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Review article

# Omics technologies as powerful approaches to unravel colorectal cancer complexity and improve its management

Zaynab Fatfat[1], Marwa Hussein[2], Maamoun Fatfat[3], and Hala Gali-Muhtasib[1,*]

[1]Department of Biology, American University of Beirut, Beirut, Lebanon, [2]Department of Biological Sciences, Faculty of Science, Beirut Arab University, Beirut, Lebanon, [3]Department of Pharmacology and Toxicology, American University of Beirut, Beirut, Lebanon

*Corresponding author. amro@aub.edu.lb

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## ABSTRACT

**Colorectal cancer (CRC) continues to rank among the deadliest and most prevalent cancers worldwide, necessitating an innovative and comprehensive approach that addresses this serious health challenge at various stages, from screening and diagnosis to treatment and prognosis. As CRC research progresses, the adoption of an omics-centered approach holds transformative potential to revolutionize the management of this disease. Advances in omics technologies encompassing genomics, transcriptomics, proteomics, metabolomics, and epigenomics allow to unravel the oncogenic alterations at these levels, elucidating the intricacies and the heterogeneous nature of CRC. By providing a comprehensive molecular landscape of CRC, omics technologies enable the discovery of potential biomarkers for early non-invasive detection of CRC, definition of CRC subtypes, prediction of its staging, prognosis, and overall survival of CRC patients. They also allow the identification of potential therapeutic targets, prediction of drug response, tracking treatment efficacy, detection of residual disease and cancer relapse, and deciphering the mechanisms of drug resistance. Moreover, they allow the distinction of non-metastatic CRC patients from metastatic ones as well as the stratification of metastatic risk. Importantly, omics technologies open up new opportunities to establish molecular-based criteria to guide the selection of effective treatment paving the way for the personalization of therapy for CRC patients. This review consolidates current knowledge on the omics-based preclinical discoveries in CRC research emphasizing the significant potential of these technologies to improve CRC screening, diagnosis, and prognosis and promote the implementation of personalized medicine to ultimately reduce CRC prevalence and mortality.**



**Keywords:** Biomarkers discovery, Colorectal cancer, Drug resistance, Omics technologies, Personalized treatment

## INTRODUCTION

Being the second leading cause of cancer-related deaths worldwide, accounting for around 10% of all cancer cases, and the third most common cancer worldwide, colorectal cancer (CRC) is a significant global health concern. Globally, in 2020, it was estimated that CRC caused more than 930,000 deaths with more than 1.9 million new cases. The prevalence of CRC will rise to 3.2 million new cases and 1.6 million deaths annually by 2040 (an increase of 63% and 73%, respectively) (World Health Organization, 2023). The rising trends in the prevalence and mortality rates of CRC could be attributed to the challenges encountered at the various stages of its management progressing from screening, diagnosis, and treatment to cancer prognosis.

CRC is asymptomatic at its early stage, and most CRCs are diagnosed at symptomatic advanced stages, presenting a poorer prognosis (Thorsen et al., 2019). Different methods are currently used for CRC screening, and they include invasive modalities such as colonoscopy and flexible sigmoidoscopy and non-invasive ones such as guaiac fecal occult blood test (FOBT), fecal immunochemical test (FIT), and multitarget stool DNA test (Mitsala et al., 2021). However, these methods have limitations that could impact their effectiveness. The invasive ones are expensive, uncomfortable, and may cause complications and poor patient compliance (Luo et al., 2020; Thorsen et al., 2019). While the FOBT and FIT have a relatively low sensitivity for detecting tumors located in the distal colon and advanced adenomas, respectively (Herring et al., 2021; Thorsen et al., 2019). The late detection of CRC could be therefore due to the lack of early, easily measurable, sensitive, and cost-effective biomarkers that delays treatment and lowers survival rates. Therefore, the discovery of early biomarkers to be used in routine screening and CRC diagnosis is

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of high importance for the early detection of CRC which was proposed to increase the 5-year survival rate to 94% (Mitsala et al., 2021; Zamorano-Leon et al., 2020).

