CHAPTER-1

INTRODUCTION
1.1. Cancer

Cancer is the uncontrolled growth of cells with the potential to invade or spread to other parts of the body. Cancer was first recorded in 1600 BC which described the breast cancer (Hajdu et al., 2011). Hippocrates used the Greek word karkinos to describe the various types of cancer (Hajdu et al., 2011). Later Celsus translated the Greek word karkinos to Latin word cancer (Hajdu et al., 2011). Cancer has become one of the major causes of morbidity during the last 40 years. Worldwide 8.2 million deaths occurred during 2012 due to cancer out of which 4.7 million were males and 3.5 million were females that accounted for 14.6% of all human deaths (Ferlay et al., 2012). Cancer cells divide uncontrollably to form lumps of tissue called tumors except that of leukemia. The cancer cells then leave the original tumor site and migrate to other parts of the body via the lymphatic system, the bloodstream, or by direct extension; a process referred as metastasis. Tumors can be of two types i.e., benign and malignant tumors.

- Benign tumors are the tumors that are localized and do not transform into cancer.
- Malignant tumors are the tumor which invade the surrounding tissue via metastases and eventually kills the host.

1.2. Types of Cancer

Cancer can be classified by the type of cells that the tumor cells resemble. These types include:

- Carcinoma: Develops from the epithelial cells, such as the tumors developing in the breast, prostrate, lung, pancreas and colon etc.
- Sarcoma: Develops from transformed cells of mesenchymal origin, such as the tumors made of fat, muscle, cartilage, bone etc.
- Germ cell tumor: Develops from the pluripotent cells.

- Lymphoma and leukemia: Develops from the hematopoietic (blood-forming) cells.

- Blastoma: Develops from precursor cells called blasts, which include retinoblastoma medulloblastoma etc.

### 1.3. Causes of Cancer

There are many causes of cancer, out of which 90-95% is due to environmental factors and the rest 5-10% are due to inherited genetics (Anand et al., 2008). The common environmental factors that lead to cancer are tobacco, radiation, diet and obesity, infections, stress, lack of physical activity, environmental pollutants etc. (Anand et al., 2008). Still it is very difficult to find the exact cause of cancer as there are so many possible chances. Other than transmission during pregnancies and organ donation, cancer is generally not a communicable disease (Tolar & Neglia, 2003). Some of the common causes of cancer are such as described below:

- Chemicals: Chemicals which leads to the formation of cancer are oftenly referred to as carcinogens. One of the most common carcinogens is tobacco which account for 90% of lung cancer. Alcohol is also reported to cause cancer of the liver and the digestive tract. Around 2-20% of all cancer cases occur due to carcinogens.

- Diet & exercise: Lifestyle has nowadays become one of the major causes of cancer. Around 30-35% of cancer cases are related to diet, physical activity and obesity.

- Radiation: Radiation whether ionizing or non-ionizing possesses high cancer risk. UV radiation from sun can lead to melanoma, so as the radio frequency radiation from electric power transmission, mobile phones etc.
Infection: Worldwide 18% of cancer death occurs due to infectious disease. Viruses with the potentiality of causing cancer are called as oncovirus such as the Epstein-Barr virus, Human Papillomavirus, Human T-cell leukemia virus-1, etc.

Physical agents: There are several physical agents which can lead to cancer such as the asbestos responsible for causing the mesothelioma. Although this form of cancer is rare but substances like wollastonite, glass wool, attapulgite, rock wool etc. do possess a possible threat.

Heredity: Heredity cancers account for only 3-10% of all forms of cancer such as the inherited mutations in the genes such as the BRCA1 and 2 can lead to breast and ovarian cancer.

Hormones: Hormones are also reported to play a significant role in the development of various cancers such as the breast, ovary, prostate etc.

1.4. Cancer Treatment

There are several treatments available for cancer which can increase the life span of the patient. The type of treatment depends on the type, grade and location of cancer. The major types of treatment that are being employed on a regular basis in clinics are as follows:

Chemotherapy: It is the treatment of cancer using chemical substances which includes two broad categories: alkylating agents and antimetabolites. It is one of the most effective forms of cancer treatment which kills the cells that divides rapidly.

