Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Review Article: Immunohistochemical Expression of Bromodomain 4 and C-MYC in Adenocarcinoma of the Colon

Fatma Atia Ali, Sabah Ahmed Fadel, Mohammed Mahmoud Mubarak, Ghada Hosny

Colorectal cancer (CRC) is the third most common cancer in terms of recognition (6.1%) and the second in terms of mortality (9.2%). More than 90% of colorectal malignancies are adenocarcinomas originating from epithelial cells of the colorectal mucosa. It was demonstrated that individuals are at increased risk for CRC if they (or their relatives) have had cancer, a history of colon polyps, inflammatory bowel diseases, diabetes mellitus, or cholecystectomy. The recognized subtypes of adenocarcinoma include adenoma-like, adenosquamous, carcinoma with sarcomatoid components, medullary, micropapillary, mucinous, serrated, signet ring cell, and undifferentiated. Good prognostic factors in adenocarcinoma of the colon are microsatellite instability and increased tumor-infiltrating lymphocytes. The most widely used immunohistochemical markers for colorectal adenocarcinoma are cytokeratin (CK) 20, CK7, and CDX2. The most common immunophenotype of colorectal adenocarcinoma is positivity for CK20 and negativity for CK7, which is a relatively specific staining pattern for colorectal origin.
Keywords: Colorectal cancer, cytokeratins, Bromodomains.

1. Introduction

Bromodomains determine the acetylated lysine molecules of histone tail(1). As bromodomain is the only protein domain whose conserved activity is to function as an acetyl-lysine binding domain(2).

BRD4 is a global regulator of gene transcription, so its inhibition would be expected to cause the global downregulation of gene activity. However, BRD4 inhibition only downregulates a few hundred genes, most of which are very important in tumorgenesis.

The dynamic role of lysine acetylation is, to some extent, attributed to the bromodomain (BRD), which is the only protein domain whose conserved activity is to function as an acetyl-lysine binding domain (3).

Bromodomain-containing protein 4 (BRD4) is emerging as a therapeutic target that acts synergistically with other targets of small-molecule drugs in cancer(4).

BRD4 promotes the expression of growth-associated genes such as c-Myc, JunB and cyclin D1(5).

The c-Myc transcription factor is a powerful regulator of cell growth, proliferation, differentiation, and apoptosis(6).

Overexpression and amplification of the c-MYC oncogene occur in approximately 70 and 10% of human primary colorectal carcinomas, respectively, indicating the importance of this gene in colorectal tumorgenesis(7).

The c-MYC protein, encoded by the c-MYC gene, acts as a transcription factor for variable cellular functions, including proliferation, differentiation, metabolism, survival, and apoptosis. The c-MYC gene can promote tumorigenesis in various malignant tumors and mediate the critical role in colorectal cancer (CRC) progression(8).

Colon

Introduction

The sigmoid colon is the terminal portion of the large intestine before reaching the rectum. It connects the descending colon with the rectum. The sigmoid colon derives its name from a Greek letter sigma. Its location is usually in the pelvis, but as it is a mobile structure with a mesentery, it can often become displaced into the abdominal cavity. The primary function of the sigmoid colon is the absorption of water, vitamins, and minerals from the undigested food particles, just like the preceding portions of the bowel; however, it does so to a lesser extent. The sigmoid colon is a hindgut structure and receives its blood supply, innervation, as well as its lymphatic drainage, similar to other hindgut structures. Various common and uncommon diseases can affect the sigmoid colon, many of which could require surgical correction if medical management fails(9).

Structure:

The average length of the sigmoid colon is 25 to 40 cm (10 to 15.75 in). The sigmoid colon is an “S” shaped portion of the large intestine that begins in front of the pelvic brim as a continuation of the descending colon and becomes the rectum at the level of the third sacral vertebrae. Unlike the descending colon, the peritoneum surrounds the sigmoid colon, and thus, it is not a retroperitoneal structure. The sigmoid mesocolon attaches the sigmoid colon to the posterior wall of the abdomen. At the level of S3, it again becomes a retroperitoneal structure(10).

Function:

The sigmoid colon receives stool that has had most of the nutrients and water reabsorbed from it at this point. Its primary purpose is to remove the final components such as water, vitamins, and minerals from the gut contents through reabsorption to make the stool solid enough to be

stored in the rectum. The sigmoid colon then pushes the firm stool into the rectum through a functional rectosigmoid sphincter with the help of strong peristaltic contractions in preparation for fecal excretion, allowing for the storage and initiation of the defecation reflex(10).

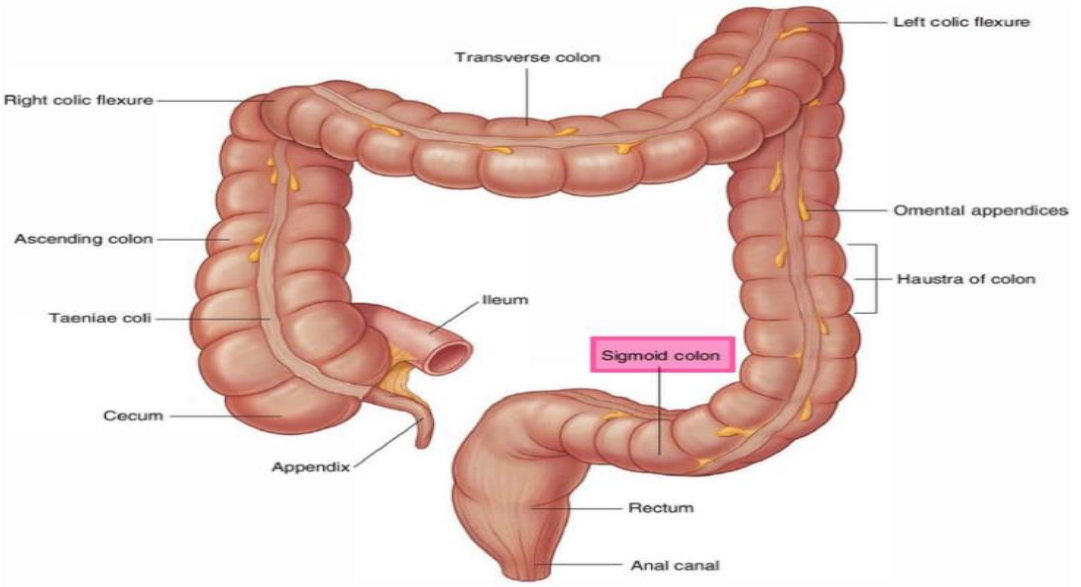

Figure 1: Diagrammatic representation of a normal healthy colon(11).

Colorectal cancer

Introduction

Colorectal cancer (CRC) is the third most common diagnosis and second deadliest malignancy for both sexes combined. CRC has both strong environmental associations and genetic risk factors. The incidence of new cases and mortality has been steadily declining for the past years, except for younger adults (younger than 50 years), possibly related to an increase in cancer screening and better therapy modalities. Approximately 5% of all CRC are attributed to two inherited syndromes, Familial Adenomatous Polyposis, and Lynch syndrome. The change of the normal colonic epithelium to a precancerous lesion and ultimately an invasive carcinoma requires an accumulation of genetic mutations either somatic (acquired) and/or germline (inherited) in an approximately 10 to 15-year period(12).