On the other hand, CRC is characterized by high inter-patient heterogeneity and high intra-tumor heterogeneity that could be responsible for the variation in the treatment sensitivity and prognosis for CRC patients (Dey et al., 2023; Molinari et al., 2018). Indeed, intra-patient inter-metastatic genetic heterogeneity was found to be a strong determinant of survival in patients with colorectal liver metastases. Patients with a high level of heterogeneity were reported to have a 3-year progression-free survival rate and overall survival rate of 5% and 18%, respectively, in comparison to 23% and 66% for those with low heterogeneity (Sveen et al., 2016). Therefore, characterization of the molecular features of CRC heterogeneity is paramount for better treatment and prognosis of this fatal disease. Additional challenges are encountered at the therapy stage of CRC management. About one-third of CRC patients who are treated with curative surgery will encounter disease recurrence (Boussios et al., 2019). In addition, although available therapies such as chemotherapy, immunotherapy, and targeted therapy increase patient survival, drug resistance for patients who are insensitive or become insensitive to these drugs limits their therapeutic potency (Wang et al., 2022a). This emphasizes the necessity of developing suitable biomarkers that can help detect residual disease as well as selecting patients that can benefit from available therapies, monitoring the patient's response to treatment, and adjusting the therapy regimen to combat any emerging resistance.

Addressing the various challenges encountered at the different levels of CRC management is complex, yet the adoption of omics technologies offers a promising avenue to overcome them.

Omics technologies have revolutionized cancer research by unraveling the intricacies of tumors at different dimensions ranging from the genomic, transcriptomic, proteomic, and metabolomic to epigenomic levels, thus deepening our understanding of tumor dynamics (Lu and Zhan, 2018). They have also uncovered the molecular signatures of cancer providing insight into tumor initiation, progression, and metastasis and allowing the discovery of diagnostic and prognostic markers in addition to potential therapeutic targets (Lu and Zhan, 2018). Moreover, omics technologies enabled recurrence risk stratification and prediction of cancer patients' survival and treatment response (Jiang et al., 2024; Malik et al., 2021; Tang et al., 2021a). Importantly, all of these advancements achieved through omics technologies are integral for the personalization of cancer treatment given its complex heterogeneous nature. The adoption of omics to elucidate a patient's genetic and molecular profile can help in selecting the most appropriate therapeutic agent from the available therapeutics which could prevent treatment failure and consequently reduce CRC deaths. In this review, we highlight the significant contributions of different omics technologies to the screening, diagnosis, prognosis, and treatment of CRC emphasizing the promising potential of the adoption of these technologies to overcome the current challenges in CRC management and achieve personalized medicine in this cancer.

## OMICS OVERVIEW

Biological sciences that end with omics including genomics, transcriptomics, proteomics, metabolomics, epigenomics, metagenomics, and pharmacogenomics refer to a novel discipline in which large-scale data or information across various omics levels of a specific biological sample are collected, analyzed, and integrated (Kang et al., 2022).

The primary idea behind these techniques is that a complicated system can be better understood when viewed as a whole. Furthermore, various omics disciplines and their associated data are like "pieces of a puzzle" that, when put together, create a more complete picture or understanding of a biological system or phenomenon. Each omics level provides a specific piece of information about the physiological mechanism or state, much like a puzzle piece contributes to the overall image. Genomics is the field of study of genomes, the complete set of genes, including their structure, function, mapping, evolution, and variation in addition to the gene description and interactions. Numerous subfields within the field of genomics exist; functional genomics explore genes function and expression (Przybyla and Gilbert, 2022), structural genomics assess genes sequencing and mapping (Burley, 2000), and comparative genomics identify similarities between genomes and the evolutionary changes across species (Little, 2004); transcriptomics study the full set of the genes expression, the mRNA (messenger RNA). Unlike genomics which is stable, transcriptomics dynamically changes depending on internal and external factors such as stage of development and environmental conditions, respectively (Song et al., 2019). Proteomics is the study of the complete set of proteins (proteome), their function, and interactions (Zhang et al., 2014). Proteins play a crucial role in biological processes, acting as enzymes, structural components, and signaling molecules. In particular, the proteome catalyzes the biochemical reactions that result in the production of metabolites like fatty acids, carbohydrates, lipids, and amino acids. The study of the metabolome is known as metabolomics. It aims to identify and quantify the small molecules (metabolites) having a molecular weight less than 1 kDa. Metabolomics is widely believed to be the closest omics to the phenotype (Guijas et al., 2018). Metagenomics is the study of DNA obtained from environmental samples to investigate the population of microorganisms present without needing isolated cultures. It enables genomic examination of the relationship between species having specific functions in a particular environment (Zhang et al., 2021a). The study of how genetic variation affects the pharmacological response for a certain drug is known as pharmacogenomics (Roden et al., 2019). By analyzing and integrating data from multiomics areas, a more comprehensive and holistic view of the underlying biology, potentially uncovering details about how genes, proteins, and molecules interact in the complex biological process.

