Updated pathological overview of colorectal lesions

Maha Ahmed Shehab *, Osama H. Labib, Magdy I. Ahmed, Aziza E. Abdelrahman
Pathology Department, Faculty of Medicine, Zagazig University, Egypt.
Corresponding author: Maha Ahmed Shehab
Email: drmahashehab12@gmail.com

Abstract

Background: Colorectal cancer (CRC) is a common and lethal disease. CRC incidence and mortality rates vary markedly around the world. Globally, CRC is the third most commonly diagnosed cancer in males and the second in females, with 1.65 million new cases and almost 835,000 deaths in 2015. Rates are substantially higher in males than in females. The genetic and environmental risk factors for colorectal cancer are briefly discussed in this review article. Familial and genetic variables, as well as lifestyle-related and environmental factors, are all known risk factors for colorectal cancer. Lifestyle factors are important because they have the potential to improve our understanding of the disease. Physical inactivity, obesity, smoking and alcohol consumption can also be addressed through therapeutic interventions. We also made efforts to systematize available literature and data on epidemiology, diagnosis, type and nature of symptoms and disease stages. Further study of colorectal cancer and progress made globally is crucial to inform future strategies in controlling the disease’s burden through population-based preventative initiatives. Cancer stem cells (CSCs) are responsible for tumor initiation, metastasis and treatment resistance leading to disease relapse following surgery and/or chemotherapy.

Key words: Colorectal cancer, pathogenesis, risk factors

Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer death worldwide, accounting for 9.2 percent of female cancer deaths and 10.0 percent of male cancer deaths. The prevalence of CRC varies from country to country, with more developed countries reporting a higher proportion of patients (55 percent) (1). According to GLOBOCAN 2020 data there were over 1.9 million new cases and 935,000 deaths in 2020, accounting for approximately one out of every 10 cancer cases and deaths worldwide. Colorectal cancer is the third most common cancer in the world, but it is the second most lethal in terms of mortality (2).
By 2030, it is expected that the global burden of CRC will be increased by 60%, with over 2.2 million new cases and 1.1 million annual deaths. This increase is expected because of economic development and environmental changes, which include a more sedentary lifestyle, increased obesity, increased consumption of processed foods, alcohol, and meat, as well as increased overall longevity (3).

I. Epidemiology of colorectal carcinoma

Colorectal cancer (CRC) is the fourth cause of cancer death world-wide. About 55% of patients are reported in more developed counties compared to 40% in developing countries (1).

Colon cancer incidence rates per 100,000 people are highest in Europe, Australia, and New Zealand, and lowest in Africa. Rectal cancer rates have a similar regional distribution, although rates in Eastern Asia rank among the highest. Rates of both colon and rectal cancer tend to be low in Africa and South Central Asia (2).

In the United States and western countries, up to 8% of those being diagnosed with the disease are under the age of 40, but in many Middle-Eastern countries up to 23% are below 40, according to a study by the American Cancer Society (AAS) published in 2009 (4).

In Egypt, the incidence rate of colorectal cancer is 5.1% in males and 4.7% in females. It represents about 33.8% of all Gastrointestinal Tract Tumors (GIT) and 6.26% of total malignancies (1).

Egypt has one of the highest rates of early onset cancer (CRC) in the world as 35% of 1,600 Egyptian patients were under 40. Young patients have threefold increased risk to die within 5 years compared to those who have CRC over the age of 50 (1).

Most diets in Egypt are rich in meat and fat, with poor fibers and cereals. As well as the lifestyle is changed to have excessive amount of daily sitting with niggling aches and pains due to lack of exercise and sleep apnea (5).

There are about 694,000 individual worldwide with CRC each year, according to World Health Organization (WHO) figures. The disease can be easily cured if diagnosed and treated. (6).
II. Risk Factors of Colorectal Carcinoma (CRC)
There are many factors which lead to the development of CRC, some of them are modifiable while others are non-modifiable:

A- Modifiable risk factors:

1) Obesity
   Obesity raises the risk of CRC in both men and women, but the association is stronger in men than women, visceral fat or abdominal obesity which is more common in men than women seem to be of greater concern than subcutaneous fat obesity in increasing the risk of CRC (7).
   Insulin and IGF1 have been shown to stimulate growth of colorectal epithelial and carcinoma cells. Tumorigenic effects of insulin may be direct through mediation of the insulin receptor in target cells, or indirect through influencing sex hormones and sex hormone reproduction (6).

2) Physical inactivity
   The risk of colorectal cancer (CRC) is associated with physical activity, rather than diet or lifestyle factors. The mechanisms by which physical activity reduces the risk of CRC are still not fully understood but some theories suggest that physical activity modifies the gastrointestinal transmit time (8).

3) Certain types of diets
   Frying, grilling, broiling, or cooking meat at high temperatures can lead to the formation of mutagenic and carcinogenic heterocyclic amines. Those substances can induce genetic alterations and form DNA adducts characteristic of colorectal tumors (9).
   The heme iron content of meats might contribute to colorectal neoplasia. The greater abundance of heme in darker meats than in white meats could increase the risk of CRC. Fish and poultry are alternative sources of protein and have been shown to reduce the risk. (10).
   Fibers can be classified according to their water solubility, which affects their action in the body. Bran fiber is insoluble; fruit and vegetable fiber tend to be more soluble. High consumption of fruits was associated with a 32% reduction in risk of CRC, while high intake of cereal fiber did not lower risk of CRC (10).
   Their protective effect of high fiber diet against CRC could be explained by the fact that their presence in meals contributes to lower transit time through the gastrointestinal tract, reducing the concentrations of intestinal carcinogens because of increased stool mass, diluting colonic contents, and enhancing bacterial fermentation, which leads to increased production of short chain fatty acids, Dietary fiber has also been proved to have an anti-inflammatory function, lowering the production of interleukin 6, tumor necrosis factor α, cyclooxygenase 2, and gene expression of inducible nitric oxide synthase (11).
Calcium and vitamin D can reduce the risk of CRC through mechanisms that decrease cell proliferation or promote cell differentiation (11).

4) **Smoking**

Smoking increases the risk for CRC. The Inhaled chemicals and toxins invite free radicals to damage DNA and mutate healthy cells. Free radicals cause the development of precancerous polyps in the large intestine, which can become cancerous and eventually cause CRC (12).

5) **Heavy alcohol use**

Alcohol intake impairs the colonic mucosal barrier and is a known risk factor for development of colorectal cancer (CRC). Alcohol itself is not a carcinogenic but metabolism of alcohol leads to formation of metabolites that can promote carcinogenesis through several mechanisms including induction of DNA damage (13).