Subtype

The 5th edition of the World Health Organization (WHO) Classification of Tumors (Digestive System) has recognized a new subtype of colorectal adenocarcinoma, called adenoma-like adenocarcinoma. Over the last few decades, several publications attempted subclassifying colorectal adenocarcinoma based on a distinctive histologic appearance, such as villous tumor, invasive papillary adenocarcinoma and villous adenocarcinoma. Based on the morphologic descriptions, it appears that some of them could be referring to this subtype that is now termed adenoma-like adenocarcinoma(13)

Etiology

Mutations in specific genes can lead to the onset of colorectal cancer, as happens in other types of cancer. Those mutations can appear in oncogenes, tumour suppressor genes and genes related to DNA repair mechanisms. Depending on the origin of the mutation, colorectal carcinomas can be classified as sporadic, inherited and familial(14).

Risk Factors

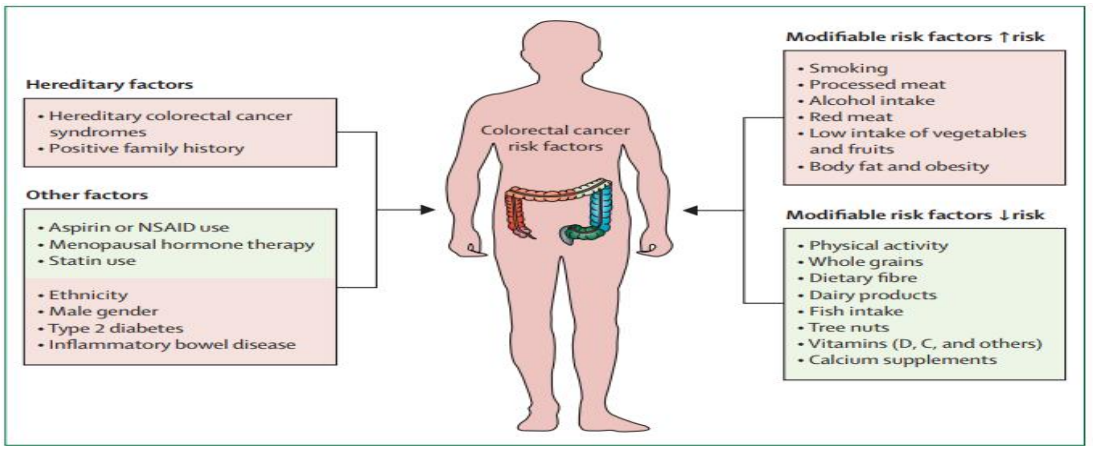

Figure 2: List of modifiable and non-modifiable risk factors for colorectal cancer Although data for some risk factors (eg, smoking and processed meat consumption) are convincing, other factors (eg, menopausal hormone therapy) exist, for which data are more suggestive. NSAIDs=non-steroidal anti-inflammatory drugs(15).

Epidemiology

Colorectal cancer is the third most popular occurring cancer in men and the second most commonly occurring cancer in women. There were over 1.9 million new cases in 2020. Colorectal cancer is the second most common cause of death from cancer, estimated to be responsible for almost 935,000 cancer deaths(16). Globally it is one of the cancers whose incidence is increasing comprising 11% of all cancer diagnoses(17). According to GLOBOCAN 2020 data there is a broad geographic variation in CRC incidence and mortality among various countries of the world(17). It has been recognized that the most significant increase in CRC incidence and mortality occurs in medium and high human development index (HDI) countries that are adopting the "western" way of life(17). Developed countries are at the highest risk of colon cancer. Obesity, sedentary lifestyle, red meat consumption, alcohol and tobacco are considered the driving factors behind the growth of CRC(18). Therefore, colorectal cancer is a disease of developed countries with a western lifestyle (19).

prevalence in Egypt

In Egypt, CRC is uncommon and represents only 3 percent of all malignant tumors (excluding tumors of the nervous system). It is the third most common tumor in males after urinary bladder and lymphohemopoietic malignancies, and in females it ranks fifth after breast, lymphohemopoietic, cervical, and urinary bladder cancers. Recent interest in Egyptian CRC

has been raised when personal observations and epidemiologic studies revealed a high incidence of the disease among the young Egyptian population(20).

Pathogenesis

Most cancers arise from a polyp. This process begins with an aberrant crypt, evolving into a neoplastic precursor lesion (a polyp), and eventual progressing to colorectal cancer over an estimated 10–15-year period. The cell of origin for the majority of colorectal cancers is currently assumed to be a stem cell or stem-cell-like cell(21). These cancer stem cells are the result of progressive accumulation of genetic and epigenetic alterations that inactivate tumor-suppressor genes and activate oncogenes. Cancer stem cells reside in the base of the colonic crypts and are essential for the initiation and maintenance of a tumor. Investigating the regulatory mechanisms that control the growth of these cancer stem cells is a promising area of investigation for possible therapeutic agents and preventive treatment(22).

Left-sided versus right-sided disease

Molecular features of right-sided (proximal) colon cancers are different when compared with left-sided (distal) colon cancers and rectal cancers (figure 4). Apart from molecular differences, embryological, biological, and anatomical differences exist between left-sided and right-sided colorectal cancer. Sidedness has a key role, particularly in the metastatic setting and is increasingly being recognised as a predictive marker of response to anti-EGFR drugs(23).

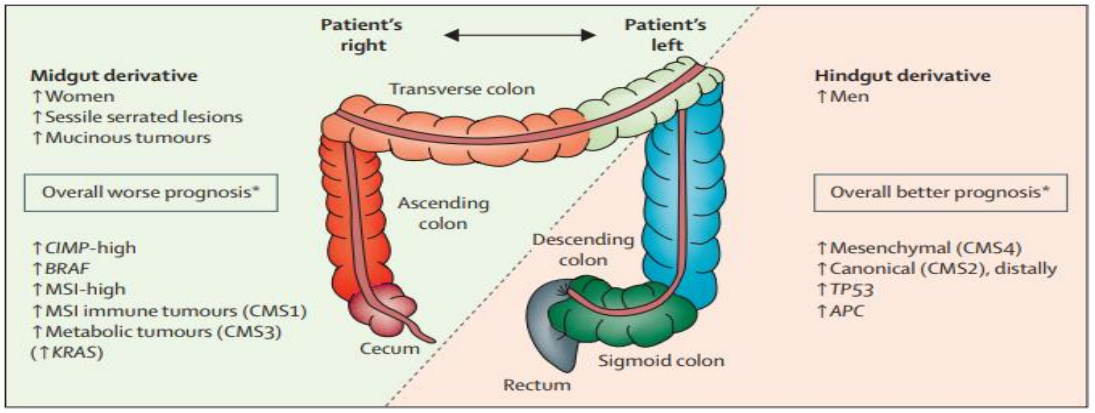

Figure 4: Differences in right-sided versus left-sided colon and rectum Simplistic schematic representation or right-sided colon (cecum, ascending colon, hepatic flexure) versus left-sided colon (splenic flexure, descending colon, sigmoid, rectosigmoid) and rectum representing a continuum of changes secondary to different embryological origin. Arbitrarily, two thirds of transverse colon are considered right-sided. The figure is a simplistic cartoon reflecting the heterogeneity and the continuum of changes seen in patients with colorectal cancers. *Please note that the prognosis of overall being worse for right-sided colon cancers does not apply to all stages of cancers and is primarily seen in metastatic setting with respect to response to anti-EGFR and anti-VEGF therapies. With more of the right-sided tumors being MSI-high, although historically these tumors had worse prognosis because of being relatively resistant to chemotherapy, they now have immunotherapy as an

