) domain. COX active site is formed by a hydrophobic channel containing tyrosine 358 and serine 530 at its top.

On the other hand, COX-2 has a molecular weight of 70 kDa and the gene for COX-2 lies on human chromosome 1 \((\text{Asgari et al, 2004})\). Its promoter contains a TATA box and is under control of the various transcription factors like nuclear factor κB (NFκB), the nuclear factor for interleukin-6 expression (NF-IL-6) and cAMP response element binding (CREB) protein \((\text{Appleby et al, 1994})\). Its expression is controlled by several inflammatory mediators such as cytokines, for instance, IL-1, IL-2, tumor necrosis factor (TNF-α) and endotoxins etc. \((\text{Hillario et al, 2006})\).

COX-2 shares 60% homology with COX-1 and their three dimensional structure is more or less similar \((\text{Kam and See, 2000})\). However, the active site of COX-2 is 17% larger than that of COX-1 \((\text{Hinz and Brune, 2002})\).

**Figure 2.13:** Schematic depiction of the structural differences between the substrate-binding channels of COX-1 and COX-2 that allowed the design of selective inhibitors. The amino acid residues, Val434, Arg513, and Val523, form a side pocket in COX-2 that is absent in COX-1. (a): Nonselective inhibitors have access to the binding channels of both isoforms. (b): The more voluminous residues in COX-1 i.e. Ile434, His513, and Ile532, obstructs access of the bulky side chains of the selective COX-2 inhibitors. \((\text{Grosser et al, 2006})\).
Furthermore, COX-1 has bulky isoleucine residues at positions 434 and 523 whereas COX-2 has smaller valine residues at these sites (Figure 2.13). This small difference in amino acid composition along with several conformational changes accounts for the differences in specificity of COX-1 and COX-2 inhibitors. The presence of valine residues at these sites in COX-2 allows a side pocket to form that is part of the active site of COX-2 selective drugs.

**COX-2 as a tumor promoter and a good candidate for cancer therapy**

Over-expression of COX-2 has been detected in a number of tumors, such as colon, breast, pancreatic as well as lung cancers (DuBois et al, 1998; Cao and Prescott, 2002; Ristimaki et al, 2002; Secchiero et al, 2005), where it correlates with a poor prognosis. Data suggested that COX-2 may play a significant role in different steps of cancer progression, by increasing proliferation of mutated cells (Cao and Prescott, 2002) thus favoring the tumor promotion as well as by affecting programmed cell death or apoptosis. Consequently, it affect the efficacy of various anticancer therapies (Philip et al, 2004; Palayoor et al, 2005; Chan et al, 2007; Johnson et al, 2008; Zrieki et al, 2008) for example, by affecting the apoptosis induced by loss of cell anchorage (anoikis) (Choi et al, 2005). Furthermore, COX-2 induction or overexpression is associated with an increased production of PGE₂, one of the major products of COX-2, known to modulate the cell proliferation, cell death and tumor invasion in many types of cancers including colon. Therefore, both host and tumor cells could contribute to the PGs generation within the tumor microenvironment (Cha and DuBois, 2007).

Eberhart et al, 1994 were the first to recognize the considerable rise in COX-2 expression in 85% of human colorectal carcinomas and approximately 50% of colorectal adenomas. Two independent groups have further repeated these results (Kargman et al, 1995; Sano et al, 1995). COX-2 is also observed to be overexpressed in adenoma from Apc\textsuperscript{Min} mice (Williams et al, 1996) and carcinoma samples from the colon of AOM-treated rats (DuBois et al, 1996). Further, it was illustrated that stimulated macrophages can alter rat intestinal epithelial cells into a more neoplastic phenotype in a paracrine fashion via a COX-2 dependent mechanism (Ko et al, 2002). This signifies that the augmented COX-2 expression in intestinal cells is an early event which regulates surrounding epithelial cells to transform
Review of literature

Towards malignancy. Lately, colon cancer cells have been shown to induce COX-2 expression in monocytes/macrophages via mucin production (Inaba et al, 2003).

