1. Introduction

Cancer is the second leading cause of death in the world. The word cancer came from a Greek word “karkinos” to describe carcinoma tumors by a physician Hippocrates (460–370 B.C). The Roman physician, Celsus (28–50 BC), later translated the Greek term into cancer. Galen (130–200 AD), another Greek physician, used the word oncos (Greek term for swelling) to describe tumors \(^1\).

1.1 Colorectal cancer

Colorectal cancer (CRC) starts in the colon or rectum portion of large intestine in the gastrointestinal (GI) tract \(^2\). CRC is a common malignancy associated with significant mortality. Colorectal carcinogenesis generally occurs in a slow and stepwise process of accumulating mutations under the influence of genetic and environmental factors, the so-called adenoma-carcinoma sequence \(^3\) as depicted in Figure 1.1 \(^4\).

**Figure 1.1 The genetic paradigm of colorectal cancer**

The genetic changes that accompany the stepwise transformation of normal colonic mucosal tissues to carcinoma are depicted in the model. Both mutational inactivation of tumor suppressor genes (light shaded) and activation of oncogenes (dark shaded) are involved. ACF-aberrant crypt foci, APC-adenomatous polyposis coli, COX-2-cyclooxygenase-2, MSH2- MutS protein homolog 2, MLH1- MutL homolog 1, TCF- T cell transcription factor.
1.2 Epidemiology of colorectal cancer

CRC is the third most common cancer worldwide and is the second commonest cause of cancer-related mortality \(^{(5)}\). Almost 55% of the cases occur in more developed regions. There is wide geographical variation in incidence across the world and the geographical patterns are very similar in men and women. The incidence rates vary ten-fold in both sexes worldwide. In India colorectal cancer ranks 5\(^{th}\) in both sexes. There were 64,332 new cases of CRC, accounting for 6.3\% of all cancer in India. There were an estimated 48,603 death from CRC in India (7.1\% of the total number of cancer death). The 5-year prevalence of CRC in 2012 was estimated 86,650 (9.8 CRC survivors per 100,000 population) \(^{(6)}\). According to the data available on Madras Metropolitan Tumour Registry (MMTR) as on 2012–2013, colorectal cancer has crude incidence rate (CIR) of 8.2 per 100,000 and an age-standardized rate (ASR) of 8.4 per 100,000 in males, whereas the CIR and ASR for females are 5.6 and 5.3 per 100,000 respectively \(^{(7)}\).

1.3 Causes for colorectal cancer

CRC risk is increased or decreased by many known factors. It includes age, hereditary factors, environmental and lifestyle risk factors which may play an important role in the development of colorectal cancer. Nonmodifiable risk factors include a personal or family history of colorectal cancer or adenomatous polyps, and a personal history of chronic inflammatory bowel disease \(^{(8)}\). Modifiable risk factors that have been associated with an increased risk of colorectal cancer include physical inactivity, obesity, high consumption of red or processed meats, smoking, and moderate-to-heavy alcohol consumption \(^{(9)}\) (Figure 1. 2).
1.4 Pathogenesis of colorectal cancer

The pathogenesis of CRC is complex and includes genetic and epigenetic alterations. The loss of genomic stability plays a key molecular step in cancer formation \(^{(10)}\). The hereditary cancer syndromes frequently correspond to germ line forms of key genetic defects whose somatic occurrences drive the emergence of sporadic colon cancers \(^{(11)}\).

CRC presents in one of three patterns: inherited, familial, and sporadic. Sporadic colon cancer makes up 80–85% of all cases of CRC and occurs in average risk patients, age 50 and older without obvious predisposing risk factors. Only about 15–20% of all cases are familial and occurs in moderate risk patients with a family history of colorectal adenomas or cancer \(^{(12)}\). The distribution of pattern of CRC is shown in Figure 1.3 \(^{(3)}\).
Colorectal cancer syndromes. Relative number of cases that are considered sporadic, familial and hereditary. FAP: Familial Adenomatous polyposis, HNPCC: Hereditary Nonpolyposis Colorectal cancer.