## GENOMICS IN CRC

Numerous genomic approaches have been widely adopted to provide novel insights into the complex and dynamic mutational

**Table 1.** Various oncogenic alterations detected by DNA sequencing in CRC patients from different populations

| Population | Oncogenic alterations | References |
|---|---|---|
| N/A | Top-4 mutated genes: KRAS, SMAD4, TP53, and ZNF717 that is a novel mutation<br>1,057 Insertion/Deletion, including 128 exons with 17 frame-shift and 3 nonframe-shift insertions and 92 frame-shift and 11 nonframe-shift deletions<br>Significantly mutated genes: KRAS, SMAD4, TP53, APC, ZNF717, FBXW7, ZNF493, CDR1, the Armadillo repeat-containing 4 (ARMC4), and sulfate-modifying factor2 (SUMF2)<br>Most frequently mutated genes: KRAS, SMAD4, and TP53; the novel ones ZNF493, CDR1, ARMC4, and SUMF2<br>Frequently arm-level changes including gains in 1p, 1q, 2p, 10q, and 11p. Frequently deleted chromosomes: 1q, 3q, 4q, and 11q<br>Frequent amplification at 10q25.3, followed by 1p31.1, 1q44, 10q23.33, 11p15.4, and 20q13.33. Most recurrent aberrations with deletions occurring at 3q21.3 and 3q29<br>Top-6 recurrent pathway alterations: PI3K-Akt, MAPK, Rap1, regulating pluripotency of stem cells, Wnt, and oxytocin pathways | Liang et al. (2019a) |
| White, Black, Asian, Native Hawaiian, and Others | Recurrently mutated genes: APC, TP53, KRAS, PIK3CA, and SMAD4<br>Potentially novel recurrently mutated genes in MSS CRC, which included PTPRS, PIK3CG, FLT4, MAP2K4, IKZF1, JUN, TBX3, FOXP1, INHBA, and CDKN1B | Yaeger et al. (2018) |
| Taiwanese | Recurrent mutations in genes such as APC, TP53, KRAS, and FBXW7<br>Novel mutations in NRAS, PIK3CA, SOX9, APC, SMAD4, MSH3, MSH4, PMS1, PMS2, AXIN2, ERBB2, PIK3R1, TGFBR2, and ATM<br>RTK-RAS, PI3K, TGF-β, WNT, and P53 pathways, Notch signaling pathway, and alterations in cell adhesion molecules | Chang et al. (2019) |
| Malaysian | Top-ten most frequently mutated genes: APC, TP53, KRAS, MUC4, TCF7L2, CCDC168, FAT3, KMT2C, LRP1B, PCLO, SCN1A, and SPEG<br>Top significantly mutated genes: APC, TP53, KRAS, TCF7L2, and ACVR2A<br>Novel, nonsynonymous mutation that led to amino acid substitutions in 3 genes: KDM4E, MUC16, and POTED | Mohd Yunos et al. (2022) |
| Chinese | Top high-confidence significantly mutated genes: TP53, APC, KRAS, FBXW7, and CTNNB1<br>Eight novel high-confidence significantly mutated genes: LYST (lysosomal trafficking regulator), DAPK1 (death-associated protein kinase 1), CR2 (complement receptor type 2), KIF16B (kinesin family member 16B), NPIPB15 (nuclear pore complex-interacting protein family member B15), SYTL2 (synaptotagmin-like protein 2), ZNF91 (zinc finger protein 91), and KIAA0586 (encodes the protein TALPID3, a centrosomal protein that is essential for primary cilia formation)<br>Recurrent somatic copy number alterations including gains at chromosomes 1q, 7, 8q, 13q, and 20q and losses at 1p, 4, 5q, 8p, 14q, 15q, 17p, and 18p and recurrent alterations in focal regions, including the gain at 10q and loss at 20p | Zhao et al. (2022) |

landscapes of colorectal tumors which are clinically relevant for prognostic and therapeutic purposes.