**B. Non-modifiable risk factors**

1. **Aging**

The risk of CRC increases with old age. Although it could happen with young age, it's much more common after age 50 (14).

2. **Previous history of colorectal polyps or colorectal cancer**

The risk of developing colorectal cancer (CRC) increases if there is a history of polyps in the colon or rectum, says the American Cancer Society (ACS) (15).

3. **A family history of CRC**

People with a history of CRC in a first-degree relative are at increased risk. The risk is even higher if that relative was diagnosed with cancer when he was younger than 45 years of age or if more than one first-degree relative is affected (16).

4. **Genetic mutations**

The risk of developing hereditary non-polyposis CRC increases with age. About 5% of people who develop CRC have inherited mutations that cause family cancer syndromes, such as Lynch syndrome and familial adenomatous polyposis (17).

5. **Racial and ethnic background**

Variations in survival within a nation can be race and ethnicity dependent. In the US, African Americans and Native Americans have a higher incidence of CRC and suffer lower survival among all stages of CRC (3).

6. **Type 2 diabetes**

People with type 2 (non-insulin dependent) diabetes have an increased risk of developing colorectal cancer (CRC). Diabetes may play a role in the development and growth of cancer, according to research published in the Journal of Clinical Oncology (18).
Factors decreasing the risk of CRC

1) Screening
   Early detection of CRC by screening test before the appearance of signs and symptoms can provide an early successful treatment (19).

2) Eating lots of vegetables, fruits, and whole grains.
   Diets that include lots of vegetables, fruits, and whole grains have been linked to a decreased risk of colon or rectal cancer (20).

3) Exercise.
   Increasing physical activity helps reducing the risk of CRC (8).

4) Weight control.
   Eating healthier and increasing the physical activity can help in control the body weight and consequently reducing the risk of CRC that is related to obesity and physical inactivity (21).

III. Premalignant lesions of the Colon

A. Colorectal adenoma:
   Premalignant lesions, such as adenomas, are frequently found as general indicators of their presence. About 70% of all polyps found in the colon are caused by this type of growth. As previously stated, conventional adenomas (tubular, villous, and tubulovillous) are classified as follows: sessile serrated adenoma, and traditional serrated adenoma (22).

   1) Tubular adenomas
      Tubular adenomas are small (less than ½ inch). They are the most common histological subtype, constituting approximately 65–80% of all polyps removed. They are most often pedunculated and have the histological appearance of branched tubular glands with less atypia than villous adenomas (23).

   2) Villous adenomas
      It is estimated that only 5-10 percent of all neoplastic polyps are villous adenomas. They are more commonly sessile and have cauliflower-like protrusions that appear as long, finger-like projections when viewed under the microscope. Villous adenomas are more likely than other types of adenomas to exhibit severe atypia or dysplasia (23).

   3) Tubulo-villous adenomas:
      They account for 10–25% of the total number of neoplastic polyps. Both tubular and villous adenomas have elements that can be seen under a microscope (24).

   4) Traditional serrated adenomas:
      They make up only 0.56 to 1.9 percent of all neoplastic polyps, depending on their size. Adenomas with traditional serrated growth patterns have a villiform growth pattern with slit-like serrations and are composed of pseudostratified columnar epithelial cells with eosinophilic cytoplasm and dark,
penicillate, dysplastic-like nuclei, as opposed to adenomas without traditional serrated growth patterns (25).

5) Sessile serrated adenomas:

Neoplastic polyps with sessile serrated adenomas account for 1.7 percent of all neoplastic polyps. Because of this, it has a sessile or flat appearance. Similarly, to hyperplastic polyps, the lining exhibits sawtooth serrations of the epithelium as well as an abundance of mucin (25). If left untreated, up to 10% of these polyps can develop into cancer within a 10-year period if they are not detected or treated (26).

B. Chronic inflammatory bowel disease:

For the purposes of this definition, "inflammatory bowel disease" (IBD) is a group of chronic, recurrent, and incurable diseases of unknown etiology that affect the gastrointestinal tract and can ultimately result in the destruction of normal intestinal architecture. The etiopathogenesis is complex and multifactorial, with a significant influence associated with genetic susceptibility as well as immunological and environmental factors, all of which interact and result in dysregulation of the mucosal immune system of the gut, resulting in uncontrolled inflammation (27).

In terms of clinical manifestations, there are two main subtypes of IBD: Crohn's disease (CD), in which inflammation can develop anywhere in the gastrointestinal tract from the mouth to the anus, and ulcerative colitis (UC), in which inflammation is limited to the colonic mucosa (28).

CRC is more common in patients with long-standing ulcerative colitis and Crohn's disease (CD) than in the general population. Even though IBD-associated CRC accounts for approximately 2 percent of all CRC cases, the mortality rate associated with CRC in IBD patients ranges between 10 and 15 percent (29).

Cytokines such as TNF-α, IL-6 and IL-1 secreted in response to infection and inflammation during IBD may influence several stages of cancer initiation and progression. Tumor cells can react to host-derived cytokines that promote growth, attenuate apoptosis, and facilitate invasion and metastasis (29).

Tumor necrosis factor-alpha (TNF-alpha) is a protein that promotes tumor progression and angiogenesis while also maintaining chronic inflammation. TNF-alpha stimulates the activation of signaling pathways and transcription factors such as NF-B, among others. In turn, NF-B is known to play a significant role in the development of CRC and colitis associated carcinoma (CAC) tumors (27).
C. Hereditary syndromes:
Including:
1. Polyps of the gastrointestinal tract, as well as hyperpigmentation around the lips, genital mucosa, buccal mucosa, feet, and hands, are all associated with Peutz-Jeghers syndrome (30).
2. Typically, juvenile polyps are characterized by an abundance of edematous lamina propria with inflammatory cells and cystically dilated glands lined by cuboidal to columnar epithelium in the subcutaneous tissue. In many cases, the polyps have a frond-like growth pattern with less stroma, less dilated glands, and more proliferative smaller glands than in other cases (31).
3. Familial adenomatous polyposis (FAP) syndromes are caused by a mutation in the APC gene that is passed down through families. According to Vasen et al. (32), there are syndromes caused by a germline mutation in the APC gene, which results in FAP. The following are examples of these syndromes:
   - Gardner syndrome is characterized by colonic polyposis, which is characteristic of FAP, as well as osteomas (bony growths, which most commonly occur on the skull and mandible), dental abnormalities, and soft tissue tumors, among other things.
   - Turcot syndrome is characterized by colonic polyposis that is characteristic of FAP, as well as tumors of the central nervous system (medulloblastoma).
   - When compared to classic FAP, attenuated adenomatous polyposis coli (AAPC) is characterized by fewer colonic polyps (average number of polyps, 30-35) and a shorter duration of infection (32).