option. Therefore, the outcomes for these tumors are evolving and changing. CMS=consensus molecular subtypes. MSI=microsatellite instability(24).

Diagnosis

Clinical symptoms

Patients can present with a wide range of signs and symptoms such as occult or overt rectal bleeding, change in bowel habits, anemia, or abdominal pain. However, colorectal cancer is largely an asymptomatic disease until it reaches an advanced stage. By contrast, rectal bleeding is a common symptom of both benign and malignant causes, and therefore additional risk factors might be needed to help identify those people who should undergo further investigation by colonoscopy. New onset rectal bleeding should generally prompt colonoscopy in individuals aged 45 years or older. In younger patients, additional factors are used to identify those at highest risk for colorectal cancer (eg, having a family history of colorectal cancer, change in bowel habits, unexplained weight loss, and blood mixed with the stool as opposed to blood on the surface of the stool)(25).

Colonoscopy

Colonoscopy is the gold standard for diagnosis of colorectal cancer. It has a high diagnostic accuracy and can assess the location of the tumour. Importantly, the technique can enable simultaneous biopsy sampling and, hence, histological confirmation of the diagnosis and material for molecular profiling. Colonoscopy is also the only screening technique that provides both a diagnostic and therapeutic effect. Removal of adenomas using endoscopic polypectomy can reduce cancer incidence and mortality. Indeed, the efficacy of colonoscopy for reduction of colorectal cancer incidence and mortality was well demonstrated by the US National Polyp Study. 20-year follow-up data from this study showed a reduction in colorectal cancer-related mortality of 53%, an encouraging result that has been echoed by a more-recent study(26).

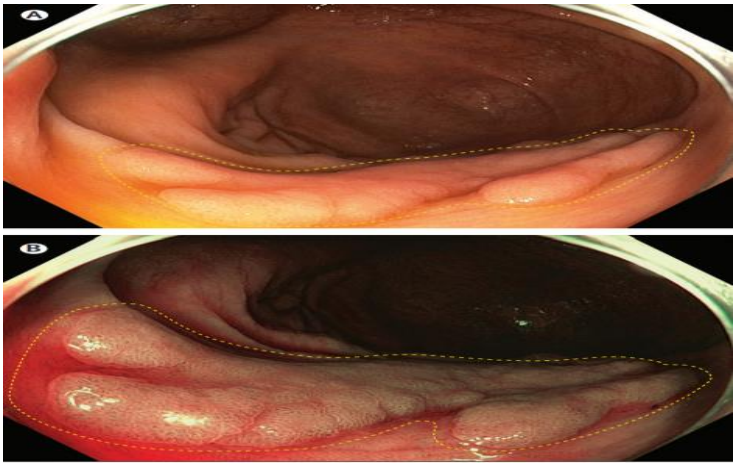

Figure 5: High-definition images of a flat, lateral-spreading polyp (A) High-definition white light image. (B) Close-up view, using narrow band imaging—an endoscopic imaging

technique that highlights the mucosal surface and can help with detection and differentiation of colonic lesions. The yellow dotted lines indicate the borders of the lesion(27).

CT colonography

CT colonography uses low-dose CT scanning to obtain an interior view of the colon. The technique is well established as a diagnostic modality for colorectal cancer(28).

CT colonography has been recommended as one of the options for colorectal cancer screening in guidelines in the United State and Europe. In many countries, CT colonography has replaced double-contrast barium enema (the conventional X-ray-based imaging modality for the colon) examination and is increasingly being used as an alternative to conventional colonoscopy. However, CT colonography has not readily been accepted in Europe because of radiation exposure, costs, burden to patients and high colonoscopy referral rates. In the Asia–Pacific region, CT colonography is not recommended for colorectal cancer screening unless in those for whom total colonoscopy is not possible(29).

Histopathologic diagnosis of colorectal carcinoma

More than 90% of colorectal carcinomas are adenocarcinomas originating from epithelial cells of the colorectal mucosa. Other rare types of colorectal carcinomas include neuroendocrine, squamous cell, adenosquamous, spindle cell and undifferentiated carcinomas. 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. In practice, most colorectal adenocarcinomas (~70%) are diagnosed as moderately differentiated. Well and poorly differentiated carcinomas account for 10% and 20%, respectively(30).

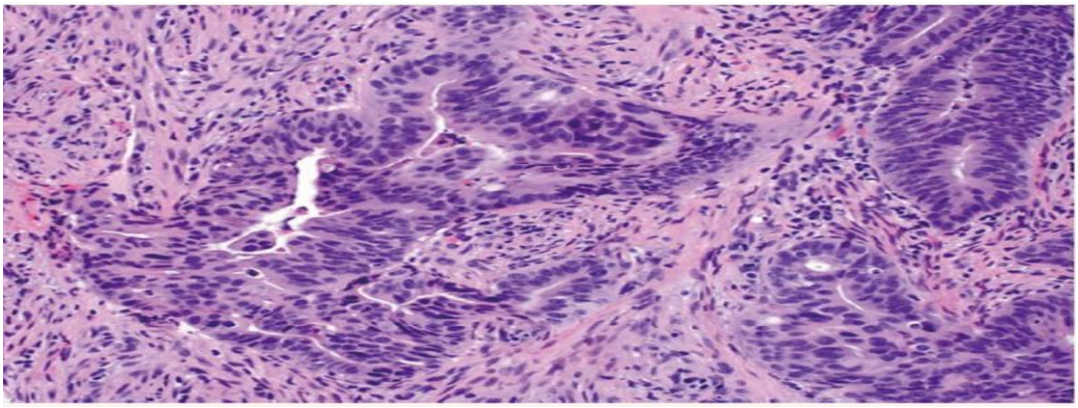

Figure 6: An example of moderately differentiated adenocarcinoma showing complicated glandular structures in a desmoplastic stroma (original magnification ×200)(31).