Chemoprevention of colon cancer by COX inhibitors

Chemoprevention presents a plausible approach to reduce the incidence and mortality from cancer (Tanaka, 1997a; Tanaka, 1997b). It involves the long-term use of a variety of oral agents that can delay, prevent or even reverse the development of adenoma in the colon and interfere with the multi-step progression from adenoma to carcinoma (Tanaka, 1997a). Chemoprevention is thus of particular importance to genetically predisposed patients and to those patients who are mainly susceptible to the environmental causes of cancer (Tanaka, 1997b). Colon cancer is particularly suitable for prevention strategies because it is prevalent and associated with considerable morbidity and mortality (Tanaka and Mori, 1996). It has a natural history of transition from normal crypts through adenoma (a benign epithelial neoplasm) to overt adenocarcinoma (a malignant epithelial neoplasm) occurring over an average of 10–20 years, thereby providing a window of opportunity for effective intervention and prevention.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the oldest and most commonly used chemopreventive drugs in human history (Vane and Botting, 1990) (Figure 2.14). Aspirin-like drugs, now referred as NSAIDs, have been used to treat arthritis since 1899, when the analgesic and anti-inflammatory effects of aspirin were first recognized. In spite of their potential gastrointestinal and renal toxicities, they are among the most widely used therapeutic classes of compounds primarily because they are generally effective for the relief of pain and inflammation. A previous report revealed that aspirin and indomethacin inhibited prostaglandin production by blocking COX enzymatic activity which could form the basis of their analgesic and anti-inflammatory properties (Vane, 1971). Since that report, it has been found that NSAIDs can directly affect COX activity, either by covalently modifying the enzyme (for instance in case of aspirin and the selective COX-2 inhibitor APHS), or by competing with the substrate for the active site (as mostly with all other NSAIDs) (Williams et al, 1999). Later it was found out that aspirin inhibits the COX activity covalently through the acetylation of Ser-530 in COX-1, and Ser- 516 in COX-2, while other NSAIDs mostly compete with arachidonate for active site binding (Wennogle et al, 1995).
Evidences in later years collectively stated that NSAIDs (Figure 2.14) are the promising anticancer drugs (Taketo, 1998a; Taketo 1998b; Janne and Mayer, 2000). Moreover, NSAIDs have also been shown to stimulate apoptosis while they inhibit angiogenesis, the two mechanisms that contribute in inhibiting the malignant transformation as well as tumor growth. Plentiful epidemiologic (nonrandomized) studies have shown that long-term users of aspirin or other NSAIDs have experienced reduced risk of colorectal cancer than the nonusers (Thun et al, 2002). Further, randomized clinical trials have verified that two NSAIDs, such as the prodrug sulindac (Labayle et al, 1991; Giardiello et al, 1993; Nugent et al, 1993) and the selective COX-2 inhibitor celecoxib (Steinbach et al, 2000) efficiently restrain the adenomatous polyp growth and cause regression of existing polyps in patients with the unusual hereditary condition called familial adenomatous polyposis (FAP).

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<tr>
<th>Salicylates</th>
<th>Arylalkanoic acids</th>
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<tr>
<td>• Acetylsalicylic acid (aspirin)</td>
<td>• Diclofenac</td>
<td>• Ibuprofen</td>
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<td>• Amosiprin</td>
<td>• Aceclofenac</td>
<td>• Alminoprofen</td>
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<td>• Benorylate/Bootenilate</td>
<td>• Acemithracan</td>
<td>• Carprofen</td>
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<td>• Choline magnesium salicylate</td>
<td>• Alclofenac</td>
<td>• Dextubuprofen</td>
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<td>• Diflunsal</td>
<td>• Bromfenac</td>
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<td>• Ethnansuline</td>
<td>• Etdololac</td>
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<td>• Faislamime</td>
<td>• Indomethacin</td>
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<td>• Methyl salicylate</td>
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<td>• Magnesium salicylate</td>
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<td>• Salicylic acid</td>
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<td>• Salicylamide</td>
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<th>Pyrazolidine derivatives</th>
<th>Oxicams</th>
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<td>• Phenybutazone</td>
<td>• Pirowasam</td>
<td>• Celecoxib (FDA alert)</td>
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<td>• Ampryone</td>
<td>• Droxicam</td>
<td>• Etoricoxib (FDA withdrawn)</td>
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<td>• Azapropazone</td>
<td>• Loroxicam</td>
<td>• Lumiraxicox (TGA cancelled</td>
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<td>• Clocezone</td>
<td>• Meloxicam</td>
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<td>• Kebuzone</td>
<td>• Tenoxicam</td>
<td>• Parecoxib (FDA withdrawn)</td>
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<td>• Metamizole</td>
<td>• Piropfen</td>
<td>• Rofecoxib (withdrawn from</td>
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<td>• Morfebuzonazou</td>
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<td>• Oxyphenbutazone</td>
<td>• Tiaprofenic acid</td>
<td>• Valdecoxib (withdrawn from</td>
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<td>• Phenazonie</td>
<td>• Sulfinpyrazone</td>
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Figure 2.14: Classification of NSAIDs. Image adopted from a website (www.dimensionsofdentalhygiene.com)
Piroxicam

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) (Figure 2.15) that non-selectively and reversibly inhibits both isoforms of COX i.e. COX-1 and COX-2 \((\text{IC}_{50} = 1.57 \text{ and } 1.69 \ \mu\text{M for COX-1 and COX-2, respectively})\) (Pairet et al, 1998). It has an extended half-life of about 40 hours, which allows one time daily administration leading to sustained plasma and tissue levels. In addition to its anti-inflammatory effects, piroxicam is known to be an effective analgesic that has been extensively used in the treatment of arthritis. It has also been used to inhibit COX activity for treatment of various cancers in animals (Pairet et al, 1998).