IV. Molecular basis of Colorectal Carcinoma (CRC)
Approximately 65% of CRC cases are sporadic with no family history or apparent genetic predisposition. The remaining cases are familial, arising from moderately penetrant inherited susceptibility, possibly interacting with environmental factors (33).

A. Sporadic colorectal cancer pathogenesis:
The progression from colorectal adenoma to carcinoma is caused by three major pathways: Chromosomal instability, microsatellite instability and cytosine-phospho-guanine (CpG) island methylator phenotype (CIMP) pathway (34).
CRC development can be divided into two distinct morphologic multistep paths. 80 percent of CRCs are caused by the suppressor pathway, which is characterized by chromosomal instabilities. The serrated adenoma route is likely responsible for the remaining 15 to 20 percent of CRCs, and this pathway is commonly linked to epigenetic silencing of the mismatch repair gene MLH1. When tumor suppressor genes are significantly hypermethylated in CpG islands, it means that the genes are inactivated, and this promotes tumor formation. The CpG island methylator phenotype (CIMP) may be a precursor to the serrated adenoma pathway. (34).
1. Chromosomal Instability (CIN) pathway

CIN refers to a high rate of gains or losses of whole, or large portions of chromosomes. Chromosomal instability is observed in 85% of adenoma-carcinoma transitions and is the most common type of genetic instability in the disease (34).

Colorectal cancers (CIN) are caused by the accumulation of mutations in specific oncogenes. Mutations include loss of adenomatus polyposis coli APC, tumor protein p53 loss and loss of heterozygosity for the long arm of chromosome 18q (34).

Inactivation of APC occurs as the first event, followed by oncogenic KRAS mutations in the adenomatous stage. The transition to malignancy begins with deletion of chromosome 18q and inactivation of tumor-suppressor gene TP53 on chromosome 17p (34).

2. Microsatellite Instability (MSI) pathway

Microsatellite instability is caused by defects in DNA mismatch repair proteins. It manifests as an abnormal (increased or decreased) length of microsatellite repeats. The presence of instability can be inherited or sparkle multiple-generational problems (35).

The National Cancer Institute guidelines for MSI testing recommend a panel of five microsatellite loci, including three dinucleotide repeat markers (D2S123, D5S346, D17S250) and two mononucleotide repeat markers (BAT 25 and BAT 26). This panel is known as the Bethesda panel (35).

Microsatellite stable (MSS) status is established when none of the five markers shows instability. Sporadic MSI-H CRCs, as well as those with Lynch syndrome, are characterized by right-sided location, mucinous or medullary type, and better prognosis (36).

3. The cytosine-phosphate-guanine (CpG) Island methylator phenotype (CIMP) and the serrated pathway

About 20% of cancer cases are related to the CIMP pathway. Hypermethylation of numerous promoter cytosine-phospho-guanine CpG islands, leading to loss of gene expression, is the mechanism behind many types of cancer (37).

No consensus is reached regarding the optimal panel of CpG sites for CIMP determination. The classic panel consists of CpG sites in (MLH1, cyclin-dependent kinase inhibitor 2A (CDKN2A, p16), and (methylated in tumors 1, 2, and 31). CIMP can classified in two groups (CIMP positive (CIMP+) and CIMP negative (CIMP−)) or in three groups (CIMP-high (CIMP-H), CIMP-low (CIMP-L), and CIMP-negative (CIMP-N) (38).

The progression of serrated adenoma into serrated carcinoma has been described. This is the less common molecular pathway of sporadic colorectal cancer (CRC) called the serrated pathway. BRAF
mutation and gene promoter hypermethylation are the target mechanisms in this pathway (38).

B. Hereditary colorectal cancer pathogenesis:

1. **Hereditary non-polyposis colorectal cancer (HNPCC)**

   Hereditary non-polyposis colorectal cancer or Lynch syndrome is the most common inherited colon cancer syndrome. It is caused by a germline mutation in one of several mismatch repair (MMR) genes. HNPCC defects in DNA MMR genes leads to microsatellite instability (MSI) (39).

   It is characterized by an increased risk for CRC and endometrial cancer as well as a fewer risk of some other cancers (ovary, gastric, small intestine, hepatobiliary tract, upper urinary tract, brain and skin) (39).

2. **Familial adenomatous polyposis (FAP)**

   Familial adenomatous polyposis (FAP) accounts for approximately 1% of all colon and rectum cancers (CRCs). FAP is an autosomal dominantly inherited condition where hundreds to thousands of adenomas develop throughout the colon (40).

   Familial adenomatous polyposis is a result of germline mutations in adenomatous polyposis coli (APC) gene. APC is a tumor suppressor gene located on locus 5q21-22. It plays a central role in the Wnt signaling pathway, especially with regards to the degradation of β-catenin within the cell cytoplasm. If APC is mutated, the β-catenin-Tcf complex is not suppressed and leads to constitutive activation of several genes and oncogenes controlling cell growth and division. Mutations in APC affect the ability of the cell to maintain normal growth and function, which results in cell overgrowth/adenoma formation (40).

   **Tomlinson et al., (2013)** described adenomatous polyposis syndromes as of four types, namely:

   A. **Classic FAP**

      This group refers to patients who are diagnosed with FAP due to the development of more than 100 adenomatous colorectal polyps from early childhood (typically around the age of 16) and who harbor an APC germline mutation. Other gastrointestinal manifestations include fundic gland polyps, adenomatous polyps in the duodenum and periampullary region, as well as small bowel adenomas (41).

      Extra-colonic manifestations are common but rarely malignant and include; desmoid tumors (benign soft-tissue tumors), cutaneous lesions such as fibromas, lipomas, sebaceous and epidermoid cysts, brain tumors (mainly medulloblastoma, hepatoblastoma, dental abnormalities, cancer of the pancreas, thyroid, gallbladder, bile duct and adrenal glands (41).
B. MUTYH-associated polyposis (MAP)

Patients usually present with less than 100 colorectal polyps at an average age of 50 years and a high risk of CRC. It is an autosomal recessive disease caused by biallelic mutations in the repair gene MUTYH, this gene is necessary in fixing the DNA damage caused by reactive oxygen species prior to cell division. MAP has been associated with malignancies of the duodenum, ovaries, urinary bladder and skin (42).

C. NTHL1-associated polyposis (NAP)

It is a recently described autosomal recessive polyposis condition. Patients have germline homozygous or compound heterozygous mutations in the repair gene NTHL1. Due to its recent discovery the clinical picture is not yet set, but it points to an extended spectrum of cancer diagnosis in these patients (43).