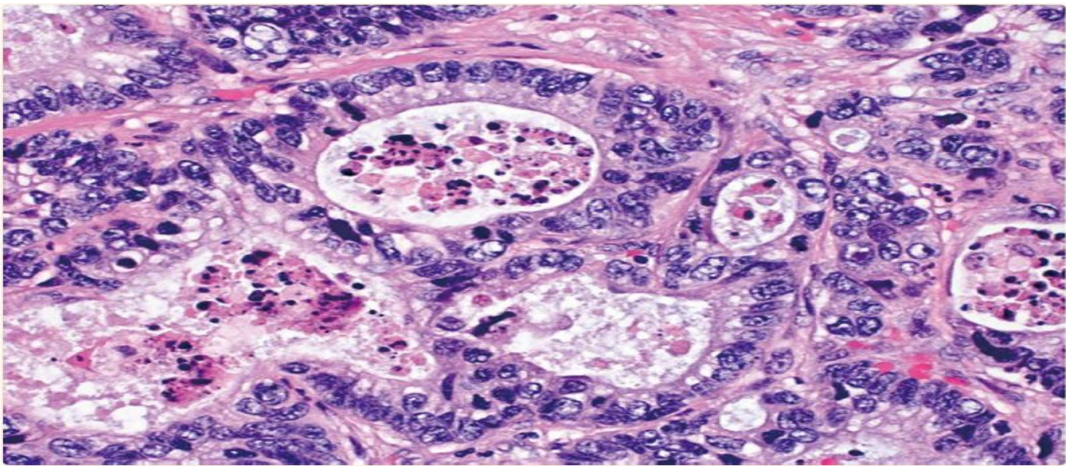

figure7:Necrotic debris ("dirty necrosis") within the lumina of adenocarcinomatous glands (original magnification ×400)(31).

Treatment

For many years, cancer patients have been treated with surgery and chemotherapy as the initial lines of defense against the disease. However, individuals with metastatic disease have historically had a poor prognosis for CRC. Primary and adjuvant therapy advancements have improved CRC survival time. In usual cases, surgery is required to remove the tumor (32)altogether. Nearly a quarter of CRC cases are diagnosed at the advanced stage, and 20% of the remaining cases acquire metachronous metastases; therefore, curative surgical control alone is often challenging, resulting in tumor-related mortality(33). Notably, chemotherapy or radiotherapy may be used before or after surgery to help shrink or stabilize the tumor (32). Current chemotherapy comprises single-agent therapy (primarily fluoropyrimidine (5-FU)) and multiple-agent regimens, including oxaliplatin (OX), irinotecan (IRI), and capecitabine (CAP or XELODA or XEL). The combined therapy regimens FOLFOX (5-FU + OX), FOXFIRI (5-FU + IRI), XELOX or CAPOX (CAP + OX), and CAPIRI (CAP + OX) remain the mainstream approaches in first-line treatment. Patients with poor performance or low risk of deterioration are recommended single-agent therapy. Choosing additive agents appears to be similar in efficacy, with only side effects varying(34)

Endoscopic treatment

Some early cancers are amenable to local treatment only. The incidence of these early colorectal cancers have increased because of colorectal cancer screening programmes. Upon diagnosis, malignant polyps might be resected endoscopically in an en-bloc manner, thus allowing for a precise assessment of high-risk features (submucosal invasion depth, differentiation, lymphatic invasion, and tumor budding) and deep and lateral margins by the pathologist. The decision on adjuvant surgery with mesenteric lymphadenectomy is challenging and depends on the estimated oncological and operative risk, and the preferences of the patient. Depending on its size, appropriate endoscopic resection techniques for T1 cancers (figure 8) are en-bloc endoscopic mucosal resection, endoscopic submucosal dissection, and endoscopic full-thickness resection. The last two techniques should be

considered when there is a high suspicion of superficial submucosal invasion, to be assessed on the basis of mucosal pit pattern analysis, polyp morphology, and other endoscopic aspects of the colorectal lesion(35).These resection techniques require substantial technical skills(36)and should be done in centers with such expertise(37).

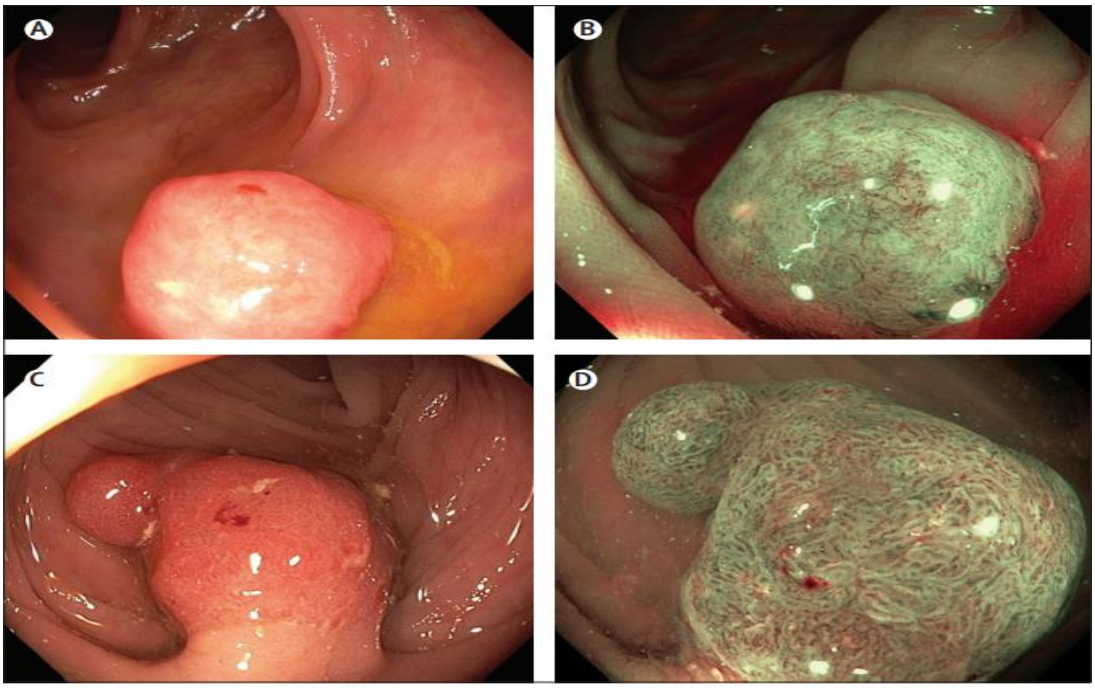

Figure 8: White light and narrow band imaging of T1 colorectal cancer Corresponding photographs of T1 colorectal cancers with white light (A and C) and narrow band imaging (B and D). Narrow band imaging can help with differentiating between the different subtypes of colonic lesions (adenomas, hyperplastic polyps), and can also help with finding areas within polyps that are suspicious for invasive growth (eg, T1 cancer)(38).

Surgical treatment

Surgery is the cornerstone of curative intent treatment. Quality of colorectal cancer resection is crucial and can be assessed with objective parameters. Postoperative imaging studies have shown that surgical quality could be further optimised, stressing the importance of training and specialisation of surgeons(39).