Radiation: It uses ionizing radiation to cure cancer. It generally works by damaging the DNA of the rapidly dividing cells leading to cell death.
Surgery: It is the primary method of treatment, where the lump of cancer tissue is surgically removed from the patient.

Palliative care: It is a form of treatment where more care is taken to reduce the physical, spiritual, emotional, and psycho-social distress experienced by people with cancer.

Immunotherapy: It’s a form of treatment where the immune system is evoked to treat the cancer.

1.5. Colon Cancer

Colon cancer ranks as second in cancer related deaths in the western countries and accounts for approximately 10%-15% of all cancers (Boring et al., 1991). Colon cancer is the type of cancer which occurs in the tissues of the colon (longest part of the large intestine). Generally colon cancer starts with the formation of a small, benign clump of cells called adenomatous polyps, which over time become colon cancers. Polyps are generally small and hardly produce any symptoms. The common symptoms of colon cancer are weight loss, blood in the stool, change in bowel movements, weakness, persistent abdominal discomforts, etc. Nowadays the increase in colon cancer cases is because of the changed lifestyle, increasing age and genetic disorders. More than 95% of colon cancer is sporadic occurring without a significant hereditary risk (Watson & Collins, 2011). The main risk factors are old age, intake of large quantities of fat, alcohol, red meat, smoking, obesity etc. People who suffer from ulcerative colitis and Crohn's disease have a high risk of colon cancer (Jawad et al., 2011). The risk is greater when the person had the disease for a longer time and worse based on the severity of the inflammation (Xie & Itzkowitz, 2008; Triantafillidis et al., 2009). Inherited gene mutations that increases the risk of colon cancer, constitute a small percentage of colon cancers. Based on inherited gene mutation
there are two common types of colon cancer syndromes which are familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) also known as Lynch syndrome. There are various types of colon cancer such as:

- **Adenocarcinomas:** It constitutes about 95% of all the colon cancer. It generally starts in cells leads to gland formation that produces mucus which in turn lubricates the inside of the colon.

- **Carcinoid tumors:** Tumors which originates from hormone-producing cells in the intestine are known as carcinoid tumors.

- **Gastrointestinal stromal tumors (GISTs):** These tumors originate from the walls of the colon.

- **Lymphomas:** These cancers generally start in the lymph nodes but can also start in the colon, rectum, or other organs.

- **Sarcomas:** These types of tumors can occur in muscle, blood vessels as well as connective tissue in the wall of the colon and rectum.

### 1.6. Molecular Basis of Colon Cancer

Colon cancer generally arises due to the frequent mutation of the Wnt signaling pathway, which can be inherited or acquired. The most common type of mutation in colon cancer is the *APC* gene, which produces the Adenomatous polyposis coli (APC) protein. APC protein generally acts as a brake on the accumulation of the β-catenin proteins, in the absence of which β-catenin accumulates and moves into the nucleus and leads to the transcription of genes that are responsible for cell proliferation (Markowitz & Bertagnolli, 2009). Mutation in *TP53* gene,
producing the p53 protein can also lead to cancer due to the inability to perform cell cycle arrest (Markowitz & Bertagnolli, 2009). Inactivation of TGFBR2 by somatic mutation leads to the inactivation of the TGF-β tumor suppressor pathway leading to cancer (Markowitz & Bertagnolli, 2009). Inactivation of genes such as the MLH1 and MSH2 responsible for the repair of base-base mismatches in C leads to genomic instability and ultimately cancer (Markowitz & Bertagnolli, 2009). Several oncogenes play a major role in causing colon cancer such as the RAS and BRAF which activates the mitogen-activated protein kinase (MAPK) signaling pathway responsible for cancer (Bos et al., 1987; Davies et al., 2002; Markowitz & Bertagnolli, 2009). It was observed that one third of the colorectal cancer have somatic mutation in PI3KCA gene responsible for encoding the catalytic subunit of phosphatidylinositol 3-kinase (PI3K) (Markowitz & Bertagnolli, 2009). Increased level of COX-2 and loss of 15-prostaglandin dehydrogenase (15-PGDH) have been found in many cases of colon cancer (Markowitz & Bertagnolli, 2009). Vascular endothelial growth factor (VEGF) activation has also been reported in various cases of colon cancer needed for the purpose of angiogenesis (Hurwitz et al., 2004; Markowitz & Bertagnolli, 2009). Increased activity of MYC has also been reported in colon cancer cases (Muzny et al., 2012).