![Figure 2.15: Structure of piroxicam, 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide.](http://en.wikipedia.org/wiki/Piroxicam)

In the human colon carcinoma cell line HCA-7, piroxicam has been shown to reduce the relative level of COX-2 mRNA and also PGE\(_2\) production (Sherratt et al, 2003). Furthermore, in several experiments including an ApcMin/+ mouse model, piroxicam was able to consistently reduce the intestinal tumor number by 95% (Rao et al, 1991; Jacoby et al, 1996; Edelmann et al, 1999).

Current clinical trials have shown that the traditional non-steroidal anti-inflammatory drugs \([\text{t NSAIDs}]\) (Grosch et al, 2006) such as piroxicam and sulindac are able to cause regression of adenomas in patients with FAP by up to 100% (Steinbach et al, 2000). Studies however, exposed that although the selective COX-2 inhibitors are very effective anti-neoplastic agents, they also suffer from cardiovascular, renal and other side effects (Komers et al, 2001a).

Therefore the hunt is for more amenable compounds and in this context piroxicam, which is having analgesic, anti-inflammatory, antipyretic as well as the anti-tumor properties, via presumably inhibiting the COX activity is more appropriate (Jacoby et al, 1996, 2000b). It was also found to have growth inhibitory effects toward premalignant and malignant epithelial cell lines via COX/PGE\(_2\)-independent mechanisms targeting signaling pathways regulating cell cycle progression.
C-phycocyanin

C-phycocyanin is one of the major biliproteins of *Spirulina platensis*, a blue-green algae and is a selective inhibitor of COX-2 ([Reddy et al., 2000](#)). It is indeed a more potent (IC$_{50}$ 180 nM) inhibitor of COX-2 activity than celecoxib (IC$_{50}$ 255 nM) and rofecoxib (IC$_{50}$ 401 nM), two well known selective COX-2 inhibitors. The human whole blood COX-2 assay further provided a more relevant measure of COX-2 inhibition selectivity, under a pathophysiological environment rich in plasma protein and cells ([Patrignani et al., 1994; Brideau et al., 1996](#)). The chromophore of c-phycocyanin such as reduced c-phycocyanin and phycocyanobilin, conversely, are the poor inhibitors of COX-2 without COX-2 selectivity, which suggested that apoprotein in c-phycocyanin may perhaps play an essential role in the selective inhibition of COX-2 ([Reddy et al., 2000](#)). Studies illustrated that c-phycocyanin significantly reduces R-(-)-pulegone (a potent hepatotoxin) and CCL$_4$-induced hepatotoxicity in rats ([Vadiraja et al., 1998](#)). One of the process involved in CCL$_4$-induced hepatotoxicity is the free radical catalyzed lipid peroxidation ([Slater et al., 1985](#)) which is accompanied by activation of COX and increased synthesis of PGs ([Reddy et al., 2000](#)). Also, one of the end products of lipid peroxidation such as 4-hydroxy-2-nonenal, a breakdown product of hydroperoxy fatty acid, is a specific inducer of COX-2 expression ([Kumagai et al., 2000](#)), which implies that during CCL$_4$-induced hepatotoxicity the COX-2 level gets elevated. Since, inhibition of COX-2 activity is a favorable condition for treating inflammatory arthritis and preventing cancer ([Kawamori et al., 1998](#)), therefore, the anti-inflammatory property of c-phycocyanin ([Remirez et al., 1999](#)) can be explained in part by the specific inhibition of COX-2. Recently it was demonstrated that oral administration of c-phycocyanin exerted anti-inflammatory effects in arthritis induced by zymosan in mice ([Remirez et al., 1999](#)). It was suggested that the anti-inflammatory activity of c-phycocyanin could be due to its ability to inhibit arachidonic acid metabolism and to scavenge oxygen free radicals ([Remirez et al., 1999; Gonzalez et al., 1999](#)).

In comparison to the toxicities associated with the currently available anti-inflammatory drugs and their activity as COX-2 inhibitors, c-phycocyanin will possibly provide safer therapeutic alternatives since it is as efficacious as currently used drugs (NSAIDs), and most importantly it is extracted from a natural source and seemingly least toxic. The mechanism of inhibition of COX activity by c-phycocyanin appears to be similar
to those reported for COX-2 selective inhibitors, occurring via a time dependent mechanism leading to a possible formation of a tightly bound inhibitor complex (Copeland et al, 1994; Marnett and Kalgutkar, 1999). Evidences also revealed that the active site of COX-2 is larger than that of COX-1 so that COX-2 can accommodate larger structures (Luong et al, 1996), therefore c-phycocyanin which is significantly bigger in size (37.5 kDa) than NSAIDs and hence its three dimensional structure perhaps may facilitate the proper binding with the active site of COX-2.