Surgery for rectal cancer is more complex related to the accessibility and intricate anatomy of the pelvis. Total mesorectal excision is the standard oncological approach to rectal cancer, and extent of resection further depends on involvement of the sphincter complex and other surrounding structures. In rectal cancer, the role of conventional multiport laparoscopy is still debated(40).Transanal minimally invasive total mesorectal excision and robot-assisted laparoscopic total mesorectal excision might improve results for mid-rectal and distal rectal cancer, but these techniques require a high degree of expertise and still have to prove additional value(41).

Prognosis

Microsatellite Instability

MSI is a particular molecular change as a hallmark of averagely 15% of CRCs. At first, these molecular changes were named “dispersed somatic mutations” in simple tandem repeats or a replication error phenotype (RER). Due to a defect in DNA-mismatch repair (MMR) system, microsatellites or short tandem repeats, repetitive sequences containing 1–6 nucleotide units up to 100 times, are prone to accumulation of mutations. It is mainly attributed to a failure inefficient DNA polymerases attachment to these repetitive sequences during DNA replication(42).

MSI is a molecular change in some different tumors such as colorectal, stomach, endometrium, ovarian, sebaceous carcinoma, glioblastomas, and lymphomas. Most of MSI CRC tumors are sporadic usually due to epigenetic silencing of MLH1 promoter because of somatic hypermethylation. These contain about 12%–15% of all CRCs in which lack of MLH1 function could lead to fast accumulation of mutations in other genes like TGF-β and BAX resulting in tumor development. Meanwhile, a somatic hereditable hypermethylation of MSH2 gene promoter has been also recently reported which is rarely occurred by some large deletion mutations in last exon of EPCAM, a gene located next to MSH2, or EPCAM-MSH2 locus. A few of MSI-CRC tumors including about 2%–3% of the all CRC tumors is related to Lynch syndrome (LS), a hereditary predisposing cancer syndrome, which is mainly due to a germline mutation in one of the four DNA-MMR genes: MLH1, MSH2, PMS2, and MSH6(42).

Tumor infiltrating lymphocytes

In the past decade immunotherapy has achieved impressive success in eradicating malignant cells by harnessing the inherent mechanisms of the host immune system, transforming the therapeutic landscape for a variety of solid and hematological malignancies. Among cancer immunotherapy strategies, immune checkpoint blockade has shown significant benefits. It is the most thoroughly studied class of immunotherapy to date, increasing the overall survival (OS) rates of patients with advanced melanoma, non-small-cell lung cancer (NSCLC), urothelial cancer(43). Immune checkpoint therapy rejuvenates T cells and allows the adaptive immune system to block immune escape caused by cascade activation of tumor-specific immune checkpoints, such as those controlled by programmed cell death protein (PD-1), programmed death-ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated protein 4 (CTLA-4). In the treatment of CRC, the PD-1 inhibitors pembrolizumab and nivolumab, which have been approved by the Food and Drug Administration (FDA), led a to durable response in patients with metastatic CRC that is mismatch-repair-deficient (dMMR) and microsatellite instability-high (MSI-H) (dMMR-MSI-H). Another inhibitor, ipilimumab, a fully-humanized monoclonal antibody that blocks CTLA-4, has also been approved by the FDA for combination with nivolumab in patients with dMMR-MSI-H CRC who have previously received chemotherapy(44).

Cytokeratin

Cytokeratins (CKs) belong to a group of approximately 20 cytoskeletal structural proteins present in epithelia and tumors derived from epithelia. CK expression is usually maintained

by neoplastic cells; therefore, specific anti-CK antibodies are widely used in routine histopathology diagnostics to determine tumor origins, particularly in metastases. CK7 is present in various ductal and glandular epithelia, including the lung, breast, skin appendages, salivary gland, pancreas, ovary, and endometrium. CK20 is widely expressed in mucosal cells of the gastrointestinal and urinary tract. Expression of CK7 is seen in most adenocarcinomas, except for those arising from the colon, prostate, kidney, thymus, carcinoid tumors, and Merkel cell tumors of the skin. CK 20 positivity is seen in most CRC cases and Merkel cell tumors. CK 20-positive staining is also observed in a subset of pancreatic carcinomas, gastric carcinomas, cholangiocarcinomas, and transitional cell carcinomas. The CK7 − /CK20 + immunoprofile has been shown to be characteristic of CRC5. It is taken as a good distinguishing marker for primary lung cancer and CRC metastatic spread to the lungs, but not all CRCs lack CK7 expression. Various studies have shown that the rate of CK7-positivity can vary from 0 to 22%(45).