1.7. Therapies available for Colon Cancer

There are several treatments available for the treatment of colon cancer and to improve the life span of the patient. Scientists worldwide are coming up with various, new type of treatments to cure colon cancer. Some of the basic types of colon cancer treatment are as follows.

1.7.1. Surgery
Surgery can be curative for patients suffering from localized form of colon cancer (Cunningham et al., 2010). Preferred treatment is complete surgical removal of the tumour done with adequate margins, and lymphadenectomy (Cunningham et al., 2010). Generally 12 but not less than 10 lymph nodes are taken to analyze the appropriate nodal staging with a recommended distal margin of 5cm (Cunningham et al., 2010). The surgery can be done either by open laparotomy or sometimes laparoscopically. Laparoscopic surgery is a modern surgical technique performed far from their location by creating small incisions (usually 0.5-1.5 cm) elsewhere in the body. Laparoscopic colectomy is the best for left-sided colon cancer (Cunningham et al., 2010). If metastasis occurs then liver and lungs is the most common site for the recurrence of the cancer, which may also be removed to improve the life-span of the patients (Cunningham et al., 2010).

### 1.7.2. Radiation Therapy

Radiation therapy uses rays of high energy such as the X-rays or particles to eliminate cancer cells by damaging the chromosomes in the cell so that the cells cannot multiply (Cunningham et al., 2010). Radiation therapy is mainly employed when the cancer is attached to an internal organ or the lining of the abdomen and the surgeon is not certain whether all the cancer has been removed, and hence radiation therapy can be used to try to eliminate any cancer cells which might be left behind after surgery. Radiation therapy is also used to treat colon cancer that has spread to the bones or brain. There are generally four types of radiation therapy:

- **External-beam radiation therapy:** In this type of radiation therapy the radiation is given from a machine outside the body.
- **Brachytherapy (internal radiation therapy):** In this type of radiation therapy small pellets of radioactive material is used which is placed into a catheter or tube that was placed next to or directly into the cancer limiting the effects on surrounding healthy tissues.

- **Radioembolization:** In this type of radiation therapy radiation is given during embolization procedure.

The problem with radiation therapy is that it also harms the normal cells. The possible side effects of radiation therapy include skin irritation, nausea, bladder irritation, sexual problems, fatigue etc.

### 1.7.3. Targeted Therapy

There are certain genes and protein whose differential expression inside the cell lead to cancer. Hence scientists have developed new drugs targeting these changes. These drugs are different from chemotherapeutic drugs and have lesser side effects. Some common targeted drugs are such as:

- **VEGF targeted drugs:** Bevacizumab and Ziv-aflibercept are some of the drugs that are used in colon cancer for targeting the vascular endothelial growth factor (VEGF). VEGF is a protein responsible for the angiogenesis helping the tumors to form new blood vessels to get nutrients.

- **EGFR targeted drugs:** Drugs such as Cetuximab and Panitumumab are used in the treatment of metastatic colon cancer. These are both monoclonal antibodies that specifically target the epidermal growth factor receptor (EGFR). EGFR is protein molecule found in high amounts on the surface of cancer cells and helps them grow.
Other targeted drugs: There are other drugs such as Regorafenib used to treat advanced form of colon cancer. It’s a type of kinase inhibitor targeting the kinases found on or near the surface of a cell transmitting signal necessary for tumor cells to grow or help form new blood vessels to feed the tumor.

1.7.4. Chemotherapy

Cancer treatment which uses chemical substances especially one or more anti-cancer drugs is known as chemotherapy. Chemotherapy is generally given by two ways:

- Systemic chemotherapy: In systemic chemotherapy the drug is injected into the vein or given in mouth, which then enters the bloodstream and reaches every parts of the body. This procedure is employed when the cancer has metastasized from the region of origin.

- Regional chemotherapy: In this procedure, the drug is injected directly into the artery that leads to the tumor reducing the side effects by limiting it from reaching the rest of the body. For example hepatic artery infusion is employed for the treatment of colon cancer that has spread to the liver.

Chemotherapy can be used at different phases of colon cancer treatment such as:

- Adjuvant chemo: Adjuvant chemo is used to remove the cancer after surgery and also prevents cancer recurring.