D. Polymerase proofreading-associated polyposis (PPAP)

It is associated with mono-and biallelic mutations in the genes POLE and POLD1. Both genes are part of the mismatch repair (MMR) pathway. PPAP is an autosomal dominantly inherited CRC predisposition (44).

Both POLE and POLD1 have been associated with an increased risk of endometrial cancer. POLD1 has been associated with breast and brain tumors in addition to CRC and endometrial cancer. Tumors in colon, pancreas, ovaries, small intestine have been seen in POLE mutation carriers (44).

3. Peutz-Jeghers syndrome (PJS)

It is a very rare autosomal dominant genetic disorder. Patients with PJS have a germline mutation of the serine threonine kinase 11 (STK-11). This is a tumor suppressor gene, characterized by multiple hamartomatous polyps of the gastrointestinal tract, most often found in the small intestine. The most characteristic extra-intestinal manifestations are mucocutaneous lesions causing patches of hyperpigmentation in the mouth and on the hands and feet, which usually occur in infancy and fade in late adolescence. Adults with PJS not only have a high risk of developing gastrointestinal cancer, but also non-gastrointestinal cancers, especially breast cancer (45)

4. Serrated polyposis syndrome (SPS)

Serrated polyps often have BRAF and KRAS activating mutations and the CpG island methylator phenotype. About 3% of adenocarcinomas have mutations affecting proofreading domain of POLE that confer very high mutation rates (46).

A patient is diagnosed with SPS if at least one of the following criteria is present: 1) at least 5 serrated polyps proximal to sigmoid, two of which >10 mm; 2) serrated polyps proximal to sigmoid in an individual who has a first-degree relative with SPS; and 3) >20 serrated polyps (any size)
distributed throughout the colon (46).

V. Pathology of Colorectal Carcinoma (CRC)

Grossly, there are variable presentations of colorectal carcinoma, tumors may appear as a flat lesion, exophytic fungating mass in the lumen, endophytic ulcerating lesion mainly growing intramurally, annular lesion growing circumferentially within the wall and may lead to obstruction or grow as infiltrative mass. Microscopically, the majority of CRC are conventional adenocarcinoma or adenocarcinoma not otherwise specified (47).

Conventional adenocarcinoma is characterized by glandular formation, which is the basis for histologic tumor grading. In well differentiated adenocarcinoma >95% of the tumor is gland forming. Moderately differentiated adenocarcinoma shows 50-95% gland formation. Poorly differentiated adenocarcinoma is mostly solid with <50% gland formation. The invasion through the muscularis mucosa into the submucosa is a defining character for CRC, A limited carcinoma within the mucosa is called “intramucosal adenocarcinoma (47).

VI. Histopathological variants of colorectal carcinoma

There are various histopathological types of CRC. These include:

1) Conventional adenocarcinoma:

Nearly 90% have this type of CRC. It comes from the colon's mucus-secreting glands (48). Desmoplasia, particularly at the tumor's edge, is common in this type of well-differentiated gland-forming carcinoma. Lymph nodes may be clogged with necrotic debris, which is known as "dirty necrosis". Both in the primary tumor and in the metastatic deposit, inflammatory cells and scattered neuroendocrine cells predominate (47).

Fig 1: Conventional adenocarcinoma: The prototypic colorectal cancer is a well to moderately differentiated adenocarcinoma consisting of tubular, anastomosing, and branching glands in a desmoplastic stroma (49).
2) **Mucinous adenocarcinoma:**

Nearly a tenth of all colorectal carcinomas are mucinous adenocarcinomas. The presence of extracellular mucin accounting for at least 50% of tumor volume defines them arbitrarily, but new research shows that the degree of mucinous differentiation is not linked to prognosis. The growth of mucin-filled adenocarcinomas is expansive and pushing, with tumor cells arranged in strips, clusters, or singly. Adenocarcinoma with mucinous features or mucinous differentiation is the term used for tumors with a significant mucinous component greater than 10% but less than 50%. Tumor cells, such as signet ring cells, can range in number (50).

3) **Signet ring cell carcinoma:**

Only 1% of colorectal carcinomas are Signet ring cell carcinomas, but they are more common in people under 40, especially those with inflammatory bowel disease. Most of the mass in these tumors is composed of discohesive, medium- to large-sized cells with abundant mucinous cytoplasm and hyperchromatic eccentric nuclei. Mismatch repair deficiency is found in about one-third of patients with signet ring cell carcinomas, but it does not appear to affect biological behavior. Even when present in small numbers, Signet ring cells are linked to a poor prognosis in both mismatch repair-proficient and mismatch repair-deficient carcinomas. Signet ring cell differentiation therefore justifies the designation of high-grade carcinoma, regardless of whether mismatch repair is active or not (51).

![Fig 2: Mucinous adenocarcinoma](image)

Collectionsof extracellular mucin comprising at least 50% of the tumor. The glandular component is well differentiated and consists of irregular, branching or anastomosing glands lined by tall columnar epithelium with minimal cytologic atypia and low mitoticactivity (52).
5) Medullary carcinoma:
One in a million people will be diagnosed with a medullary carcinoma. Approximately 5-8 cases of colorectal cancer per 10,000 people (87). Medullary carcinomas are benign, solid tumors that have little or no glandular content in their structure. Their nuclei have large, prominent nucleoli and have eosinophilic cytoplasm as well as large vesicular nuclei. Lymphocytes, both intra- and peritumor, are frequently found and may be linked to lymphoid aggregates of the Crohn type as the tumor edge advances (51).

6) Micro-papillary adenocarcinoma:
Only 1 in 100,000 cases have pure micropapillary colorectal cancer and about 10% of all colorectal cancers contain some degree of micropapillary growth. Lacunar spaces in micropapillary carcinomas contain tumor cells but lack a supporting stroma. The apical surfaces of tumor cells are oriented toward the cluster's periphery, indicating reverse polarity. Eosinophilic cytoplasm and high nuclear features with frequent lymphovascular and perineural invasion and deep penetration into the bowel wall make it have poor prognosis in comparison to the conventional type of the tumor (51).
Fig 4: **Medullary carcinoma:** Diffuse proliferation of markedly pleomorphic tumor cells occurring in a background of intense peri and intra tumoral lymphocytic infiltration. The tumor nuclei are vesicular with frequent prominent nucleoli, and surrounded by abundant eosinophilic cytoplasm (54).

Fig 5: **Micropapillary adenocarcinoma:** The tumor cells infiltrate as small nests surrounded by retraction artefact, simulating lymphovascular space involvement. The cells have eosinophilic cytoplasm and moderate nuclear atypia (47).