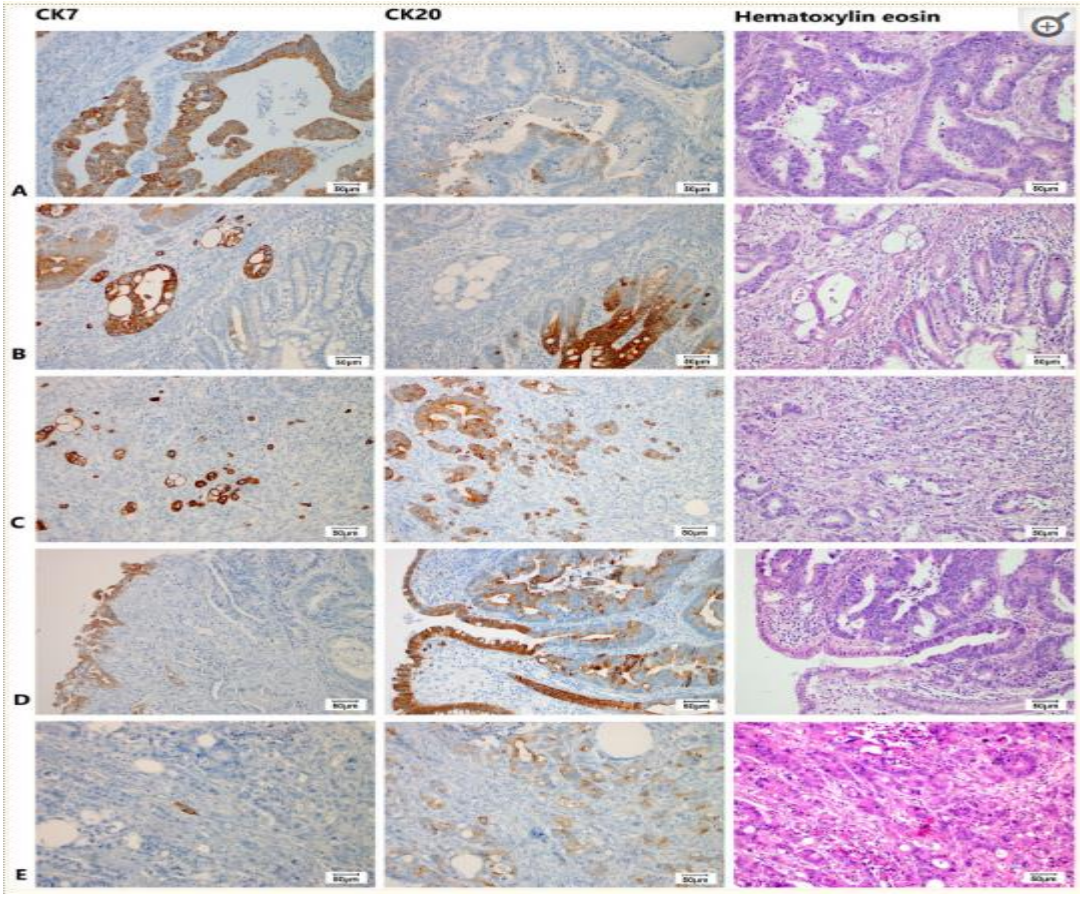

Figure 9:Histological images (magnification 200x) showing: (A) diffusely strongly CK7 + adenocarcinoma with a weak focal CK20 staining regarded as CK20- tumor; (B) diffusely strongly CK7 + and CK20- adenocarcinoma of cecum infiltrating CK7- /CK20 + non-neoplastic mucosa; (C) tumor budding cells with strong CK7 + /CK20 + ; (D) adenocarcinoma showing moderate CK7 + in ca 20% of cells and CK20 + in ca 80% of cells;

(E) adenocarcinoma with CK7 expression in isolated single cells (< 1%), and weak CK20 staining in ca 40% of cells, regarded as CK7-/CK20 + . The hematoxylin eosin images document glandular arrangement of colorectal adenocarcinoma, in line c with tumor budding(46).

Bromodomains

The regulation of gene transcription has been done by Bromodomains (BRDs) as they are recognizing this acetyl marking present in histone tails that have been targeted by chromatin-modifying enzymes and other proteins that are site-specific for chromatin. Bromodomains (BRDs) are known as “readers” as recognizing this acetyl marking in histone tails (2).

BRD4

BRD4 is a universal gene transcription regulator so the inhibition of BRD4 would be predicted to attempt universal down-regulation of gene functionality. Inhibition of BRD4 is of prime importance as it is regulating several hundred genes essential for tumorigenesis (Figure 11)(2).

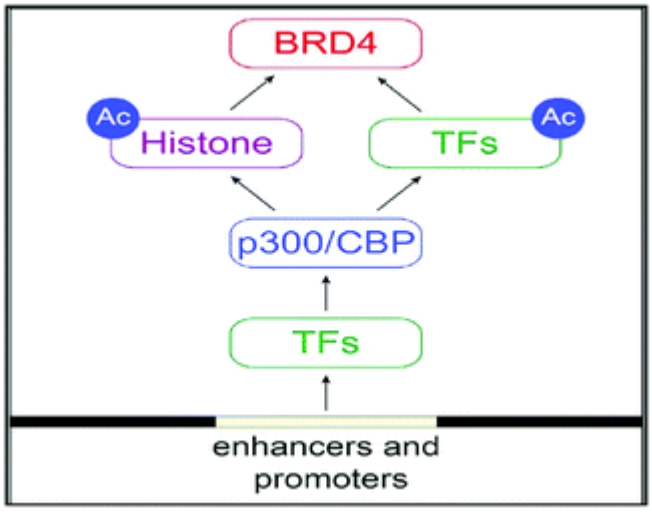

Figure 11:The BET protein BRD4 required for the functional output of an ensemble of lineage-specific transcription factors(1).

Myc

Myc is an essential gene and overexpressed in over 50% of all malignancies. The Myc family consists of three transcription factors encoded by C-MYC (MYC), L-MYC (MYCL), and N-MYC (MYCN)(47). Myc regulates gene expression via sequence-specific binding to the E-box and sequence-independent binding to RNA polymerase II-bound promoters under stress(48). Myc protein has a short half-life of 30 min and is degraded by the ubiquitin-proteasome pathway. The amino-terminal transactivation domain (TAD), the core region, and the carboxy-terminal basic-helix-loop-helix-leucine zipper (bHLH-LZ) domain are all crucial for Myc biological activity . The bHLH-LZ domain is responsible for dimerization with its critical partner Max and sequence-specific DNA binding. The TAD and core regions of Myc are the primary protein-protein interaction (PPI) areas, which include six highly conserved

regions known as Myc homology boxes (MB0, MBI, MBII, MBIIIa, MBIIIb, MBIV) (MBs). MBI regulates the proteasome-mediated breakdown of Myc protein; MBII engages in chromatin remodeling and modification; MBIIIa has a role in gene repression; MBIIIb interacts with WD repeat domain 5 (WDR5) as a glue binding to chromatin; and MBIV controls chromatin access to regions of apoptosis and cell cycle arrest regulators. Deregulation of Myc and its targets in cellular transformation is complex and context-dependent(49).

c-MYC

During CRC carcinogenesis, stabilized β-catenin exerts its oncogenic role by activating the transcription of several regulatory genes, including c-MYC. Surprisingly, in 2007, Sansom et al. found that, after APC loss, nuclear β-catenin was insufficient to induce any phenotype in the absence of c-MYC. Indeed, over half of the Wnt targets that showed significant induction after APC loss were no longer upregulated in the absence of c-MYC. These data indicate that the phenotypes acquired after APC loss in the intestine greatly depend on c-MYC target gene expression. c-MYC plays a major role in the onset and progression of CRC, where it is overexpressed in up to 80% of sporadic cases(50)

Roles of c-Myc in the Regulation of Cancer Stem Cell-Related Signaling

The denomatous polyposis coli gene (APC), a tumor suppressor gene, is usually downregulated in the CRC and is known to be a central hub in early CRC. Mutations in the APC gene often lead to altered β-catenin regulation via the Wnt signaling pathway. When the Wnt signaling pathway is deactivated, a low cytoplasmic level of β-catenin is maintained. Contrarily, when the Wnt signaling pathway is activated, the concentration of β-catenin increases in the cytoplasm and migrates to the nucleus, where it serves as transcriptional machinery for c-Myc and cyclin D1. The prognostic value of Wnt signaling for CRC patients remains debatable. Recent studies reported that CRC patients with marked Wnt and c-Myc signaling activation by gene expression-based CRC classifications show better prognosis (superior survival after relapse) (51). Another study also reported that overexpression of c-Myc protein is significantly correlated with better survival of CRC patients(52). Contrarily, a meta-analysis revealed that the accumulation of nuclear β-catenin could be a biomarker for late phase and worse survival of CRC.

In CRC, mutations of Wnt pathways induce transactivation of T cell factor-4 (TCF-4) target genes, a main transforming episode in CRC. Perturbation of β-catenin/TCF-4 activity in CRC cells produces a prompt G1 arrest and stops a physiologically active genetic program in the proliferative compartment of colon crypts. c-Myc gene is targeted in this signaling pathway, as it plays a major role in this switch by direct repression of the p21 promoter. After disruption of β-catenin/TCF-4 activity of CRC cells, the declined expression of c-Myc results in the transcription of p21, which in turn triggers G1 arrest and differentiation. Therefore, the β-catenin/TCF-4 complex represents the major switch that regulates proliferation and differentiation in healthy and malignant intestinal epithelial cells (53).