- Neoadjuvant chemo: Sometimes to shrink the cancer, chemo is given before surgery to make surgery easier.

- Chemo for advanced cancers: In this scenario, chemo is used to shrink the tumor and relieve the symptoms of cancer that have metastasized. It helps patients to live longer.
Generally chemotherapy works by impairing mitosis, causing damage to the DNA, inhibiting the cellular machinery involved in cell division etc.

There are various types of chemotherapeutic molecules based on their mechanism of action such as:

- **Alkylating agents:** This is the group of the mostly used chemotherapeutic drugs nowadays. These compounds alkylate many molecules including the DNA, RNA and proteins. They impose their anti-cancer effect by covalently binding to DNA using their alkyl group (Lind, 2008). These compounds may bind to the single or double stranded DNA causing DNA break, leading to apoptosis (Damia et al., 1998; Siddik, 2005). Alkylating agents can be further classified into cisplatin, nitrogen mustards, tetrazines, aziridines, nitrosoureas. Cisplatin derivative includes cisplatin, oxaloplatin, carboplatin (Damia et al., 1998; Lind, 2008). The various types of nitrosoureas are N- carmustine (BCNU), Nitroso-N-methylurea (MNU), lomustine (CCNU) and semustine (MeCCNU), streptozotocin and fotemustine. Different types of nitrogen mustards are cyclophosphamide, mechlorethamine, busulfan, melphalan, chlorambucil and ifosfamide. Different types of tetrazines are such as dacarbazine, temozolomide and mitozolomide. Similarly various types of aziridines include diaziquone (AZQ), thiotepa, mytomycin.

- **Anti-metabolites:** These groups of drugs block the DNA and RNA synthesis. Many of the anti-metabolites mimic the structure of a nucleotide, the building blocks of DNA and RNA. These compounds exert their anti-cancer effect by inhibiting the enzymes involved in DNA synthesis or by mis-incorporation in DNA or RNA. Mis-incorporation in DNA leads to DNA damage and thereby apoptosis. Anti-metabolites can be further subdivided
into anti-folates, fluoropyrimidines, deoxynucleoside analogues and thiopurines (Lind, 2008). Anti-folates include methotrexate and pemetrexed while deoxynucleoside analogues include clofarabine, cytarabine, fludarabine, gemcitabine, decitabine, Vidaza, nelarabine, cladribine and pentostatin. Thiopurines include thioguanine and mercaptopurine while fluoropyrimidines include fluorouracil and capecitabine (Lind, 2008).

- **Anti-microtubule agents:** These are basically plant derived compounds which blocks the cell division by inhibiting the microtubule formation (Rowinsky *et al*., 1991). There are two main categories of anti-microtubule agents such as Vinca alkaloids and taxanes. Taxanes prevent microtubule disassembly while vinca alkaloids prevent the microtubules formation. Some of the common anti-microtubule drugs of this group are paclitaxel, vindesine etc.

- **Topoisomerase inhibitors:** This category of drugs affects two enzymes topoisomerase I and topoisomerase II. Inhibition of these enzymes affects both the transcription and the replication process (Goodsell, 2002; Lodish *et al*., 2000). Some of the commonly used topoisomerase inhibitors are irinotecan, topotecan, etoposide etc.

- **Cytotoxic antibiotics:** These groups of drugs vary in their mechanism of action. These groups can be classified into anthracyclines and other drugs including plicamycin, bleomycin, actinomycin and mitomycin. The most commonly used anthracyclines are doxorubicin and daunorubicin.
1.8. 5-Fluorouracil

5-Fluorouracil (5-FU) is a pyrimidine analog drug that is used for the treatment of cancer. It is trademarked by various names such as Adrucil, Carac, Efudex, etc. It belongs to the antimetabolites family of drugs and is considered as a suicide inhibitor that works by irreversible inhibition of thymidylate synthase and also by incorporation into DNA and RNA. It has been listed as one of the most essential medicine for the basic health system according to WHO (World Health Organization). The drug was first discovered by Charles Heidelberger in 1957, which he later patented (Heidelberger et al., 1957; Chu, 2007). 5-FU is a uracil analogue having fluorine atom at the C-5 position instead of hydrogen. The structure of 5-FU is shown in Figure 1.1.