6) **Serrated adenocarcinoma:**

CRCs with serrated adenocarcinomas make up about 7.5%–9% of all CRCs (54). A lack of necrosis and glandular serration are the defining characteristics of this condition. It may also have mucinous, cribriform, or trabecular areas. They are filled with abundantly green-colored eosinophilic cytoplasm and have preserved polarity and a low nucleo-cytoplasmic ratio in condensed nuclei (51).

7) **Squamous cell carcinoma (SCC):**

There is a greater incidence of squamous cell carcinoma in women than in men (55). Tumor with malignant glandular and squamous components that can metastasize was the original description. Colon and upper rectum SCCs may be poorly differentiated squamous cell cancers, according to recent research (56).
8) Adenosquamous carcinoma:
Both squamous cell carcinoma and adenocarcinoma characteristics can be found in this cancer. Herxheimer was the first to describe adenosquamous carcinoma in 1907, a tumor with a low frequency of colorectal origin (about 0.06 percent) (57). It is distinguished by the presence of intercellular bridges, keratin pearl formations, glandular differentiation, and keratinization of individual cells (58).

9) Cribriform comedo type adenocarcinoma:
As the name suggests, the growth of cancerous epithelial cells appears as large nests punctured by numerous rounds, irregularly shaped holes, which are called cribriform lesions in histopathology (59). In all, 7.3% of colon cancer cases are caused by this tumor (60). Methylation on the MSI and CPG islands is frequently found in adenocarcinomas of the cribriform comedo type (59). Kim et al. (61) found that colorectal carcinomas with a cribriform component that had a high level of microsatellite instability (MSI-H) had a poor prognosis, with patients living with the disease for a shorter period (61).

Fig 6: Serrated adenocarcinomas contain angulated, serrated glands lined by cells with abundant eosinophilic cytoplasm. They often show marked desmoplasia and tumor budding with luminal neutrophils (51).
Fig 7: Cribriform comedo-type carcinoma resembles ductal carcinoma in situ. Comedo-type necrosis is surrounded by a rim of tumor cells with cribriform architecture (51).

10) Small and large cell carcinomas

Neuro endocrine cell tumors are the source of these cancers. Neuroendocrine tumors are first classified according to how aggressive they are, with two types: benign and malignant:

- Neuro endocrine tumors, both large and small cell varieties, are notoriously aggressive; they spread quickly and aggressively into nearby organs and tissues.
- Colon carcinoid tumors are a non-cancerous condition. They are less aggressive and grow more slowly than large cell and small cell neuro endocrine tumors.

VII. Prognostic Factors of Colorectal Carcinoma (CRC)

1. Tumor grading:
Tumor grade is the description of a tumor differentiation. It is an indicator of how quickly a tumor is likely to grow and spread, grading systems differ depending on the type of cancer (62). If a grading system for a tumor type is not specified, the TNM system is generally used:

- **GX:** Grade cannot be assessed (undetermined grade)
- **G1:** Well differentiated (low grade)
- **G2:** Moderately differentiated (intermediate grade)
- **G3:** Poorly differentiated (high grade)
- **G4:** Undifferentiated (high grade) (63).

Grades 1 to 3 are applied only to adenocarcinomas, whereas grade 4 or undifferentiated carcinoma is only used for tumors that don’t start in gland cells or make mucus, including neuroendocrine tumors and squamous cell carcinomas.

Grading has deep clinical impact, in that the loss of differentiation during tumor progression has repeatedly been associated with tumor aggressiveness, thereby indicating poor outcome (64).
2. Pathological staging:

A. TNM staging system:
The staging system most often used for colorectal cancer is the American Joint Committee on Cancer (AJCC) TNM system, which is based on 3 key pieces of information:

1. The extent (size) of the tumor (T): Determines how far the cancer has grown into the wall of the colon or rectum.
2. The spread to regional lymph nodes (N).
3. The spread (metastasis) to distant sites (M): Determines the cancer spread to distant lymph nodes or distant organs such as the liver or lungs.

The system described below is the most recent AJCC system. It uses the pathological stage which is determined by examining tissue removed during an operation (65).

Primary tumor (pT)
- PTX: primary tumor cannot be assessed
- PT0: no evidence of primary tumor
- PTis: carcinoma in situ, intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)
- PT1: tumor invades submucosa (through the muscularis mucosa but not into the muscularis propria)
- PT2: tumor invades muscularis propria
- PT3: tumor invades muscularis propria into the pericolorectal tissues
- PT4:
  - PT4a: tumor invades through the visceral peritoneum (including gross perforation of the bowel by the tumor and continuous invasion of tumor through areas of inflammation to the surface of the visceral peritoneum)
  - PT4b: tumor directly invades or adheres to other adjacent organs or structures

Regional lymph nodes (pN)
- PNX: regional lymph nodes cannot be assessed
- PN0: no regional lymph node metastasis
- PN1: metastasis in 1 - 3 regional lymph nodes
  - PN1a: metastasis in 1 regional lymph node
  - PN1b: metastasis in 2 - 3 regional lymph nodes
  - PN1c: no regional lymph nodes are positive but there are tumor deposits in the subserosa, mesentery or nonperitonealized pericolic or perirectal / mesorectal tissues
- PN2: metastasis in 4 or more regional lymph nodes
  - PN2a: metastasis in 4 - 6 regional lymph nodes
  - PN2b: metastasis in 7 or more regional lymph nodes (66)

Distant metastasis (pM)
- PM0: no distant metastasis by imaging; no evidence of tumor in other sites or organs (this category is NOT assigned by pathologists)
- PM1: distant metastasis
  - PM1a: metastasis confined to 1 organ or site without peritoneal metastasis
  - PM1b: metastasis to 2 or more sites or organs is identified without peritoneal metastasis
  - PM1c: metastasis to the peritoneal surface is identified alone or with other site or organ metastases.
DUKES staging system:
In 1932 the British pathologist Cuthbert Dukes devised a classification system for colorectal cancer (63).

- Dukes A: Invasion into but not through the bowel wall
- Dukes B: Invasion through the bowel wall penetrating the muscle layer but not involving lymph nodes
- Dukes C: Involvement of lymph nodes
- Dukes D: Widespread metastases

Another modification of the original Dukes classification was made in 1935 by Gabriel, Dukes and Bussey (66). This staging system was noted to be prognostically relevant to rectal and colonic adenocarcinoma.