1.5 Pathogenetic pathways in colorectal cancer

There is significant heterogeneity in the pathogenetic pathways leading to CRC and three major pathways have been proposed. The most common is the chromosomal instable (CIN) group, characterized by an accumulation of mutations in specific oncogenes and tumor suppressor genes. The second is the microsatellite instable group, caused by dysfunction of DNA mismatch repair genes leading to genetic hypermutability. The CpG Island Methylation phenotype is the third group, distinguished by hypermethylation (Figure 1.4) \(^{(13)}\).
The wnt signaling pathway is disrupted by either mutations or deletions of the APC gene or by mutations in the β-catenin gene. Tumor formation can proceed through the CIN pathway, characterized by multiple allelic losses of tumor-suppressor genes and mutations of the oncogene, k-ras. Alternatively, mutations of MMR genes lead to the MSI phenotype as in the Lynch syndrome or with acquired inactivation of the hMLH1 gene. The CpG island methylator phenotype (CIMP), which silences genes through promoter methylation. CIMP can progress through silencing the hMLH1 gene causing the MSI phenotype (CIMP+/MSI+). Alternatively, a variety of tumor-suppressor genes other than hMLH1 can be silenced through promoter methylation (CIMP+/MSI-).

CIN pathway

CIN is the commonest genomic instability accounting for 80–85% of all cases (14). It defines the accumulation of numerical (aneuploidy) or structural chromosomal abnormalities, resulting in karyotypic variability from cell to cell (15) and is characterized by frequent loss-of-heterozygosity (LOH) at tumor suppressor gene loci, telomere stability and the DNA damage response (16).
MSI Pathway

The MSI pathway represents a form of genomic instability involved in the genesis of approximately 15% of sporadic colorectal cancer and >95% of Hereditary Non Polyposis Colorectal Cancer (HNPCC) syndrome. MSI is caused by the inactivity of the DNA Mismatch Repair (MMR) system. MMR system is composed of multiple interacting proteins including the human MutS homologue (MSH) 2, and human MutL homologue (MLH) 1.

CpG island methylator phenotype (CIMP)

CIMP consists of the aberrant hypermethylation of CpG dinucleotide sequences localized in the promoter regions of genes involved in cell cycle regulation, apoptosis, angiogenesis, DNA repair, invasion and adhesion. The promoter hypermethylations cause the loss of gene expression. CIMP is found in approximately 20–30% of CRC.

1.6 Molecular signalling pathways in colorectal cancer

The various molecular signalling pathways involved in colorectal cancer are as shown in Figure 1.5.

APC/Wnt/β-catenin

Adenomatous polyposis coli (APC) participates in the regulation of a myriad of cellular functions including proliferation, differentiation, apoptosis, adhesion, migration and chromosomal segregation. APC serves as a crucial member of the Wnt/β-catenin signaling pathway, which is an important determinant of cell proliferation, differentiation and apoptosis. APC’s ability to regulate cytoskeletal proteins including F-actin and microtubules, thus allowing it to regulate adhesion, migration and mitosis. The Wnt/β-catenin signalling
pathway is also called the canonical Wnt pathway and is essential for controlling intestinal epithelial cell proliferation\(^{(25,26)}\).

Germline mutations in \(APC\) result in FAP or one of its variants, Gardner’s syndrome, attenuated FAP, Turcott’s syndrome, or the flat adenoma syndrome\(^{(27,28)}\). \(APC\) is mutated in up to 70\% of all sporadic colon adenocarcinomas, a high \(APC\) mutation frequency unique to colorectal cancer\(^{(29,30)}\).

**K-RAS**

*Kirstein rat sarcoma (K-RAS)* is a member of the RAS family of genes and present one of the most prominent proto-oncogenes in colon carcinogenesis. The major function of the RAS protein family is to couple growth factors to the Raf-mitogen-activated protein (MAP) kinase, kinase- MAP kinase signal transduction pathway, which leads to the nuclear expression of early response genes\(^{(31)}\).