![Figure 1.1. Structure of 5-Fluorouracil](image)

5-FU has been widely used for the treatment of various types of cancers such as colorectal, breast, aerodigestive tract etc. (Longley et al., 2003). Although the combination of 5-FU with other chemotherapeutic drugs has increased the survival rates in breast and head & neck cancer, but it has the highest impact in colorectal cancer (Longley et al., 2003). The response rate of 5-FU treatment as a first line of therapy for advanced colorectal cancer is only 10-15% but in combination with other drugs such as oxaliplatin and irinotecan, the survival rates increases upto
40-50% (Douillard et al., 2000; Giacchetti et al., 2000; Johnston & Kaye, 2001). Inspite of all these advancements in the treatment of colon cancer, new therapeutic regimes are desperately needed as resistant to 5-FU has been frequently observed in clinics. Thus understanding the mechanism of 5-FU action and the process by which tumours get resistant to 5-FU is of utmost importance.

1.9. Mechanism of 5-FU Action

5-FU enters the cells using the same mechanism as that of uracil, i.e. by facilitated transport (Wohlhueter et al., 1980). 5-FU after entering the cells is converted into three active metabolites – fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) which disrupts RNA synthesis and the action of thymidylate synthase (TS), ultimately leading to DNA damage. The rate limiting step of 5-FU catabolism is the conversion of 5-FU into dihydrofluorouracil (DHFU) mediated by dihydropyrimidine dehydrogenase (DPD) (Figure 1.2) (Diasio & Harris, 1989). 5-FU after being administered is catabolised mainly in liver where DPD is expressed abundantly (Diasio & Harris, 1989).

The main mechanism of 5-FU action is its conversion to fluorouridine monophosphate (FUMP) by orotate phosphoribosyltransferase (OPRT) (Figure 1.2) (Longley et al., 2003). FUMP is then converted to fluorouridine diphosphate (FUDP) which can either convert to fluorodeoxyuridine diphosphate (FdUDP) by the enzyme ribonucleotide reductase (RR) or can get phosphorylated to form the active metabolite fluorouridine triphosphate (FUTP) (Figure 1.2) (Longley et al., 2003). Furthermore, FdUDP can either get phosphorylated or dephosphorylated to form the active metabolites FdUTP and FdUMP, respectively (Figure 1.2) (Longley et al., 2003). Another pathway involves the conversion of 5-FU to fluorodeoxyuridine (FUDR) by the enzyme
thymidine phosphorylase which is further phosphorylated by thymidine kinase (TK) to form the active metabolite FdUMP (Figure 1.2) (Longley et al., 2003).

**Figure 1.2.** 5-FU mechanism of action.

The main mechanism of 5-FU action can be categorized into two major categories:

- **TS inhibition**: TS is a 36 kDa protein which functions as a dimer for the reductive methylation of dUMP to dTMP with the help of 5,10-methylenetetrahydrofolate (CH$_2$THF) playing the role of methyl donor (Longley et al., 2003). This reaction is the source of thymidylate necessary for DNA replication and repair (Longley et al., 2003). The active metabolite of 5-FU, FdUMP binds to TS and forms a stable ternary complex with TS and CH$_2$THF blocking the binding of the normal substrate dUMP, thereby...
inhibiting the synthesis of dTMP (Santi et al., 1974; Sommer & Santi, 1974). Reduction in the cellular level of dTMP leads to the depletion of deoxythymidine triphosphate (dTTP) causing perturbations in the levels of the other deoxynucleotides by various feedback mechanisms disrupting DNA synthesis and repair (Jackson & Grindley, 1984; Yoshioka et al., 1987; Houghton et al., 1995). Furthermore TS inhibition leads to the accumulation dUMP which increases the level of dUTP, and together dUTP and FdUTP misincorporated into DNA (Longley et al., 2003). Due to the presence of high (F)dUTP/dTTP ratios, repair of uracil and 5-FU-containing DNA by the enzyme uracil-DNA-glycosylase (UDG) is futile and only results in further false nucleotide incorporation (Longley et al., 2003). These cycles of excision, misincorporation and repair leads to DNA damage and ultimately cell death (Longley et al., 2003).