Function of c-phycocyanin as a selective inhibitor of COX-2 makes it a good candidate for preventing inflammation induced cancer. Morcos et al, 1988 have shown its photodynamic properties as well as its use in cancer treatment. They have shown that, c-phycocyanin specifically binds to cancer cells, and thus can be used for anatomical imaging of tumors in vivo (Morcos et al, 1988). Recently, its hepatoprotective (Vadiraja et al, 1998), anti-oxidant (Romay et al, 2000a) radical scavenging (Vadiraja and Madyastha, 2000) and anti-inflammatory properties have also been demonstrated (Romay et al, 1998a; b).

Various researchers investigated the combinations of NSAIDs with several chemopreventive drugs which showed that combinatorial strategies in cancer therapy can provide remarkable development in safety and efficacy over monotherapy regimens (Agarwal et al, 1999; Torrance et al, 2000; Reddy et al, 2003). For instance, the clinical trial of the combination treatment with DFMO and sulindac serves as effective agents for the prevention of recurrent colon polyps (Gerner and Meyskens, 2009). Furthermore, the combined use of JTE-522, a selective COX-2 inhibitor with conventional anticancer drugs was shown to exhibit significant enhancement in the treatment efficacy of lung cancer not only in vitro but also in vivo (Miyaishi et al, 2002). The present work thus focuses on a combination treatment of piroxicam, a t NSAID and c-phycocyanin, a specific COX-2 inhibitor in the chemoprevention of colon cancer and the underlying molecular mechanisms involved.
Phosphatidyl inositol 3-Kinase (PI3-K)/ Akt (protein kinase B) pathway

Phosphatidyl inositol 3-Kinase (PI3-K) consists of a regulatory subunit (p85) that binds to an activated growth factor/cytokine receptor and undergoes phosphorylation, which results in the activation of its catalytic subunit (p110) (Figure 2.16) (Rodriguez-Viciana et al, 1996). A product of PI3-K phosphorylation i.e., phosphatidylinositol 3,4 -bisphosphate may facilitate the recruitment of the serine-threonine protein kinase Akt (also known as protein kinase B or Aurora kinase) to the plasma membrane (Franke et al, 1997a). Akt is then considered to be stimulated via the recently identified protein kinases such as 3’-phosphoinositide-dependent kinases (PDKs) (Alessi et al, 1997). Overexpression of constitutively activated forms of PI3-K or Akt results in a declined apoptosis rate in response to serum/growth factor deprivation, UV-B irradiation or loss of matrix attachment (Kauffmann-Zeh et al, 1997; Kennedy et al, 1997; Khwaja et al, 1997; Kulik et al, 1997). Because apoptosis often arises in the presence of cytokines and growth factors that stimulate the apoptotic pathway modulated by PI3-K (Franke et al, 1997b), cells undergoing apoptosis possess a mechanism that can inhibit PI3-K.

Akt further mediates many of the downstream effects of PI3-K and consequently plays a central role in both normal and pathological signaling by the PI3-K pathway (Okano et al, 2000). Akt phosphorylates a variety of substrates involved in the regulation of key

![Figure 2.16: PI3-K/Akt pathway. Image adopted from a website (http://www.assay protocol.com/index.php page=PI3KAkt)](http://www.assay protocol.com/index.php page=PI3KAkt)
cellular functions including cell growth and survival, glucose metabolism and protein translation. These targets include glycogen synthase kinase 3 (GSK3), IRS-1 (insulin receptor substrate-1), PDE-3B (phosphodiesterase-3B), Bcl2 associated death promoter (BAD), human caspase 9, Nuclear factor xB (NFkB) transcription factor, nitric oxide synthase (NOS) and p21/WAF1 (Altiok et al, 1999; Datta et al, 1999; Galetic et al, 1999; Zimmermann and Moelling, 1999; Montagnani et al 2001; Zhou et al, 2001).

One common mechanism through which Akt-mediated phosphorylation results in substrate inhibition is through the regulation of subcellular localization and interaction with 14-3-3 proteins. 14-3-3 proteins are cytoplasmic proteins that bind specifically to phosphoproteins and retain them in the cytoplasm (Yaffe et al, 1997) away from their targets. In particular the Akt consensus phosphorylation site is also a consensus 14-3-3 binding site (Yaffe et al, 2001). For example, BAD phosphorylation by Akt inhibits its proapoptotic effects. In the unphosphorylated state, BAD is targeted to the mitochondria where it forms a complex with Bcl2 or BclXL, inhibiting their anti-apoptotic activity.