\(K\)-RAS mutations have been found in 37–41\% of colon carcinomas and appear to occur relatively early in colon-cancer formation\(^{(32,33)}\). 20\% of colorectal tumors with wild type K-RAS bear activating mutations in B-RAF\(^{(34)}\). The complementarity of mutations between K-RAS and B-RAF implies a central role for the RAS RAF-MAPK pathway in the pathogenesis of colorectal cancer\(^{(35,36)}\).

**TP53**

TP53 is located on chromosome 17p and encodes a transcription factor that is a tumor suppressor and master regulator of hundreds of genes involved in DNA metabolism, apoptosis, autophagy, cell cycle regulation, senescence, angiogenesis, immune response, cell differentiation, motility and migration.
Apoptosis is controlled by p53, activates a large number of genes that contribute to apoptosis (37). Several p53-regulated genes (BAX, NOXA, PUMA) enhance secretion of cytochrome-C from the mitochondria into the cytoplasm, which leads to activation of caspases and subsequent apoptosis (37). This is the intrinsic apoptotic pathway initiated by various stress signals that activate p53. In addition to the intrinsic pathway, p53 also regulates several genes (Fas ligands, killer DR receptor) that are involved in the extrinsic apoptotic pathway.

p53 dysfunction is almost universal in human tumors and loss of p53 function is reported in 4–26% of adenomas, 50% of adenomas with foci of carcinoma and 50–75% of CRC (38).

During the progression of CRC pathogenesis, mutations in different cyclin-dependent kinases (CDKs) are also involved. p53, through the AMPK pathway, up-regulates the CDK inhibitor 1A (CDKN1A or p21), which is involved in regulating the cell cycle (39).

The loss of p53 function occurs in the later stages of colorectal tumorigenesis (40). Mutation of p53 coupled with loss of heterozygosity (LOH) of the wild-type allele was found to coincide with the appearance of carcinoma in an adenoma (41,42).

The most common mechanism to disrupt the p53 pathway is through a missense mutation that inactivates its ability to bind specifically to its cognate recognition sequence. However, there are several other ways to achieve the same effect, including amplification of the gene encoding MDM2, a ubiquitin ligase that binds and degrades p53 (43), inactivation of p14/p19ARF that binds and inactivates MDM2 (44), and infection with DNA tumor viruses whose products (such as the E6 protein of the human papilloma-virus) binds and inactivates p53 (45). p53 also interacts with Cyclooxygenase-2 (COX-2), which plays a role in promoting inflammation and cell proliferation in CRC (46).
PIK3CA and PTEN

The PI3ks are a family of lipid kinases that regulate the activity of kinases such as AKT and p70S6K, which ultimately affects cell proliferation, and cell motility that are commonly deregulated in cancer \(^{(47)}\). PI3Ks, is somatically mutated in 15–30\% of CRCs, most commonly in exons 9 (E532K, E545K) and 20 (H1047R) \(^{(48)}\). 10\% of sporadic CRCs exhibit somatic PTEN mutations, and loss of PTEN likely enhances PIP3-mediated activation of AKT, which in turn acts on downstream antiapoptotic factors and the mTOR pathway.

Cyclooxygenase-2 (COX-2)

COX-2 plays a role in the early carcinogenic stages of CRC. It serves as an interface between inflammation and cancer. In response to various external stimuli, such as pro-inflammatory cytokines, bacterial LPS and ROS, COX-2 is transiently elevated in certain tissues. Abnormally elevated COX-2 causes promotion of cellular proliferation, suppression of apoptosis, enhancement of angiogenesis and tumour invasiveness, which accounts for its oncogenic potential. Evidence suggests an interaction between COX-2/PGE2 and the oncogenic APC/β- catenin/TCF pathway in colorectal neoplasia. This transactivation is affected by inhibition of GSK-3β \(^{(49,50)}\). The COX2 gene is overexpressed in 43\% of adenomas and 86\% of carcinomas \(^{(51)}\).

TGF-β Type II Receptor

TGF- β is a multifunctional cytokine that can induce growth inhibition, apoptosis and differentiation in intestinal epithelial cells \(^{(52,53)}\).