- RNA misincorporation: FUTP, the active metabolite of 5-FU misincorporates into RNA at high rates inhibiting normal RNA processing and function (Longley et al., 2003). Misincorporation inhibits the processing of pre-rRNA into mature rRNA as well as disrupts the post-transcriptional modification of tRNAs and also the splicing of pre-mRNA (Longley et al., 2003). 5-FU has also been found to inhibit the post-transcriptional conversion of uridine to pseudouridine, a modified base, present in all forms of RNA (Samuelsson, 1991). Polyadenylation of mRNA is also inhibited in the presence of 5-FU (Carrico & Glazer, 1979).

1.10. Modulation of 5-FU

5-FU for the last 40 years has been extensively used in clinics for the treatment of colorectal cancer, but its effect is quite limited (approximately 10–15%) (Longley et al., 2003). Hence new
strategies has been developed to improve the efficacy of the drug and to overcome clinical resistance such as:

- **Leucovorin:** Leucovorin (LV, 5′-formyltetrahydrofolate) is used to increase the intracellular level of CH₂THF necessary for optimal binding of FdUMP to TS (Longley *et al*., 2003). LV when employed with 5-FU, has been observed to increase the cytotoxic effect of the drug in cancer cells both *in vitro* and *in vivo* (Longley *et al*., 2003).

- **Inhibitors of dihydropyrimidine dehydrogenase:** The bioavailability of 5-FU is poor due to the rapid degradation of DHFU by DPD (Diasio & Harris, 1989). Hence, DPD inhibitors, such as 5-chlorodihydropyrimidine (CDHP) and eniluracil are being studied. Reports suggests that eniluracil increased the efficacy of 5-FU from 3% to 94% in rat model (Spector *et al*., 1995).

- **Methotrexate:** Methotrexate (MTX) is the inhibitor of dihydrofolate reductase (DHFR) responsible for the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF) (Gorlick & Bertino, 1999). THF is the precursor of CH₂THF and is necessary for dTMP synthesis. Pretreatment with MTX has showed to increase the antitumor activity of 5-FU which is correlated with increased 5-FU incorporation into RNA (Leyland-Jones & O’Dwyer, 1986).

- **Interferon:** Interferons (IFNs) are the cytokines which imposes a negative regulatory effect on the growth of cells. Reports suggested that IFN-α enhanced the 5-FU mediated DNA damage in colon cancer cells (Houghton *et al*., 1993). Similarly IFN-γ has been found to enhance the activity of 5-FU by upregulating the activities of the 5-FU anabolic enzymes TP and UP (Eda *et al*., 1993). Hence new studies are being conducted to use the
combination of IFN-α and 5-FU/LV as adjuvant therapy for colon cancer treatment (Wolmark et al., 1998).

1.11. Problems of 5-FU Based Chemotherapy

5-FU has many therapeutic advantages including its mild side effects, ability to use in combination with other drugs and easy administration (Mader et al., 1998). The main disadvantage of 5-FU treatment is its limited activity due to frequent resistance in patients. Resistance to 5-FU can arises due to various reasons such as enhancement of drug inactivation, alteration of drug influx and efflux, mutation of the drug target as well as enhancement of anti-survival proteins (Zhang et al., 2008). There are various others factor that may lead to resistance such as increased expression of TS (Van Triest et al., 1999), higher level of deoxyuridine triphosphatase (Grem et al., 2005), overexpression of Bcl-2 & Bcl-XL (Violette et al., 2002), methylation of the MLH1 gene (Arnold et al., 2003), overexpression of Mcl-1 (Shi et al., 2002), over expressions of cell adhesion molecule, etc. Several investigators have tried different mechanism to overcome the resistance such as use of multiple drug combinations, use of an encapsulated formulation, use of DNA microarray to identify the resistance genes, knockdown of Bcl-XL protein levels by small interfering RNA (siRNA), down-regulation of MDR1, MRP1 and LRP expression, etc. but these studies showed very poor clinical outcome due to insufficient knowledge about the mechanism of 5-FU resistance, lack of proper model to study resistance, conflicting knowledge about the action of 5-FU in cells, sample size and wide variation of techniques, etc. (Rougier et al., 1994; de Gramont et al., 2000; Patel et al., 2008; Wang et al., 2011). Hence, inspite of the advances in screening and treatment options to reduce mortality, the prognosis of advanced forms of colon cancer remains very poor (Wyatt & Wilson, 2009). Thus, a better model of 5-FU resistance is required to understand the mechanism of resistance, new
chemotherapeutic regimes to overcome resistance, identification and knockdown of genes responsible for conferring resistance and a better understanding about the action of 5-FU. 1.12.