In addition to the inhibition of pro-apoptotic factors, Akt can also activate the transcription of anti-apoptotic genes through the activation of the transcription factor NFkB (Kane et al, 1999; Ozes et al, 1999; Romashkova and Makarov, 1999). When bound to its inhibitor, termed IxB, NFkB localizes to the cytoplasm to which associates Akt and in turn activates the IxB kinases (IKKs). Activated IKKs further phosphorylate IxB and therefore target it for the degradation by the proteosome. This allows NFkB to translocate to the nucleus and activate transcription of a variety of substrates including anti-apoptotic genes such as the inhibitors of apoptosis. Current study is thus aimed to identify whether piroxicam and c-phycocyanin targeted the PI3-K and Akt signaling molecules, which may contribute to the consideration of its chemopreventive usefulness in colon carcinogenesis.
GSK3β regulated Wnt/β-catenin signaling pathway

Large body of evidences demonstrated that the PI3-K/Akt signaling pathway is often a major regulator of glycogen synthase kinase 3 (GSK3) (Grimes and Jope, 2001). GSK-3 is a constitutively active and ubiquitously expressed serine-threonine kinase (Woodgett, 1990). In addition to playing a well-defined role in suppressing the canonical Wnt/β-catenin signaling pathway (Figure 2.17) (van Amerongen and Nusse, 2009; MacDonald, 2009), its functions in numerous growth factor signaling has been shown (Hur and Zhou, 2010). The canonical Wnt signaling pathway functions by stabilizing the β-catenin and in the absence of Wnt, it is located in the cytoplasm by a multiprotein cascade, which includes the APC protein, axin1, axin2, casein kinase 1 and GSK-3β (Baryawno et al, 2010). GSK-3β then resulted in the β-catenin phosphorylation which is then targeted for proteolytic degradation (Fodde and Brabletz, 2007).

Nitric oxide synthase (NOS) signaling

Nitric oxide (NO) is an important signaling molecule in numerous physiological and pathological conditions (Abramson et al, 2001). This free diatomic radical is produced when nitric oxide synthases (NOSs), a family of enzymes, catalyze the conversion of L-arginine to L-citrulline. There are three isoforms of NOS, two of them, i.e., endothelial NOS and neuronal NOS are calcium-dependent enzymes, constitutively expressed and responsible for low levels of NO production (pico molar to nano molar) for short periods (minutes). However, the inducible NOS (iNOS), is a calcium-independent, which is not expressed in most of the tissues under normal conditions, but can be induced by LPS and various
cytokines. It can produce large quantities of NO (mM) over extended periods (days to weeks) (Abramson et al, 2001). NO is reported to have an anti-tumor as well as pro-tumor characteristics and its effects may be concentration and tissue dependent (Wink et al, 1998). Low concentrations of NO can stimulate cell growth and protect many cell types from apoptosis, whereas its higher levels can restrain cell growth along with apoptosis stimulation (Kim et al, 2001). Several studies have shown the role of NO in either promoting or inhibiting the tumor growth of colorectal cancer cells (Jenkins et al, 1995; Siegert et al, 2002) and the effect was suggested to be concentration-dependent. Clinical evidences showed the association among overexpressed iNOS levels and increased colon cancer progression with less or no expression in normal colonic tissue (Ambs et al, 1998; Kojima et al, 1999; Yagihashi et al, 2000), whereas other studies reported lower iNOS expression in colon cancer as compared to significantly higher expression in normal colonic tissue (Chhatwal et al, 1994; Hao et al, 2001; Bing et al, 2001; Roberts et al, 2001). In animal models, induction of iNOS is correlated with colorectal cancer regression (both in situ and metastatic) (Xie et al, 1995; Onier et al, 1999), while NOS inhibitors are reported to prevent colonic ACF formation (Rao et al, 1999; Kawamori et al, 2000; Rao et al, 2002). Numerous studies have been done to investigate the interaction between COX-2 and iNOS in various cell systems, particularly inflammatory models. In these systems, NO is shown to have a regulatory effect, either stimulatory or inhibitory, on the COX-2 expression and activity (Weinberg, 2000; Perez-Sala and Lamas, 2001). However, their relationship in colon cancer has not been studied yet.

Role of peroxisome proliferator-activated receptors (PPARs) in cancer

During the last decade, a group of nuclear receptors, known as peroxisome proliferator-activated receptors (PPARs), was identified (Issemann and Green, 1990), which belong to the superfamily of steroid hormone receptors, and are activated by fatty acids, eicosanoids and numerous structurally dissimilar xenobiotics, collectively known as peroxisome proliferators (Youssef and Badr, 2002). Three related PPAR isotypes have been identified till date such as PPAR α, PPAR β/δ and PPAR γ (Desvergne and Wahli, 1999; Willson et al, 2000). Reports demonstrated the presence of several human forms of PPARα (hPPARα) (Sher et al, 1993; Mukherjee et al, 1994) and PPARγ (hPPARγ) (Greene et al,
Furthermore, the tissue distribution pattern of hPPAR α mRNA is related to that of the rat PPAR α. In both species PPAR α is highly expressed in brown adipose tissue, skeletal muscle, liver, heart as well as kidney, whereas in the brain and lung, it is found to be expressed at lower level (Mukherjee et al, 1994; Su et al, 1998). Alternatively, the principal site of the expression of PPARγ is the adipose tissue, but this receptor is also expressed, although at lower levels, in several other tissues and cell types such as the retina, some parts of the immune system, mammary and colonic epithelium (Mueller et al, 1998). PPAR δ subtype is found in higher amounts than PPAR α and PPAR γ in almost all tissues except the adipose tissue (Michalik and Wahli, 1999). PPARδ is expressed ubiquitously in the rat cerebellum, thalamus and cerebellar cortex (Cullingford et al, 1998), and specific PPAR δ agonists, and to a significant lesser extent those of PPARγ, stimulated the oligodendrocyte differentiation in vitro (Saluja et al, 2001).