TGF-β type II receptor ( \(TGFβIIIR\) or \(TGFBRII\) ) mutation are found in approximately 25\% of CRCs, principally in those with MSI. In addition to MSI-associated tumors, somatic
TGFβIIR mutations are found in 15% of microsatellite stable (MSS) tumors. TGF-β-mediated receptor phosphorylation regulates the function of the SMAD2 and SMAD3 proteins (54).

Figure 1.5 Molecular pathways in CRC

A. Wnt signalling pathway-Loss of APC protein allows for cytoplasmic accumulation of β-catenin, which then complexes with DNA-binding proteins of the TCF/LEF family, and translocates to the nucleus, where it drives transcription of multiple genes with TCF DNA-binding sites involved in tumor growth and invasion. B. K-RAS (MAPK pathway)- Mutation in KRAS result in constitutive activation of the signalling cascade resulting in uninhibited cellular and tumor growth. C. TGFBI/II pathway-Mutation in TGFBI/II altered the SMAD dependent signalling, inhibits effects of TGFB and suppressed apoptosis.
D.p53 pathway – p53 mutation increases the CDK inhibitor increases the level of Bcl-2 with loss of cell cycle which lead to cell proliferation.

**Telomeres**

Telomeres are hexameric DNA repeats (TTAGGG in humans) that protect the ends of eukaryotic chromosomes from fusing and breaking during segregation. A portion of telomeric DNA is lost after each round of DNA replication due to the inability of DNA polymerase to completely synthesize the 3’ end of chromosomes. Cells with sufficiently shortened telomeres are targeted for senescence and apoptosis by DNA damage checkpoints. Cells that survive the checkpoint activate telomerase, which elongates telomeres ($^{55,56}$).

Telomere shortening results in aberrant crypt foci, adenomas and gastrointestinal tumors ($^{55,56}$). 77–90% of CRCs harbor shorter telomeres, compared to adjacent normal tissue, but increased telomerase activity has also been reported ($^{57–63}$). Figure 1.6 depicts the telomeres in normal and cancer cells ($^{64}$).

**Figure 1.6 Telomeres in normal and cancer cells**
Cells encountering oncogenic signals are exposed to telomeric replication stress, which leads to stochastic telomere attrition and telomere dysfunction in cells that lack telomerase activity. In normal somatic cells, telomere dysfunction results in cellular senescence, thereby preventing malignant cancer progression. In contrast, telomere dysfunction is suppressed in cells with high telomerase activity allowing these cells to continue proliferating. Similarly, in cells with compromised senescence responses, telomere dysfunction generates chromosomal instability, an event that is associated with reactivation of telomerase and malignant cancer progression.

1.7 Stages of colorectal cancer

The staging system most often used for colorectal cancer is the American Joint Committee on Cancer (AJCC). CRC are classified according to local invasion depth (T stage), lymph node involvement (N stage) and presence of distant metastases (M stage). These stages are combined into an overall stage definition, which provides the basis for therapeutic decisions.\(^{(65)}\).

Numbers or letters after T, N, and M provide more details about each of these factors. Higher numbers mean the cancer is more advanced. Once a person’s T, N, and M categories have been determined, usually after surgery, this information is combined in a process called stage grouping and an overall stage of 0, I, II, III, or IV is assigned. The five stages of CRC is shown in Table 1.1\(^{(66)}\) and Figure 1.7\(^{(67)}\).

**Table 1.1 TNM Classification and Duke's Classification**

<table>
<thead>
<tr>
<th>Stages</th>
<th>T</th>
<th>N</th>
<th>M</th>
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<td>B2</td>
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<td>M0</td>
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<td>Any T</td>
<td>Any N</td>
<td>M1</td>
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Stage 0 – Cancer cells are located only in the inner lining of the colon or rectum. Typically, this is confined to the surface of a polyp (a growth that protrudes from a mucous membrane). It is also known as carcinoma in situ.

Stage 1 – Cancer cells have spread from the inner lining into the middle layers of the muscular wall of the colon or rectum.

Stage 2 – Cancer has spread to the outside surface of the colon or rectum and may involve nearby tissues but not the lymph nodes.