- Stage A: Limited to muscularis propria; nodes not involved
- Stage B: Extending beyond muscularis propria; nodes not involved
- Stage C: Nodes involved but highest (apical) node spared
- Stage D: Distant metastatic spread

An adaptation by the Americans Astler and Coller in 1954 further divided stages B and C (67)

- Stage A: Limited to mucosa
- Stage B1: Extending into muscularis propria but not penetrating through it; nodes not involved
- Stage B2: Penetrating through muscularis propria; nodes not involved
- Stage C1: Extending into muscularis propria but not penetrating through it. Nodes involved
- Stage C2: Penetrating through muscularis propria. Nodes involved

Stage D: Distant metastatic spread (67).

3. Lymphovascular invasion

Lymphovascular invasion may be intramural vessel invasion; which is limited to vessels in the submucosal and/or muscular layer, or extramural vessel invasion; which includes vessels located beyond the muscularis propria, ie, within the pericolic or perirectal adipose tissue (67).

Both lymph and blood-vessel invasion are major prognostic variables in patients with CRC, the invasion of extramural veins is an independent predictor of unfavorable outcome and increased risk of hepatic metastasis, while the significance of intramural venous as well as lymphatic, invasion is less clear and is associated with increased risk of lymph nodal metastasis (66).

4. Perineural invasion (PNI):

PNI is defined as neoplastic invasion of nerves and/or spread along nerve sheaths. Its presence constitutes a process of cancer spread independent of blood and lymphatic vessels. In the pathogenesis of PNI, neurotropic factors and matrix metalloproteinases are involved as recent evidence has suggested that both cancers and neuronal cells upregulate neurotrophic factors, which may facilitate the growth and directional spread of cancer along nerves (67).

Ueno et al., (64) introduced a three-tiered grading system for PNI (PNI-0, PNI-1, and PNI-2) based on “intensity” (the number of PNI within a 20 high power field) and “depth” (distance from muscularis propria), PNI-0 defined as no perineural invasion, PNI-1 defined as intensity less than 5
foci and depth less than 10 mm and PNI-2 defined as 5 or more foci and 10 mm or more depth, with a 5-year disease-free survival rates of 88%, 70%, and 48%, respectively. Ueno and his colleagues also suggested the site of PNI to be a significant prognostic variable, independent of the T and N classification. Perineural invasion in CRC has been associated with poorer prognosis (67).

5. Tumor budding:

Tumor budding was defined as the presence of isolated single cells or small cell clusters of less than five cells that are detached from the main tumor mass. Tumor buddings are disturbed within the stroma at the tumor margin. They tend to lose adhesion and dissociate, and this situation causes the tumor to be aggressive (65).

Tumor budding has been studied predominantly at the invasive front and is referred to as peritumoral tumor budding. Tumor buds can also be observed within the tumor, which has been referred to as intratumoral tumor budding. Intratumoral tumor budding can be evaluated in preoperative biopsies and has also been associated with the presence of lymph node metastasis, higher tumor grade, and lympho-vascular invasion in the resection specimen, as well as distant metastasis (65).

There is a close relationship between tumor budding and the process of epithelial-mesenchymal transition. In this transitional process epithelial cells lose intracellular and cell-matrix contacts mediated by E-cadherin, resulting in invasion and ultimately metastatic cancer spread. Several trials have shown the relationship between tumor budding and prognosis in colorectal cancer. It might be closely related to poor survival and high risk of recurrence (66).

Tumor budding should be considered a promising and strong prognostic factor in colorectal cancer. Widespread reporting of tumor budding has not progressed to routine clinical practice due to a lack of consensus on scoring methods. However, routine reporting is now advocated by using the approach outlined by the International Tumor Budding Consensus Conference (ITBCC), with recommendations for the assessment and reporting of tumor budding in CRC (66).

Tumor budding is a main prognostic factor in distinct subgroups of CRC as micropapillary variant and poorly differentiated adenocarcinoma. In early lesions, it appears to be one of the strongest parameters associated with the presence of regional lymph-node spread (68).
Fig 8: (a) that is defined as single tumor cells or tumor cell clusters at up to four cells. (b) Example of poorly differentiated clusters that are defined as five tumor cells or more (68).

6. Tumor necrosis:

Tumor necrosis is a consequence of chronic ischemic injury due to rapid tumor growth and thereby reflecting the level of intra-tumoral hypoxia. Increased cellular hypoxia correlates with increased metastatic potential and worse prognosis as well as resistance to radiotherapy and chemotherapy (68).

Tumor necrosis is associated with elevated circulating interleukin (IL-6) and vascular endothelial growth factor (VEGF) concentrations, thereby modulating both local and systemic inflammatory responses, as well as angiogenesis, which in turn may promote tumor progression and metastasis (68).

7. Inflammatory response:

The overall inflammatory response at the tumor margin is characterized by lymphocytic infiltration and infiltration of the tumor stroma by eosinophils and macrophages. Eosinophilic infiltration in the tumor area, which is also called tumor-associated tissue eosinophilia, is an easily assessable parameter in routine pathology. Increased numbers of eosinophils have been associated with decreased recurrence rate and improved patient’s survival (64).

The role of macrophages seems to be more complex, because they have been attributed to both pro- and antitumor properties. It produces growth factors/cytokines that stimulate growth of epithelial cells that have spontaneously acquired cancer associated mutations. However, a high number of CD68-positive tumor-associated macrophages has been identified as a favorable morphological feature. The antitumoral inflammatory response is a distinct histological feature and a promising prognostic tool in CRC pathology where marked local inflammatory response is associated with improved survival (64).
VIII. Intestinal Stem Cells

The luminal surface of the colon consists of a single layer of columnar epithelial cells which are folded to form finger-like protrusions into the lumen. The spaces between these folds are known as the crypts of Lieberkühn, the functional unit of the intestine. The colonic epithelium contains four distinct cell lineages: enterocytes, goblet cells, endocrine cells and Paneth cells (68).

Crypt base columnar cells (CBCs) are small undifferentiated cells thought to be the true intestinal stem cells which give rise to the epithelial lineages. These stem cells possess the ability to divide asymmetrically, giving rise to identical daughter cells and also transit amplifying cells which proliferate and differentiate into enterocytes, goblet cells and endocrine cells during their upward movement through the crypt. These three epithelial cell types occupy the top half of the crypt as well as the luminal epithelial surface. Paneth cells differentiate from transit amplifying cells as they move downwards to the crypt base, where they are eventually found among the stem cell population (69).

Epithelial cells in the intestine have a lifetime of around 5 days and are continuously renewed by intestinal stem cells (ISCs) under the micro-environmental influence. Functional definition of stem cells was proposed as undifferentiated cells with (1) extensive proliferation capacity, (2) ability to self-renew, (3) potential to produce differentiated functional progenitors, (4) ability to regenerate tissue post injury (69).