Activation of Notch signaling can upregulate many signaling pathways that favor CRC cell survival. Pathways that are activated by Notch signaling include PI3K/AKT signaling, c-Myc, and epithelial growth factor receptor (EGFR). A recent study revealed that the co-activation of the Notch pathway by mastermind-like protein (MAML)-1 could transcriptionally bind to

cyclin D1 and c-Myc promoters in CRC cell lines. As cyclin D1 and c-Myc are closely associated with cell cycle progression, the anti-cancer effect of Notch inhibitors might be related to its inhibitory effect on cell cycle progression(54).

Roles of c-Myc in Resistance of Chemotherapy

cancer therapy mainly depends on conventional chemotherapeutic agents, which mostly cause cytotoxicity in rapidly proliferating cells by damaging DNA. However, cancer cells often become refractory to chemotherapeutic agents. The phenomenon known as chemoresistance is due to either intrinsic factors or acquired ones after genotoxic therapy. The mechanisms by which tumor cells increase chemoresistance could be attributable to an increase in the machinery of cellular DNA repair, suppression of apoptosis and enhanced efflux of genotoxic drugs. A major impediment to cancer chemotherapy is the emergence of multi-drug cross-resistance. Once tumor cells get resistant to conventional chemotherapy such as cisplatin, it naturally develops cross-resistance to many other chemotherapeutic agents, leading eventually to the failure of treatment in more than 90% of patients. Therefore, the main clinical strategy for effective chemotherapy is the fast eradication of tumor cells before the emergence of chemoresistance(55).

Roles of c-Myc in Colorectal Cancer Organoids

The role of c-Myc in CRC organoids growth and proliferation are summarized below. In CRC, the ectopic expression of c-Myc by retrovirus rescues the reduction of organoid-forming efficiency and proliferation in Proliferating Cell Nuclear Antigen (PCNA)-associated factor (PAF) KO $Lgr5^{+}$ organoids, suggesting that the PAF-transactivated c-Myc (PAF-Myc axis) is required for expansion of the intestinal stem and the progenitor cells during intestinal regeneration and tumorigenesis(56). However, the ectopic expression of c-Myc could not recover the organoid development in PAF KO; $Apc^{Min/+}$ state, suggesting that other additional pathways might be implicated in the PAF-influenced CRC stemness.

Myc family proteins are significant mediators of cell proliferation, apoptosis, and differentiation. Since deregulation of c-Myc genes contributes to the development of several cancers, c-Myc is an attractive target for cancer therapy. Identification of selective inhibitors of c-Myc is necessary for the establishment of more specific and less toxic therapeutic agents that could be used alone or in combination with conventional therapy. We discussed several promising strategies regarding c-Myc inhibition. Further investigations will provide new insights into the functions of c-Myc in tumorigenesis and may give the novel therapeutic agents for colorectal cancer(53).

References

1. Muddassir M, Soni K, Sangani CB, Alarifi A, Afzal M, Abduh NAY, et al. Bromodomain and BET family proteins as epigenetic targets in cancer therapy: their degradation, present drugs, and possible PROTACs. RSC Adv. 2021;11(2):612–36.
2. Pérez-Salvia M, Esteller M. Bromodomain inhibitors and cancer therapy: From structures to applications. Epigenetics. 2017;12(5):323–39.
3. Pervaiz M, Mishra P, Günther S. Bromodomain drug discovery–the past, the present, and the future. Chem Rec. 2018;18(12):1808–17.

4. Sanchez R, Meslamani J, Zhou M-M. The bromodomain: from epigenome reader to druggable target. Biochim Biophys Acta (BBA)-Gene Regul Mech. 2014;1839(8):676–85.
5. Jin W, Tan H, Wu J, He G, Liu B. Dual-target inhibitors of bromodomain-containing protein 4 (BRD4) in cancer therapy: Current situation and future directions. Drug Discov Today. 2022;27(1):246–56.
6. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang C V. MYC, metabolism, and cancer. Cancer Discov. 2015;5(10):1024–39.
7. Dang C V. MYC on the path to cancer. Cell. 2012;149(1):22–35.
8. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463(7283):899–905.
9. Ogobuiro I, Gonzales J, Tuma F. Physiology, gastrointestinal. In: StatPearls [Internet]. StatPearls Publishing; 2022.
10. Harkins JM, Sajjad H. Anatomy, abdomen and pelvis, sigmoid colon. 2019;
11. Gaifulina R. A Study of Raman Spectroscopy as a Clinical Diagnostic Tool for the Detection of Lynch Syndrome/Hereditary NonPolyposis Colorectal Cancer (HNPCC). UCL (University College London); 2017.
12. Sokic-Milutinovic A. Appropriate management of attenuated familial adenomatous polyposis: Report of a case and review of the literature. Dig Dis. 2019;37(5):400–5.
13. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36(7):1703–19.
14. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. Int J Mol Sci. 2017;18(1):197.
15. Padmanabhan S, Waly MI, Taranikanti V, Guizani N, Rahman MS, Ali A, et al. Modifiable and non-modifiable risk factors for colon and rectal cancer. Bioact components, diet Med Treat cancer Prev. 2018;121–30.
16. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2016;gutjnl-2015.
17. Wong MCS, Huang J, Lok V, Wang J, Fung F, Ding H, et al. Differences in incidence and mortality trends of colorectal cancer worldwide based on sex, age, and anatomic location. Clin Gastroenterol Hepatol. 2021;19(5):955–66.
18. Bray F, Colombet M, Mery L, Piñeros M, Znaor A, Zanetti R, et al. Cancer Incidence in Five Continents Volume XI: Cancer Today. IARC Sci Publ. 2021;(166).
19. Chetty R, Stepner M, Abraham S, Lin S, Scuderi B, Turner N, et al. The association between income and life expectancy in the United States, 2001-2014. Jama. 2016;315(16):1750–66.
20. Hassan AM, Khalaf AM, Elias AA-K. Colorectal cancer in egypt: clinical, life-style, and socio-demographic risk factors. Al-Azhar Int Med J. 2021;2(9):6–15.
21. Sullivan BA, Noujaim M, Roper J. Cause, epidemiology, and histology of polyps and pathways to colorectal cancer. Gastrointest Endosc Clin. 2022;32(2):177–94.
22. Toh TB, Lim JJ, Chow EK-H. Epigenetics in cancer stem cells. Mol Cancer. 2017;16:1–20.
23. Loree JM, Pereira AAL, Lam M, Willauer AN, Raghav K, Dasari A, et al. Classifying colorectal cancer by tumor location rather than sidedness highlights a continuum in mutation profiles and consensus molecular subtypes. Clin Cancer Res. 2018;24(5):1062–72.
24. Steele SR, Maykel JA, Lightner AL, Bleier JIS. Steele's Colon and Rectal Surgery. Lippincott Williams & Wilkins; 2022.
25. Sabry AO, Sood T. Rectal bleeding. In: StatPearls [Internet]. StatPearls Publishing; 2023.
26. Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. N Engl J Med. 2013;369(12):1095–105.
27. Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJY, Young GP, et al. Colorectal cancer