The possible role of PPARs in cancer formation in humans is still controversial (Seed, 1996). Two recent reports illustrated the role of PPARγ in promotion and development of colon cancer (Lefebvre et al, 1998; Saez et al, 1998), while a third study indicated a protective role for PPARγ agonists against colon cancer in humans (Karam and Ghanayem, 1997). Mice genetically predisposed to develop polyps in the colon exhibited an increased number of polyps when subjected to PPARγ agonists orally (Lefebvre et al, 1998; Saez et al, 1998). Conversely, it was demonstrated that human colon cancer cell lines both in culture and in nude mice respond to PPAR γ agonists with a reduced rate of growth and an increased degree of differentiation (Sarraf et al, 1998). Recently it was found that PPARγ agonists may be beneficial in combating breast cancer in humans (Badawi and Badr, 2002). Furthermore, another report suggested that inhibiting PPAR δ may be responsible for reducing the incidence of colorectal cancer caused by NSAIDs (He et al, 1999), however, the involvement of PPAR δ in colorectal cancer requires more precise studies using compounds specific to this PPAR subtype (Willson et al, 2000).

Mitochondrial pathway of apoptosis

Apoptosis or programmed cell death is a normal constituent of the development and health of multicellular organisms. Cells die in response to a variety of stimuli and during apoptosis they do so in a controlled, regulated fashion (Adopted from
www.sgul.ac.uk/depts/immunology/~dash). This property makes apoptosis distinctive from another form of cell death called necrosis in which uncontrolled cell death leads to cell lysis, inflammatory responses and potentially to serious health problems. Apoptosis, however, is a process in which cells play an active role in their own death, which is why apoptosis is often referred to as cell suicide (Adopted from www.sgul.ac.uk/depts/immunology/~dash).

To relate the kinetics of PI3-K inhibition with that of the apoptotic cell death, we decided to study the mitochondrial pathway of apoptosis, in the current study. Mitochondria may play an essential role in the regulation of cell death. It contains many pro-apoptotic proteins such as apoptosis inducing factor (AIF), cytochrome c etc which are released from the mitochondria following the pore formation in the mitochondrial membrane called the permeability transition pore or PT pore (Adopted from www.sgul.ac.uk/depts/immunology/~dash). These pores are considered to form through the action of the pro-apoptotic members of the Bcl2 proteins family which in turn are activated by apoptotic signals such as cell stress, free radical damage or growth factor deprivation. Some members of the Bcl2 protein family (such as Bcl2 and BclXL) are anti-apoptotic, while others (such as Bad, Bax or Bid) are pro-apoptotic in nature. Since, the sensitivity of cells to apoptotic stimuli depend on the balance of pro- and anti-apoptotic Bcl2 proteins, therefore, if there is an excess of pro-apoptotic proteins the cells are more sensitive to apoptosis while surplus amount of anti-apoptotic proteins render the cells more resistant to apoptotic cell death.

The pro-apoptotic Bcl2 proteins are generally found in the cytosol where they act as sensors of cellular damage or stress. Following the cellular stress they relocate to the surface of the mitochondria where the anti-apoptotic proteins are located. This association among the
pro- and anti-apoptotic proteins disrupts the normal functioning of the anti-apoptotic Bcl2 proteins and can lead to the formation of pores in the mitochondria as well as the release of cytochrome c and other pro-apoptotic molecules from the intermembrane space.

Cytochrome c release from the mitochondria is considered as a key signal that initiates the irreversible events in cell death (Figure 2.18) (Liu et al, 1996; Martinou et al, 2000). Several apoptosis inducing agents are known to trigger mitochondrial uncoupling leading to the rupture of outer membrane. This in turn causes the release of pro-apoptotic factors such as AIF, cytochrome c and the apoptosis protease-activating factor (Apaf-1) into the cytosol (Subhashini et al, 2004). Cytosolic cytochrome c is known to become associated with caspase-9, Apaf-1 and dATP to form the apoptosome complex which in turn activates caspase-9, -3 and -7 (Reubold et al, 2009). Activated caspase-3 cleaves off target substrates like poly (ADP-ribose) polymerase (PARP), a nuclear enzyme that senses DNA nicks and catalyzes the ADP ribosylation of histones and other nuclear proteins in order to facilitate DNA repair (Lazebnik et al, 1994).