DNA sequencing of colorectal tumors allowed to identify aberrant alterations in the genome of CRC patients, including the somatically acquired single-nucleotide variants, multiple nucleotide variants, structural variants, insertions/deletions, copy number variants, and significantly mutated genes in addition to the frequently altered pathways (Chang et al., 2019; Liang et al., 2019a; Mohd Yunos et al., 2022; Yaeger et al., 2018), showing that CRC displays vast and complex inter-patient genomic heterogeneity. Table 1 shows the diverse oncogenic aberrations detected by DNA sequencing in CRC patients from different populations. In a recent study, ultradeep whole-exome sequencing on 1015 patients with CRC led to the

identification of 46 high-confidence significantly mutated genes among which 8 genes have not been reported in other databases and were suggested to be involved in CRC development or progression. It also helped to identify 52 substitution hotspots in 14 genes as well as the significantly mutated pathways, namely PI3K, cell cycle, and RTK-RAS pathways (Zhao et al., 2022). In another report, nanopore long-read sequencing in CRC samples allowed to detect large-scale inversions that were reported to alter the expression of the tumor suppressor gene APC and the structure of the tumor suppressor gene CFTR. Additionally, it allowed the identification of a novel gene fusion *RNF38-RAD51B* that was demonstrated to increase the migration and invasion of CRC cells in vitro and metastasis in vivo (Xu et al., 2023).

In addition to detecting oncogenic alterations in the genome of CRC patients, DNA sequencing allowed to compare the genomic landscapes of primary tumors with their corresponding metastases. Whole-genome sequencing of lung and liver metastases and their matched primary colorectal tumors showed that their genomic landscapes are very similar but not identical. While primary tumors and matched metastases shared an average of 65% of all somatic single-nucleotide variations, an average of 15% was found specific to tumors and an average of 19% specific for metastases. Moreover, numerous pathways were found significantly enriched in metastases, namely extracellular matrix, hepatic fibrosis/stellate cell and actin cytoskeleton cascades, PI3K-Akt signaling, and focal adhesion-related pathways. Sequencing also helped to identify clinically relevant metastasis-specific mutations (Ishaque et al., 2018). Another report compared the genomic landscape of brain metastasis with the primary CRC by whole-exome sequencing and whole-genome sequencing. The results showed a more pronounced mutational signature of homologous recombination deficiency and mismatch repair deficiency in brain metastasis compared with the primary CRC. This event was suggested to progress early and independently from the gradual accumulation of numerous mutations associated with aging at the primary CRC. Analysis of DNA sequencing also helped to identify potential drivers of brain metastasis, such as voltage-gated sodium channels (SCN7A and SCN2A) that could serve as candidate biomarkers or therapeutic targets (Sun et al., 2019). In another study, targeted sequencing was adopted to evaluate differences between right- and left-sided primary microsatellite stable metastatic CRC (mCRC). Compared with the left-sided primary mCRC, the right-sided ones were found to be associated with shorter survival, older age at diagnosis, and enhanced number of mutations in KRAS, BRAF, PIK3CA, AKT1, RNF43, and SMAD4. As for left-sided primary tumors, they were found not to harbor identifiable genetic alteration in mitogenic signaling but highly expressed mitogenic ligands (Yaeger et al., 2018).

On the other hand, genetic mutations revealed by DNA sequencing can predict the overall survival of CRC patients. Detection of genomic alterations in a large CRC cohort helped in the classification of CRC into 4 subtypes having different overall survival outcomes, namely hypermutated, chromosome instability with high risk, chromosome instability with low risk, and genomic stable. While patients of the hypermutated group had the longest median overall survival, patients with chromosome instability with high risk had the poorest overall survival in comparison with the other groups (Zhao et al., 2022). Additionally, mitochondrial DNA copy number was reported to predict the survival outcome of CRCs. CRC patients with low mitochondrial DNA copy number had a better overall survival than those with extremely high mitochondrial DNA copy number (Zhao et al., 2022). In another study, Lin et al. (2021) performed targeted deep sequencing of colorectal tumors and reported that sequential and co-occurring of DNA damage response genetic mutations affected the survival of CRC patients in stage III and treated with adjuvant oxaliplatin-based chemotherapy. Evolutionary trees were constructed, and sequential somatic mutations were extracted. CRC patients harboring germline followed by ATM or BRCA2 somatic mutations or non-TP53, APC somatic mutations had a better clinical outcome compared with patients without such mutations. In addition, the occurrence of BRCA2 and TP53 somatic mutations resulted in a worse prognosis than only BRCA1 somatic mutations in CRC patients. The order of the sequential somatic mutations is also associated with clinical outcomes. The 4-year recurrence-free survival probabilities were reported to be significantly higher for patients where APC mutations occurred before KRAS or BRCA2 mutations, compared with those where APC mutations occurred after KRAS or BRCA2 mutations (Lin et al., 2021).