1.12. Aims and Objectives of the Study

Colon cancer is one of the most deadly forms of cancer with high mortality rate especially in western countries. The molecular mechanism behind tumor origin is still a matter of question. One of the most common forms of colon cancer origin is due to frequent mutation in the APC gene and to make it worse drug resistance is frequently observed in clinics to one of the main regime of chemotherapy-5fluorouracil. Hence, identification of genes responsible for drug resistance is of utmost importance and the mechanism of action of APC becomes of needs to be properly understood.

Chemotherapy still stands as the last line of defense for the treatment of colon cancer in spite of its several side effects and toxicity. Chemotherapeutic drugs target both the normal and the cancer cells, but the effect is mostly seen in the cancer cells due to their high uptake because of the fast dividing property. 5-Fluorouracil is a chemotherapeutic drug mostly used for the treatment of the advanced form of colon cancer. It belongs to the antimetabolites family of drugs and is considered as a suicide inhibitor that works by irreversible inhibition of thymidylate synthase and also by incorporation into DNA and RNA. 5-FU enters the cells using the same mechanism as that of uracil, i.e. by facilitated transport (Wohlhueter et al., 1980). 5-FU after entering the cells is converted into three active metabolites - fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) which disrupts RNA synthesis and the action of thymidylate synthase (TS), ultimately leading to DNA damage. APC is a well established tumor suppressor gene that regulates Wnt signalling and β-catenin signalling, cell-cell adhesion, apoptosis, cell migration, chromosomal instability,
cell cycle control and DNA repair (Kinzler and Vogelstein, 1996; Narayan and Roy, 2003; Aoki and Taketo, 2007; Rustagi, 2007; Brocardo and Henderson, 2008). The role of APC in relation to DNA damage is also well documented. But the involvement of APC in 5-FU mediated cell death or DNA damage is not studied.

Furthermore, drug resistance in colon cancer patients to 5-FU has become a menace making the drug ineffective. Hence identification, delineating mechanism of action and down-regulation of genes responsible for 5-FU resistance is of utmost necessary which needs to be studied. Nowadays use of combination therapy for the treatment of colon cancer has proved effective over traditional chemotherapeutic approaches. However, understanding the mechanism of action of the combinatorial regime in combating colon cancer or overcoming drug resistance is also important. Thus to systematically study the 5-FU resistance, identify gene responsible for these resistance and to develop a novel drug to increase the 5-FU sensitivity, the work is divided into following basic objectives:-

1. **Development and characterization of a 5-FU resistant cell line from a 5-FU sensitive cell line and study the mechanism of action of combinatorial drug to overcome the resistance.**

   - Development of 5-FU resistant colon cancer cell line from 5-FU sensitive colon cancer cells.
   - Characterization of the 5-FU resistant cells.
   - Study the effect of combinatorial drug in 5-FU sensitive and resistance cell lines
   - Elucidate the mechanism of action of the combinatorial drug both in the 5-FU sensitive as well as 5-FU resistant cell line.

2. **Study the mechanism of tumor suppressor protein Adenomatous Polyposis Coli (APC) mediated 5-FU cytotoxicity in colon cancer cells.**
Effect of 5-FU on APC in colon cancer cell lines expressing different variants of APC.

Study the 5-FU mediated apoptosis in colon cancer cell lines expressing different variants of APC.

Study the involvement of the DRI (DNA repair inhibitory) domain of APC in 5-FU mediated cell cytotoxicity.

Study the involvement of LP-BER (Long patch- base excision repair) in 5-FU mediated cell death in colon cancer cells.

Interaction between 5-FU and DRI domain of APC.


Identification and characterization of NECTIN-4 as a 5-FU resistance gene.

Identification of pathway involved in NECTIN-4 mediated action of 5-FU resistance.

Study the localization and role of NECTIN-4 in DNA repair.

Screening and identification of combinatorial drug to overcome 5-FU resistance by down regulating NECTIN-4.