Several reports collectively demonstrated that the cell death belongs to the numerous cell functions on which Ca2+ exerts a complex regulatory role (Berridge et al, 2000; Clapham DE, 2007; Rimessi et al, 2008). It has long been known that in neurons and other cell types an unchecked increase in cytosolic Ca2+ concentration can trigger apoptosis (Sattler and Tymianski, 2000), and likewise, agents that are able to release Ca2+ from intracellular stores have been shown to be pro-apoptotic (Mattson and Chan, 2003). Most importantly, Ca2+ is a critical sensitizing signal in the pro-apoptotic transition of mitochondria that plays a key role in the regulation of cell death (Kroemer and Reed, 2000). Mitochondrial Ca2+ overload (Figure 2.19) is one of the pro-apoptotic means to induce the mitochondrial swelling, agitation or rupture of the outer membrane, and releasing of the mitochondrial apoptotic factors into the cytosol (Giorgi et al, 2008).
Reports suggested that selective COX-2 inhibitor such as NS-398, induced apoptotic cell death in a number of colon cancer cell lines, including HT-29 (COX positive), HCT 15 (COX negative) and SW480 (COX-2 negative) by releasing cytochrome c from mitochondria, leading to the activation of caspase-9 and caspase-3 (Li et al, 1997).

Regulation of cell cycle and specific action of Cyclin-CDK complexes

Studies had demonstrated that apoptosis can be induced by various means such as growth arrest of cells overexpressing c-myc oncogene (Evan et al, 1992; Shi et al, 1992) or p53 by blockers of G1, S, or M phases of the cell cycle (Figure 2.20) (Yoish-Rouash et al, 1993; Ryan et al, 1993; Lotem and Sachs, 1993).

The typical events common to most inducers of apoptosis include membrane and nuclear blebbing (Cohen, 1993; Cohen and Duke, 1992), activation of a Ca$^{2+}$ dependent endonuclease (Peitch et al, 1993) and DNA “laddering” (Arends and Wyllie, 1991; Cohen, 1993; Cohen and Duke, 1992).

Further, it was illustrated that a dysregulation in the cell cycle machinery is another cause of tumor formation, for instance, the mutations in a number of genes such as cell cycle inhibitors retinoblastoma protein (pRb), p53 etc., may cause the cell to multiply in an uncontrolled manner, ultimately forming a tumor.

Although the duration of cell cycle in tumor cells is equal to or longer than that of normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G0 phase) in tumors is much higher than that in normal tissue (Adopted from http://en.wikipedia.org/wiki/Cell_cycle). Thus there is a net increase in cell number
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since the number of cells that die by apoptosis or senescence remains the same 

Numerous results from studies in various eukaryotes have illustrated that the progression through cell cycle is driven by the activation and inactivation of cyclin-dependent kinases (CDKs), which trigger the transition to subsequent phases of the cell cycle (Figure 2.20). CDKs are small serine/threonine protein kinases that require association with a Cyclin subunit for their activation. Cyclin D is the first Cyclin produced in the cell cycle, in response to extracellular signals (e.g. growth factors) (Adopted from http://en.wikipedia.org/wiki/Cell_cycle). It binds to CDK4, forming the active Cyclin D-CDK4 complex which in turn phosphorylates the retinoblastoma susceptibility protein (pRb). The hyperphosphorylated Rb dissociates from the E2F/DP1/Rb complex and ultimately stimulating the activity of E2F transcription factor (Adopted from http://en.wikipedia.org/wiki/Cell_cycle). Activation of E2F results in transcription of various genes like Cyclin E, Cyclin A, DNA polymerase, thymidine kinase, etc. Cyclin E thus produced, binds to CDK2 forming the Cyclin E-CDK2 complex, which pushes the cell from G1 to S phase (G1/S transition) (Adopted from http://en.wikipedia.org/wiki/Cell_cycle).

In response to DNA damage, checkpoints also arrest the cell cycle in order to provide the time for DNA repair. DNA damage checkpoints are positioned before the cell enters S phase (G1-S checkpoint) or after DNA replication (G2-M checkpoint) and also there appears to be DNA damage checkpoints during S and M phases. At the G1-S checkpoint, cell cycle arrest induced by DNA damage is p53-dependent. Usually, the cellular level of p53 is kept low but DNA damage can be resulted in a rapid induction of p53 activity (Levine, 1997) and p53 in turn stimulates the transcription of various genes such as p21 [a CDK inhibitor (CKI)], Mdm2 (Mouse double minute 2 homolog) and Bax (Bcl2 associated X protein) (Agarwal et al, 1998). The induction of p21 results in CDK inhibition and cell cycle arrest, thus preventing the replication of damaged DNA (Ko and Prives, 1996).