Stage 3 – Cancer involves the nearby lymph nodes.

Stage 4 – Cancer has spread to other distant parts of the body, such as the liver or lungs \(^{(67)}\).

**Microscope appearance**

Microscopically, the tumours may be classified into three grades \(^{(68)}\).
Grade 1 or Low grade – typically means that cancer is slow growing, also known as well differentiated.

Grade 2 or Moderate grade – also known as intermediate differentiation.

Grade 3 or High grade – means that cancer is faster growing, also known as poorly differentiated (68).

1.8 Apoptosis and cancer

Apoptosis is programmed cell death which is essential for development and survival of living organisms. It is a sequentially regulated suicidal programme where cells activate certain enzymes which dissolve their own nuclear component and various protein component of nucleus and cytoplasm. Disturbance of this regulatory pathway may lead to various diseases like autoimmune diseases, neurodegenerative diseases and cancers (69). There are two major apoptosis signalling pathways. The death receptor (extrinsic) pathway and the mitochondria (intrinsic) mediated pathway (Figure 1.8) (69).

Extrinsic pathway

The extrinsic pathway is initiated by cell surface expressed death receptors of the tumor necrosis factor superfamily. The apoptosis is initiated by cytokines, such as tumor necrosis factor-α (TNF α), Fas ligand (FasL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (70). Once the receptor is activated by Fas ligand, receptors oligomerize, recruit intracellular adaptor proteins and form scaffolding complexes, while FADD is recruited for Fas signalling (71). The complete ligand-receptor-adaptor protein complex is known as the Death-Inducing Signalling Complex (DISC). The complexes recruit one or more members of the caspase family of cell death protease, classically caspase-8. Cleavage of caspase-8 leads to the formation of an active enzyme comprising p20 and p10 heterotetramer. This activated initiator caspase cleaves downstream effector caspases, in
particular caspase-3. Caspase-3 then cleaves a large number of intracellular substrates, now numbering ~400 which culminate the morphological changes of apoptosis (72).

**Figure 1.8 Intrinsic and Extrinsic pathway**

![Intrinsic and Extrinsic pathway diagram](image)

The intrinsic pathway is initiated after mitochondrial damage and occurs in response to diverse apoptotic stimuli including many toxic chemicals, DNA damaging agents, growth factor withdrawal and irradiation. The extrinsic pathway occurs following stimulation of cell surface death receptors by CD95L, TNF or TRAIL (TNF-related apoptosis-inducing ligand).

**Intrinsic pathway**

The intrinsic pathway is initiated by anticancer drugs, growth factor withdrawal, hypoxia, or via induction of oncogenes. These stimuli induces permeabilization of the outer mitochondrial membrane and activates the mitochondrial pathway. The mitochondrial pathway is engaged by the release of apoptogenic factors like cytochrome-C from the
mitochondrial intermembrane space into the cytosol. This release of cytochrome-C into the cytosol triggers caspase-3 activation through formation of the cytochrome-C/Apaf-1/caspase-9-containing apoptosome complex \(^{(73)}\).

Mitochondria-mediated apoptosis includes downstream of ROS production and upstream of caspase activation and is regulated by Bcl-2 family proteins. The Bcl-2 family of proteins consists of antiapoptotic proteins (Bcl-2, Bcl-xL and Mcl-1), as well as a number of pro-apoptotic molecules (Bax, Bad and Bim), whereas overexpression of the anti-apoptotic protein Bcl-2, blocks mitochondrial outer membrane permeabilization and inhibits apoptosis\(^{(74,75)}\).

**Caspase and apoptosis**

Caspase belong to a group of enzymes known as cysteine proteases and main executors of the apoptotic process \(^{(76)}\). It exists within the cell as inactive pro zymogens and can be cleaved to active enzymes following the induction of apoptosis. Induction of apoptosis via death receptors typically results in the activation of an initiator caspase such as caspase-8 or caspase-10. These caspases can then activate other caspases in a cascade. This cascade eventually leads to the activation of the effector caspases, such as caspase-3 and caspase 6. These caspases are responsible for the cleavage of the key cellular proteins, such as cytoskeletal proteins, that leads to the typical morphological changes observed in cells undergoing apoptosis \(^{(77)}\).