The stem cells in various body tissues need an environment to maintain their division, which is called the niche (61).

The functional importance of the intestinal micro-environment (niche) in the advanced control of stem cell life cycle is widely accepted. The niche consists of cellular and extracellular components that ensure the optimal conditions for SC maintenance through the secretion of various cytokines, growth factors, and direct interactions. Interestingly, intestinal SCs may also be affected by components in the crypt lumen, derived from epithelial cells or from bacteria. Intestinal subepithelial myofibroblasts are key regulators of SC self-renewal and differentiation, mediate the crosstalk between epithelial and mesenchymal cells, and secrete a wide range of morphogenetic factors. Epithelial–mesenchymal interactions regulate the normal intestinal architecture and additionally define the balance between proliferation and differentiation. Wingless/Int (Wnt), Hedgehog, bone morphogenetic protein (BMP), Notch, and platelet-derived growth factor pathways are involved in these interactions (70).
The intestinal stem cells are located at the base of the mucosal crypt and undergo asymmetric division, giving rise to one identical daughter cell and one cell with the potential to differentiate into the intestinal cell, thereby maintaining tissue homeostasis and repair. They are the prime suspects as the source of reliable of most, if not all, of colorectal cancers due to their pre-existing enhanced proliferative and self-renewal properties (71).

Paneth cells may contribute to the maintenance of the stem cell niche by producing mucosal defense barriers, modulating intestinal microflora and producing growth factors and other regulatory molecules (72).

**Cancer stem cell (CSC):**

Cancer stem cell were described first in acute myeloid leukemia (AML), but soon evidenced in solid tumors. The CSCs arise by gene mutations or deregulation of genetic programs in normal stem/progenitor cells, Colorectal CSCs share the major biological characteristics of stem cells from other solid tumors, including 1) self-renewal and multi-directional differentiation potential, 2) abnormal activation of proliferating signaling pathways, such as Wnt, Notch and Hedgehog, 3) high tumorigenicity, and 4) strong drug and/or radiation resistance. The colorectal CSCs also share many features of normal intestinal stem cells, such as infinite division, telomerase activity and organ-specific differentiation (67).

The origin of colorectal CSCs remains elusive, and it is still a matter of active debate amongst scientist whether they derived from intestinal stem cells having stemness during cancer formation or are the direct progeny (differentiated cells) of mutated cells. The identification of stem cells in majority of normal tissues including colon crypts favors the hypothesis that non-neoplastic stem cells could be the possible target for cancer transforming genetic (e.g., mutations) and epigenetic (e.g. DNA promoter methylation, small RNA-mediated gene silencing, etc.) alterations and the origin of CSCs. Since intestinal stem cells and colorectal CSCs share many features such as longevity and self-renewal, intestinal stem cells might be the potential source of CSCs. In addition, CSCs could derive from cells outside a tumor. For example, bone marrow-derived mesenchymal stem cells can serve as CSC’s ancestors, Therefore, colorectal CSCs may arise from (i) intestinal non-neoplastic stem cells by accumulating genetic and epigenetic alterations, (ii) de-differentiation of cancer cells or (iii) cells outside the tumor (67).

Cancer stem cells preferentially demonstrate persistent activation of multiple signal transduction pathways for stemness maintenance and self-renewal. The abnormal signaling pathways that have been well addressed in colorectal CSCs, including Wnt/β-catenin, Notch, TGF-β and Hedgehog. The Wnt/β-catenin pathway is particularly important in stemness maintenance and drug resistance of colorectal CSCs (67).
Human colorectal CSCs were first identified and detected using CD133 (also called prominin-1) as cell surface marker. These CD133-expressing cells are capable of regenerating tumors in mice that resembled original cancer. Later on, other surface and intracellular markers for colorectal CSCs have been identified (Table 2), which in turn described several phenotypes of CSCs in colorectal cancer (67).

Cancer stem cells which are also known as tumor-initiating cells, are heterogeneous and are highly tumorigenic. The colorectal CSCs rely on different pathways including the WNT pathway, the BMP pathway, the Notch pathway, etc. to maintain their stemness and to contribute tumor progression. CSCs mediate cancer pathogenesis by driving the fundamental processes, i.e., cell proliferation, growth angiogenesis, invasion, metastatic dissemination and therapy resistance (73).

Colorectal CSCs are identified via a group of surface markers. The main colorectal CSC markers documented are CD44, CD133, CD166, Lgr5, ALDH1 and EpCAM. Other more universal CSC markers include Nanog, Sox2, Oct-4, CD51, CD24, CD26 and CD29 (67).

**Role of cancer stem cells in tumor progression**

Cancer stem cells also called tumor-initiating cells, are highly tumorigenic. They rely on distinct reprogrammed pathways to maintain stemness and to contribute tumor progression by driving the fundamental processes of tumor development, such as cell proliferation, growth, angiogenesis, invasion, and metastatic dissemination (74).

**CSCs in tumor initiation:**

Multiple oncogenic mutations and epigenetic alterations in adult stem cells and/or their progenitors or normal differentiated cells create CSCs that disrupt the tightly controlled self-renewal and differentiation processes and promote the bypass of protective mechanisms in cells. The result is uncontrolled proliferation and escape of apoptosis, resulting in cancer. For example, mutations in p53 and PTEN (phosphatase and tensin homolog) can stimulate c-Myc activation to promote self-renewal capacity and impair the differentiation of glioblastoma-initiating cells (75).

A single CSC may expand and differentiate into a large tumor. Also, multiple CSC pools may be present, each of which has the potential to initiate and develop into cancer. Different CSC subpopulations may arise during tumor progression due to the additional epigenetic modifications and mutations. These new CSC populations may exhibit more aggressive growth and drive the formation of malignancy. These CSC-derived differentiated cancer cells form major components of tumor and play an important role in sustained tumor growth (74).
CSCs and tumor vasculature:

Tumor vascularization (angiogenesis and lymphangiogenesis) is critical for tumor growth, invasion, and metastasis. CSCs can promote angiogenesis and lymphangiogenesis by secreting elevated levels of vascular endothelial growth factors (VEGFs) in a tumor and drive their progression by providing new blood and lymphatic vessels. These newly generated vessels supply nutrients for the growth and development of cancer. Previous studies have confirmed that CSCs have the potential to induce angiogenesis and produce angiogenic cells, whereas the differentiated non-CSCs tumor cells are nonangiogenic (76).