The role of CDK2 in the regulation of the cell cycle in mammalian cells is well established in several studies (Pagano et al, 1992). However, the role of cyclins D and E (G1 cyclins) in the regulation of the various phases of the cell cycle is still under extensive investigation (Lew and Reed, 1992; Xiong et al, 1992). Reports suggested that selective COX-1 or 2 as well as non selective COX inhibitors may alter the cell cycle machinery at numerous sites, which could explain their anti-proliferative or apoptotic effects. Evidences

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also explain the role of Sulindac, a COX-2 inhibitor in the inhibition of proliferation rate of HT29 colon adenocarcinoma cells, hence allowing them to accumulate in the G0/G1 phase of the cell cycle, and reduced the levels of various CDKs (Shiff et al, 1995).

**Angiogenesis**

Angiogenesis, a process that involves the formation of new blood vessels growing from the pre-existing and quiescent vascular endothelium (Figure 2.21) is one of the essential requirements for the progression of multistage carcinogenesis (Leahy et al, 2007). COX-2, an inducible enzyme is a known mediator of angiogenesis and tumor growth (Gatley and Lee, 2004). The pro-angiogenic effects of COX-2 are mediated primarily by three products of arachidonic acid metabolism i.e., thromboxane A2 (TXA2), prostaglandin E2 (PGE2), as well as prostaglandin I2 (PGI2). Moreover, downstream pro-angiogenic effects of these eicosanoid
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products include: (1) vascular endothelial growth factor (VEGF) formation, (2) stimulation of vascular sprouting, migration and tube formation, (3) enhanced endothelial cell survival via Bcl2 expression and Akt signaling, (4) activation of matrix metalloproteinases, (5) induction of epidermal growth factor receptor (EGFR)-mediated angiogenesis and (6) inhibition of interleukin-12 (IL-12) generation. Selective inhibition of COX-2 activity by several chemopreventive agents has been shown to suppress angiogenesis both in vitro and in vivo. Because these chemopreventive agents are safe and well tolerated, they could have clinical usefulness as anti-angiogenic agents for prevention of tumor growth. Numerous studies demonstrated that the traditional NSAIDs (t NSAIDs) can restrain cancer progression as well as angiogenesis in various experimental models (Lala et al, 1997). For example, chronic oral indomethacin treatment can delay the early growth of spontaneous mammary tumors as well reducing the vascularity in retired breeder C3H/HEJ mice, and extends the lifespan of tumor bearing mice (Lala et al, 1997). Another example of the inhibitory regulation is the topical administration of the t NSAID diclofenac (a preferential COX inhibitor) which inhibits angiogenesis in mice bearing syngeneic, subcutaneous colon-26 tumors, and resulted in apoptotic cell death (Seed, 1996; Seed et al, 1997).

Reports revealed that oral administration of a specific COX-2 inhibitor reduces the expression of potent angiogenic factors such as VEGF as well as basic fibroblast growth factor and suppressed the cell replication of the COX-2 overexpressing cancer xenografts in a dose-dependent manner. In contrast, a nonspecific COX inhibitor suppresses growth and angiogenesis of non-COX expressing cancer xenograft by inhibition of COX-1 in vascular endothelial cells. These findings may demonstrate that COX inhibitors reduce angiogenesis and tumor growth by inhibiting expression of angiogenic factors and vascular endothelial cell growth. Therefore, this supports the hypothesis that COX plays an important role in cancer growth via angiogenesis dependent pathway (Sawaoka et al, 1999).
Concluding remarks

Determination of COX-2 level is one of the molecular advances existing not only to make us possible to plan early investigation but also to target for chemoprevention of the pathway that initiates and prolongs colon carcinogenesis. The role of COX-2 is well-established, however inhibition of this enzyme cannot clarify all the observed effects, in cancer. Additional approaches in the involved pathway will enable more precise target of key molecules and will lead the future development of chemopreventive regimens. Most likely, more than one pathway is required to be investigated for the prevention of colon cancer progression.

Recent studies showed that NSAIDs, particularly COX-2 selective inhibitors were most reasonable candidate drugs for chemoprevention. However, the detection of great cardiovascular toxicity following long-term treatment of roficoxib and celecoxib prevented this class of agent being used in average risk of population. On the other hand, piroxicam, a traditional NSAID (t NSAID), owing to its preferential COX-2 selectivity can accomplish the assurance of being a suitable chemopreventive agent in colon cancer. Furthermore, a combination regimen of piroxicam as a t NSAID and c-phycocyanin, a natural COX-2 inhibitor could be a more potent chemopreventive agent in colon cancer. In this context, c-phycocyanin, a biliproteins present in *S. platensis*, a selective COX-2 inhibitor could be a more potent chemopreventive agent at a lower dose and expectedly with minimum or no side effects in colon cancer.