**Reactive oxygen species and apoptosis**

Reactive oxygen species (ROS) are produced as a by-product of cellular metabolic pathways and function as a critical second messenger in a variety of intracellular signalling pathways. ROS play an important role in apoptosis induction under both physiologic and pathologic conditions. ROS are widely generated in biological systems. Consequently
humans have evolved antioxidant defense systems that limit their production. Intracellular production of active oxygen species such as \( \cdot \text{OH}, \cdot \text{O} \text{2} \text{–} \) and \( \text{H}_2\text{O}_2 \) is associated with the arrest of cell proliferation. Similarly, generation of oxidative stress in response to various external stimuli has been implicated in the activation of transcription factors and to the triggering of apoptosis \(^{(78)}\).

**Figure 1.9 ROS generation in physiological condition**

The respiratory chain is represented showing the sites of production of reactive oxygen species (ROS) directed toward the intermembrane space (from complex III) or toward the matrix (from complexes I, III and IV), where they can be detoxified by enzymes such as manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPX) or catalase. The permeability transition pore complex (PTPC) is a polyproteic channel involved in the control of matrix homeostasis. It plays a role in ADP/ATP translocation. It is regulated by Bcl-2, membrane parameters, and matrix parameters (pH, Ca\(^{2+}\), Mg\(^{2+}\), volume and redox state) \(^{(78)}\).
1.9 Treatment

Treatment varies depending on the type of cancer and how far it has spread. Treatment of colorectal cancer depends on the location, size and extent of cancer spread, as well as the health of the patient ($^{79,80}$).

Surgery

Surgical treatment is to remove the primary tumor together with lymphnode following the course of the main arterial blood supply and any removable adjacent organ that have been directly invaded ($^{79,80}$).

Radiation therapy

Radiation therapy uses high-energy rays (such as x-rays) or particles to destroy cancer cells. It may be used to try to kill any cancer cells that may have been left behind and helps to control cancer in people who are not healthy enough for surgery or to ease (palliate) symptoms in people with advanced cancer causing intestinal blockage, bleeding, or pain. It also helps to treat cancer that has spread to other areas, such as the bones or brain ($^{79,80}$).

Chemotherapy

The anticancer drugs are used for chemotherapy treatment. The different ways of administration of chemotherapy treatment are Systemic chemotherapy, Regional chemotherapy and Hepatic artery infusion. Chemotherapy may be used at different times during treatment for colorectal cancer ($^{79,80}$).
**Adjuvant chemotherapy**

Chemotherapy can be given after surgery. The goal is to kill any cancer cells that might have been left behind at surgery because they were too small to view, as well as cancer cells that might have escaped from the main tumor and settled in other parts of the body. This helps to lower the chance for recurrence of cancer\(^{(79,80)}\).

**Neoadjuvant chemotherapy**

Chemotherapy is given (sometimes with radiation) before surgery to try to shrink the cancer cells and make surgery easier\(^{(79,80)}\).

**Chemotherapy for advanced cancers**

Cancers that have spread to other organs, such as the liver, chemotherapy can also be used to help shrink tumors and relieve symptoms. Although it is not likely to cure the cancer, it often helps people live longer\(^{(79,80)}\).

**Drugs used to treat colorectal cancer**

The most commonly used chemotherapy drugs utilized to treat colorectal cancer are fluorouracil (5-FU, Adrucil®), folinic acid (Leucovorin®, which is added to increase the effectiveness of 5-FU), oxaliplatin (Eloxatin), irinotecan (Camptosar®, CPT-11), capecitabine (Xeloda®) (this is an 5-FU in pill form) and raltitrexed (Tomudex®) (may be used instead of 5-FU for patients with advanced colorectal cancer who are unable to tolerate 5-FU)\(^{(79,80)}\).