Experimental results indicate that epithelial to mesenchymal transition (EMT), a process in which polarized epithelial cells are converted to the motile mesenchymal cells, is involved in cancer cells, invasion and metastasis to distant sites. Interestingly, CSCs can acquire the EMT properties and can interconvert themselves between epithelial and mesenchymal phenotype and execute the metastatic processes. With the acquisition of EMT, CSCs attain increased capacity for migration, resistance to apoptosis, enhanced production of extracellular matrix degrading enzymes, and higher invasiveness (77).

Thus, by undergoing EMT, CSCs possess the characteristics, such as loss of polarity, cell cell adhesion, and migratory and invasive properties, that promote cancer metastasis, which leads to tumor progression. In addition, CSCs can promote progression of cancer by escaping immune surveillance, dysregulation of cellular metabolism, and inducing genomic instability, which in turn reconstitutes the cancer in new organs or recipients (77).

Cancer stem cells in therapy resistance:

Despite the continuous improvements of therapeutic modalities, cancer recurrence and resistance to conventional chemoradiation therapies occur in patients with advanced-stage cancer. Previous cancer studies using cell cultures, animal models, and cancer patients found that CSCs are responsible for therapy resistance and cancer relapses in patients with cancer (78).

Cancer stem cells can become selectively resistant to the conventional chemotherapy-induced apoptosis, whereas the differentiated cancer cells will undergo the process of apoptotic cell death. Furthermore, the surviving CSCs can reestablish the culture and are responsible for resistance to chemotherapy (79).

Cancer stem cells isolated from different cancers, such as liver cancer, lung cancer, pancreatic cancer, breast cancer, leukemia, and brain cancers (glioblastoma), were resistant to conventional chemotherapeutic drugs such as gemcitabine, cisplatin, 5-fluorouracil, and imatinib (73).

Current conventional adjuvant treatment strategies to cancer are designed to target and eliminate all the differentiated cancer cells within a cancer. (80).
IX. Epithelial mesenchymal transition

Epithelial–mesenchymal transition (EMT) is a cellular process during which epithelial cells acquire mesenchymal phenotypes and behavior following the downregulation of epithelial features. EMT is triggered in response to signals that cells receive from their microenvironment. The epithelial state of the cells in which EMT is initiated is characterized by stable epithelial cell–cell junctions, apical–basal polarity and interactions with basement membrane. During EMT, changes in gene expression and post-translational regulation mechanisms lead to the repression of these epithelial characteristics and the acquisition of mesenchymal characteristics (81).

EMT have been classified into three types depending on the biological context in which they occur. Type I EMT is observed during embryonic development, type II EMT occurs during wound healing and tissue regeneration, and type III EMT occurs during carcinoma progression. It remains to be seen whether these classes of EMT program are truly distinct from one another or whether they are different manifestations of a common cellular process (80).

A number of distinct molecular processes are engaged in order to initiate an EMT and enable it to reach completion. These include activation of transcription factors, expression of specific cell-surface proteins, reorganization and expression of cytoskeletal proteins, production of ECM-degrading enzymes, and changes in the expression of specific microRNAs. In many cases, the involved factors are also used as biomarkers to demonstrate the passage of a cell through an EMT (82).

EMT is orchestrated by EMT-inducing transcription factors (EMT-TFs), which act pleiotropically and in various combinations to induce the expression of genes that promote the mesenchymal cell state and repress the expression of genes that maintain the epithelial state. The EMT-TFs that have major roles in orchestrating EMT program include ZEB1 and ZEB2, SNAIL, SLUG and the basic helix–loop–helix factors TWIST1 and TWIST2 (80).

These EMT-TFs regulate the expression of one another, and in different combinations, they induce the expression of hundreds of genes associated with the mesenchymal state and repress genes associated with the epithelial state (80).

EMT in cancer progression and metastasis

Epithelial-mesenchymal transition orchestrated by the EMT-TFs can impart several traits that are essential to the malignant progression of carcinoma cells, including tumor-initiating properties, motility, the ability to disseminate and elevated resistance to chemotherapeutics. The detection of many of the EMT-associated protein markers can be used by pathologists as highly specific indicators of high-grade malignancy (80).
EMT is very rarely activated as a cell-autonomous process in carcinoma cells. Signals deriving from the tumor-associated macrophages and fibroblasts within the stroma act on carcinoma cells to induce the expression of EMT-TFs, which in turn orchestrate the expression of various components of the EMT program (81).

Sequestration of β-catenin in the cytoplasm is important for the preservation of epithelial features of cancer cells, and acquisition of the mesenchymal phenotype correlates with the movement of β-catenin to the nucleus, where it becomes part of Tcf/LEF complexes. Such β-catenin accumulation in the nucleus, which is often associated with loss of E-cadherin expression, correlates with susceptibility to enter into an EMT and acquisition of an invasive phenotype. Thus, cells that lose cell surface E-cadherin become more responsive to induction of an EMT by various growth factors (83).

Epithelial cell adhesion complexes reorganize and cell proliferation is suppressed when the full-length or the cytoplasmic portion of E-cadherin (containing the β-catenin binding site) is expressed in cells that have passed through an EMT, causing such cells to lose their mesenchymal phenotype (84).

TGF-β, EGF, PDGF and HGF are responsible for the induction of EMT-induced transcription factors in cancer cells. Macrophages and activated resident fibroblast release chemokines induce basement membrane damage, β-catenin accumulation in the nucleus, which is often associated with loss of E-cadherin expression, correlates with susceptibility to enter into an EMT and acquisition of an invasive phenotype (85).

Invasion of cells into the extracellular matrix is considered one of the first steps in metastatic cascade. The cells acquiring the ability to migrate and invade matrix have long been considered a hallmark of EMT and have been used as a surrogate to describe the role of EMT in metastasis. Distinct mechanisms are involved including cytoskeletal reorganization, altered expression of cell adhesion molecules, degradation of basement membrane via activation of MMP-2 and MMP-9 as well as sustained autocrine growth factor signaling to evade apoptosis and/or anoikis (86).

Mesenchymal epithelial transition (MET) is reciprocal changes in cellular phenotype that reverse EMT-induced phenotypes, during which mesenchymal-like cells may acquire apical–basal polarity, reorganize their cytoskeleton, and exhibit increased cell–cell adhesion, resulting in an organized epithelium, MET can occur as a direct consequence of the shutdown of expression of various EMT-TFs (86).

MET seems important for the completion of some of the final steps of malignant progression. Carcinoma cells often undergo an MET after dissemination to distant tissue sites in order to form metastases at these sites. MET is thought to occur because of cell-intrinsic changes in signaling
cascades and epigenetic alterations leading to the repression of mesenchymal traits and re-expression of epithelial markers such as E-cadherin ultimately resulting in metastatic colonies that resemble the primary tumor from which they arise (86).

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**References**


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