screening: a global overview of existing programmes. Gut. 2015;64(10):1637–49.
28. Bryce C, Bucaj M. CT Colonography for Colorectal Cancer Screening. Am Fam Physician. 2021;103(1):55–6.
29. Helsingen LM, Kalager M. Colorectal cancer screening—approach, evidence, and future directions. NEJM Evid. 2022;1(1):EVIDra2100035.
30. Li C, Zheng H, Jia H, Huang D, Gu W, Cai S, et al. Prognosis of three histological subtypes of colorectal adenocarcinoma: a retrospective analysis of 8005 Chinese patients. Cancer Med. 2019;8(7):3411–9.
31. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol. 2012 Sep;3(3):153–73.
32. Messersmith WA. NCCN guidelines updates: management of metastatic colorectal cancer. J Natl Compr Cancer Netw. 2019;17(5.5):599–601.
33. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019;16(12):713–32.
34. Xie Y-H, Chen Y-X, Fang J-Y. Comprehensive review of targeted therapy for colorectal cancer. Signal Transduct Target Ther. 2020;5(1):22.
35. Backes Y, Schwartz MP, Ter Borg F, Wolfhagen FHJ, Groen JN, tot Nederveen WH de V, et al. Multicentre prospective evaluation of real-time optical diagnosis of T1 colorectal cancer in large non-pedunculated colorectal polyps using narrow band imaging (the OPTICAL study). Gut. 2018;gutjnl-2017.
36. Bhurwal A, Bartel MJ, Heckman MG, Diehl NN, Raimondo M, Wallace MB, et al. Endoscopic mucosal resection: learning curve for large nonpolypoid colorectal neoplasia. Gastrointest Endosc. 2016;84(6):959–68.
37. Ferlitsch M, Moss A, Hassan C, Bhandari P, Dumonceau J-M, Paspatis G, et al. Colorectal polypectomy and endoscopic mucosal resection (EMR): European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. Endoscopy. 2017;49(3):270–97.
38. Vişovan II, Tanțău M, Pascu O, Ciobanu L, Tanțău A. The role of narrow band imaging in colorectal polyp detection. Bosn J Basic Med Sci. 2017;17(2):152.
39. Ramirez-Rodriguez J-M, Martinez-Ubieto J, Munoz-Rodes J-L, Rodriguez-Fraile J-R, Garcia-Erce J-A, Blanco-Gonzalez J, et al. Surgical treatment for colorectal cancer: analysis of the influence of an enhanced recovery programme on long-term oncological outcomes—a study protocol for a prospective, multicentre, observational cohort study. BMJ Open. 2020;10(10):e040316.
40. Cleary RK, Morris AM, Chang GJ, Halverson AL. Controversies in surgical oncology: does the minimally invasive approach for rectal cancer provide equivalent oncologic outcomes compared with the open approach? Ann Surg Oncol. 2018;25:3587–95.
41. Jayne D, Pigazzi A, Marshall H, Croft J, Corrigan N, Copeland J, et al. Effect of robotic-assisted vs conventional laparoscopic surgery on risk of conversion to open laparotomy among patients undergoing resection for rectal cancer: the ROLARR randomized clinical trial. Jama. 2017;318(16):1569–80.
42. Zeinalian M, Hashemzadeh-Chaleshtori M, Salehi R, Emami MH. Clinical aspects of microsatellite instability testing in colorectal cancer. Adv Biomed Res. 2018;7.
43. Rosenberg JE, Hoffman-Censits J, Powles T, Van Der Heijden MS, Balar A V, Necchi A, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet. 2016;387(10031):1909–20.
44. Bai Z, Zhou Y, Ye Z, Xiong J, Lan H, Wang F. Tumor-infiltrating lymphocytes in colorectal cancer: the fundamental indication and application on immunotherapy. Front Immunol. 2022;12:808964.
45. Droy-Dupré L, Bossard C, Volteau C, Bezieau S, Laboisse CL, Mosnier J-F. Hierarchical

clustering identifies a subgroup of colonic adenocarcinomas expressing crypt-like differentiation markers, associated with MSS status and better prognosis. Virchows Arch. 2015;466:383–91.
46. Hrudka J, Fišerová H, Jelínková K, Matěj R, Waldauf P. Cytokeratin 7 expression as a predictor of an unfavorable prognosis in colorectal carcinoma. Sci Rep [Internet]. 2021 Dec 1 [cited 2023 May 16];11(1):17863. Available from: /pmc/articles/PMC8429687/
47. Dang C V. A time for MYC: metabolism and therapy. In: Cold Spring Harbor symposia on quantitative biology. Cold Spring Harbor Laboratory Press; 2016. p. 79–83.
48. Baluapuri A, Wolf E, Eilers M. Target gene-independent functions of MYC oncoproteins. Nat Rev Mol Cell Biol. 2020;21(5):255–67.
49. Dang C V, Reddy EP, Shokat KM, Soucek L. Drugging the'undruggable'cancer targets. Nat Rev Cancer. 2017;17(8):502–8.
50. Lepore Signorile M, Grossi V, Fasano C, Forte G, Disciglio V, Sanese P, et al. c-MYC Protein Stability Is Sustained by MAPKs in Colorectal Cancer. Cancers (Basel). 2022;14(19):4840.
51. Guinney J, Dienstmann R, Wang X, De Reynies A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med. 2015;21(11):1350–6.
52. Toon CW, Chou A, Clarkson A, DeSilva K, Houang M, Chan JCY, et al. Immunohistochemistry for myc predicts survival in colorectal cancer. PLoS One. 2014;9(2):e87456.
53. Elbadawy M, Usui T, Yamawaki H, Sasaki K. Emerging roles of C-Myc in cancer stem cell-related signaling and resistance to cancer chemotherapy: a potential therapeutic target against colorectal cancer. Int J Mol Sci. 2019;20(9):2340.
54. Zhou B, Lin W, Long Y, Yang Y, Zhang H, Wu K, et al. Notch signaling pathway: Architecture, disease, and therapeutics. Signal Transduct Target Ther. 2022;7(1):95.
55. Cavalcanti IDL, Soares JCS, Cavalcanti IDL, Soares JCS. Conventional cancer treatment. Adv Cancer Treat From Syst Chemother to Target Ther. 2021;29–56.
56. Kim MJ, Xia B, Suh HN, Lee SH, Jun S, Lien EM, et al. PAF-Myc-controlled cell stemness is required for intestinal regeneration and tumorigenesis. Dev Cell. 2018;44(5):582–96.