**Combination therapy**

Chemotherapy combinations are used to treat advanced or metastatic colorectal cancer which include

1) FOLFOX: folinic acid (Leucovorin), fluorouracil (5-FU, Adrucil), oxaliplatin (Eloxatin)
2) FOLFIRI: folinic acid (Leucovorin), fluorouracil (5-FU, Adrucil), irinotecan (Camptosar, CPT-11)

3) FOLFOX together with bevacizumab (Avastin®) (a targeted biological therapy) as first or second choice therapy

4) FOLFIRI taken together with bevacizumab

5) Capecitabine (Xeloda) taken instead of 5-FU combination treatments. In exceptional circumstances, raltitrexed (Tomudex) may be taken instead of 5-FU for people who can’t tolerate 5-FU. Cetuximab (Erbitux®) is another type of targeted biological therapy, which may be taken alone, or in combination with irinotecan if previous chemotherapy treatments have failed (79,80).

**Targeted therapy**

Targeted therapy is a treatment that targets the cancer’s specific genes, proteins, or the tissue environment that contributes to cancer growth and survival. This type of treatment blocks the growth and spread of cancer cells while limiting damage to healthy cells (79,80).

**Anti-angiogenesis therapy**

Anti-angiogenesis therapy is a type of targeted therapy. It is focused on stopping angiogenesis. Bevacizumab is a type of a monoclonal antibody used in anti-angiogenesis therapy called (79,80).

**Epidermal growth factor receptor (EGFR) inhibitors**

An EGFR inhibitor is a type of targeted therapy. Researchers have found that drugs that block EGFR may be effective for stopping or slowing the growth of colorectal cancer. Cetuximab and panitumumab are monoclonal antibodies that block EGFR (79,80).
1.10 5-Fluorouracil

5-FU belongs to the family of drugs called the antimetabolites. It is a heterocyclic aromatic organic compound with a structure similar to that of the pyrimidine molecules of DNA and RNA. It is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen (Figure 1.10). It rapidly enters the cell using the same facilitated transport mechanism as uracil (81).

Figure 1.10. 5-Fluorouracil

5-Fluorouracil (FU) has been the treatment of choice for both advanced colon cancer and adjuvant therapy for the early stage of disease for the past 40 years (82). The main mechanism of 5-FU activity involves inhibition of thymidylate synthase (TS), which is the rate-limiting enzyme in the synthesis of thymine nucleotides (83,84). The response rates for 5-FU-based chemotherapy as a first-line treatment for advanced colorectal cancer are only 10–15% (85).
These are often associated with deleterious side-effects caused by inadvertent drug-induced damage to healthy cells, tissues and multidrug resistance. Moreover, these drug need to penetrate target cells but cancer cells can develop resistance by cellular changes that include increased expression of multi-drug resistant proteins, altered interactions between the drug and its target, an increased ability to repair DNA damage, and defects in the cellular machinery that mediate apoptosis. The development of a new class of anticancer drugs that lack the toxicity of conventional chemotherapeutic agents which are unaffected by common mechanisms of chemoresistance would be a major advancement in cancer treatment.

1.11. Antimicrobial peptide

AMPs are endogenous polypeptides produced by multicellular organisms in order to protect a host from pathogenic microbes. These peptides are produced by several species including bacteria, insects, plants, vertebrates and they have been recognized as ancient evolutionary molecules that have been effectively preserved in mammals. AMP showed broad spectrum of antimicrobial activities, anticancer and antiviral activities.

AMPs are generally defined as peptides of less than 100 amino acid residues with an overall positive charge (generally +2 to +9), imparted by the presence of multiple lysine and arginine residues and a substantial portion (≥30% or more) of hydrophobic residues. They are amphipathic molecules of variable length, sequence and structure, serve an important role as effector molecules in inflammation, immune activation and wound healing. In general, when AMPs are folded in membrane mimetic environments, one side of AMPs is positively charged (mainly due to lysine and arginine residues) and the other side contains a considerable proportion of hydrophobic residues.
AMP exhibited a broad spectrum of activity, such as antibacterial, antifungal, antiviral and anticancer activity \(^{(97)}\). It exerts anticancer activity by a membranolytic mode of action or via interaction with intracellular targets, or through use of a combination of the two \(^{(98)}\). Their anticancer activity depends on cancer types. The interactions between AMPs and cancer cells influence apoptotic or other pathways and can result in cell death \(^{(99)}\).