In addition to tumor biopsies, researchers rely on liquid biopsies that allow the evaluation of circulating tumor DNA (ctDNA). Interestingly, sequencing of ctDNA from patients with mCRC was demonstrated to reflect the somatic mutational landscape of primary tumors. Serial ctDNA profiling during first-line therapy revealed genomic temporal dynamics and heterogeneity of mCRC and their correlations with the clinical outcomes highlighting the importance of subsequent and real-time DNA sequencing in making informed treatment decisions and improving patients' survival (Wang et al., 2022b). In another report, early change in the ctDNA level was suggested to predict the chemotherapeutic effectiveness and clinical outcomes in mCRC patients. Eight weeks after initiation of chemotherapy, patients with lower ctDNA levels were reported to have significantly longer progression-free survival and overall survival than the patients with greater ctDNA levels using amplicon-based deep sequencing with a molecular barcode (Osumi et al., 2019). In addition, the adoption of whole-exome sequencing and a personalized, tumor-specific, multiplex polymerase chain reaction (PCR)-based next-generation sequencing allowed to detect postoperative ctDNA in CRC, monitor the efficacy of adjuvant chemotherapy, detect residual disease before and after adjuvant chemotherapy in addition to early detection of cancer recurrence, and identification of actionable mutations (Reinert et al., 2019).

## TRANSCRIPTOMICS IN CRC

RNA sequencing allowed to detect differentially expressed genes, lncRNAs, 5′-tRNA-derived small RNA fragments, circRNAs, and Piwi-interacting RNAs (piRNAs) in CRC compared with the matched normal tissues having promising implications in CRC staging, early diagnosis, prognosis, prevention, and treatment.

**Table 2.** Potential transcriptomic biomarkers with promising implications for CRC diagnosis and treatment

| Category | Promising transcriptomic biomarkers | References |
|---|---|---|
| Differentially expressed genes | CD44, ANXA3 | Kim et al. (2021) |
| microRNAs | miR-23b | Kou et al. (2016) |
| | miR-141 | Cheng et al. (2011) |
| | miR-210 | Wang et al. (2017) |
| Long noncoding RNAs | AC021218.2 | Yamada et al. (2018) |
| | LINC00858 | |
| | RP11-138J23.1 | |
| | RP11-435O5.2 | |
| | RPPH1 | Liang et al. (2019b) |
| | HOTTIP | Oehme et al. (2019) |
| | LINC02418 | Zhao et al. (2019) |
| 5′-tRNA-derived small RNA fragments | 5′-tRF-GlyGCC | Wu et al. (2021) |
| Circular RNAs | circRNA0000392 | Xu et al. (2020a) |
| Piwi-interacting RNAs | piR-18849, piR-19521, piR-17724 | Yin et al. (2019) |

RNA sequencing allowed the evaluation of the differentially expressed genes among high-risk adenoma, advanced CRC, and normal controls and consequently the identification of CD44 and ANXA3 as potential markers of precancerous high-risk colorectal adenoma. These markers were suggested to be associated with the adenoma-carcinoma sequence and cancer progression. Their elucidation could possibly help in the early detection of high-risk adenoma and consequently the prevention of the occurrence and progression of advanced CRC (Kim et al., 2021).

In another study, RNA sequencing allowed the identification of 72 lncRNAs that are significantly dysregulated in CRC compared with matched normal mucosae tissues. Four lncRNAs, AC021218.2, LINC00858, RP11-138J23.1, and RP11-435O5.2 were found to be upregulated in CRC which was further validated by real-time RT-PCR. It is important to note that the expression of the 4 lncRNAs was elevated in colorectal adenomas tissues, and did not increase during the course of CRC progression, suggesting that they are involved in the very early steps of the malignant process and could serve as potential biomarkers for early detection of CRC (Yamada et al., 2018). Other deregulated lncRNAs such as RPPH1 (Liang et al., 2019b), HOTTIP (Oehme et al., 2019), and LINC02418 (Zhao et al., 2019) could also serve as potential CRC biomarkers.

Moreover, Wu et al evaluated the profiles of 5′-tRNA-derived small RNA fragments (5′-tRF) in the plasma of CRC patients and healthy controls using small RNA high-throughput sequencing. Both profiles were significantly different and a significant increase of 5′-tRF-GlyGCC was reported in CRC plasma. Interestingly, their real-time PCR results further showed the upregulated expression of 5′-tRF-GlyGCC in the plasma of CRC patients, its increase with the progression and metastasis of CRC, and its significant correlation with that in the paired CRC tissues. Importantly, 5′-tRF-GlyGCC was reported to have a better diagnosis value than that of the current tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 199 (CA199) and its combination with these markers further enhanced its diagnostic value. These findings demonstrated that the plasma level of 5′-tRF-GlyGCC could be a promising diagnostic biomarker for CRC (Wu et al., 2021).