**Magainin II**

Magainin II belongs to a family of antimicrobial peptides (Figure 1.11) and was originally isolated from the skin of the African clawed frog, *Xenopus laevis* \(^{(89)}\). These peptides are important components in the innate host defense response in a wide range of organisms, from bacteria to humans \(^{(100)}\). It has an amphipathic α-helical structure with separate cationic and hydrophobic faces comprising 23 amino acid residues that enables it to target both negatively-charged and nonpolar lipid cell membranes where it can form ion-permeable channels in the membrane, leading to depolarization, irreversible cytolysis, and finally to cell death \(^{(100–102)}\).

**Figure 1.11 Magainin II**
1.12 Cell lines

Colorectal cancer cell line

Human colorectal cancer cell lines are used widely to investigate tumor biology, experimental therapy and biomarkers\(^\text{(103)}\).

**Figure 1.12 Colorectal cancer cell lines and normal cell lines**

<table>
<thead>
<tr>
<th>a. HT-29</th>
<th>b. COLO 205</th>
<th>c. COLO 320 DM</th>
<th>d. HCT-15</th>
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</table>

a-e-colorectal cancer cell lines, f and g-normal cell lines

**HT-29 CELL LINE**

The HT-29 (human colorectal adenocarcinoma) cell line was derived from a patient with colorectal cancer in 1964. Cells secretory component of HT29 cell lines are IgA, carcinoembryonic antigen (CEA), transforming growth factor beta binding protein and mucin. HT-29 cells are negative for CD4, but there is cell surface expression of galactose...
ceramide (a possible alternative receptor for HIV). c-myc, K-ras, H-ras, N-ras, Myb, sis and fos oncogenes in the cell line and p53 is mutated in codon 273 \(^{(104)}\).

**COLO 205 CELL LINE**

COLO 205 cell line was isolated from 70-year-old Caucasian male with metastatic carcinoma of the colon in 1975. CEA, keratin, interleukin 10 are the cell secretory component. The cells are CSAp negative (CSAp-) and p53 mutated cell line \(^{(105)}\).

**COLO 320 DM CELL LINE**

COLO 320 DM cell line was isolated from 55 year old Caucasian female with metastatic colorectal adenocarcinoma. The cellular products are serotonin, norepinephrine, epinephrine, adrenocorticotropic hormone (ACTH) and parathyroid hormone .The cell lines are mutated with p53 \(^{(106)}\).

**HCT-15 CELL LINE**

The HCT-15 (human colorectal adenocarcinoma) cell line was derived from a male patient with metastatic colorectal cancer. The cellular products are CEA and keratin. The cell line are mutated with p53 \(^{(107)}\).

**HCT 116 CELL LINE**

The HCT 116 (human colorectal carcinoma) cell line was derived from a male. The cell line is positive for transforming growth factor beta 1 and beta 2 expression and shows a mutation in codon 13 of the ras protooncogene. The cellular product is carcinoembryonic antigen. It is used as *in vitro* model of colon cancer to study wild p53 in colon cancer \(^{(108)}\).
Normal cell line

MDCK CELL LINE

The MDCK cell line was derived from a kidney of an apparently normal adult female Cocker Saniel on September, 1958, by S.H. Madin and N.B. Darby. This line is hyperdiploid and there is a bi-modal chromosome number distribution. There are no consistent identifiable marker chromosomes. One normal X chromosome is present in most spreads. The cells are positive for keratin by immunoperoxidase staining. MDCK cells have been used to study processing of beta amyloid precursor protein and sorting of its proteolytic products \(^{(109)}\).

VERO CELL LINE.

The Vero cell line was initiated from the kidney of a normal adult African green monkey on March 27, 1962, by Y. Yasumura and Y. Kawakita. This cell line can be used for the detection of verotoxin., efficacy testing, media testing and mycoplasma testing. This cell line is a suitable transfection host and for detection of virus in ground beef \(^{(109)}\).