Furthermore, RNA sequencing allowed to explore the differentially expressed circRNAs in CRC tissues. The expression of circRNA0000392 was significantly upregulated in CRC tissues compared with the adjacent normal tissues, which was further confirmed by qRT-PCR. Its expression was also significantly correlated with the neoplastic progression of CRC. The function of circRNA_0000392 in CRC was also elucidated; circRNA_0000392 could function as an oncogene by being the sponge of miR-193a-5p, therefore regulating the expression of PIK3R3 as well as the activation of the AKT-mTOR pathway in CRC cells. Collectively, these findings suggested that circRNA_0000392 could be a potential therapeutic target for treating CRC and a predictive marker for CRC patients (Xu et al., 2020a).

In another report, small RNA sequencing demonstrated alterations in the expression profiles of piRNAs in CRC tissues compared with adjacent non-tumor tissues. It allowed the identification of a total of 35 differentially expressed piRNAs in CRC tissues, including 33 upregulated piRNAs and 2 downregulated piRNAs. The level of expression of the 3 most upregulated piRNAs (piR-18849, piR-19521, and piR-17724) was further validated by RT-qPCR. The upregulation of piR-18849 was also reported to be associated with lymph node metastasis in CRC patients (Yin et al., 2019).

Besides using tumor and plasma samples from patients to perform RNA sequencing, Xu et al. (2022) performed transcriptome sequencing for platelets isolated from healthy donors and patients with CRC or non-cancerous intestinal diseases (ulcerative disease, Crohn's disease, polyps, and adenomas). RNA sequencing followed by bioinformatics analysis allowed to non-invasively detect CRC at an early stage and predict its staging in addition to distinguishing it from nonmalignant diseases (Xu et al., 2022).

Beyond their diagnostic and therapeutic potential, dysregulated non-coding RNAs also play a crucial role in prognosis. Increased plasma levels of miR-23b are associated with longer

survival (Kou et al., 2016), whereas high levels of circulating miR-141 (Cheng et al., 2011) and miR-210 (Wang et al., 2017) are linked to shorter survival rates (Table 2).

## PROTEOMICS IN CRC

Two biomarkers, the fibroblast growth factor 21 (FGF-21) and pancreatic prohormone (PPY) also known as pancreatic polypeptide, were identified to be associated with colon and rectal cancer, respectively, offering the opportunity to differentiate between the subtypes of CRC (Harlid et al., 2021).

In addition, the proteomic profiles of patients with CRC were shown to differ according to the age. The differences in protein expression between CRC and normal tissues were much larger in old CRC patients than in young CRC patients. NHP2 was significantly upregulated in tumors of old CRC patients to a higher extent than in tumors of young CRC patients and was associated with a poor prognosis in old patients. Additionally, proteomic analysis allowed the identification of various hallmark proteins with different expression in patients of different age groups that can be potential therapeutic targets (Gong et al., 2021).

Proteomic and phosphoproteomic analysis of the CRC and adjacent normal tissues allowed the identification of the differentially expressed proteins and phosphoproteins in CRC tissues as well as the understanding of their possible biological processes, molecular function, and signaling pathways. The combined analysis of phosphoproteomics and proteomics reported TMC5, SMC4, and SLBP as significantly upregulated differential proteins and VSIG2 and NDRG2 as significantly downregulated differential proteins within CRC tissues with respect to the adjacent normal tissues. The in vitro analysis of the expression and role of SLBP in CRC further validated the omics results. The expression of SLBP was reported to be upregulated in CRC cell lines in comparison with normal colorectal epithelium cell line. In addition, knockdown of SLBP resulted in a significant decrease of the proliferation and migration of CRC cells in vitro, suggesting that SLBP could be a candidate diagnostic biomarker or a therapeutic target for CRC (Zhu et al., 2023).

Another study reported that the protein expression pattern differs between paracancerous tissue and CRC or adenomatous polyp tissue. It also reported that the protein expression pattern of adenomatous polyps seemed to be more similar to that of CRC. Although CRC oncogenes like KRAS and NRAS or tumor suppressor genes like APC and PTEN were reported to be frequently mutated, their expression was not significantly changed at the protein level. This highlights the importance of personalized diagnosis and screening at the protein level and not only at the genomic level. Moreover, this study reported 3 proteins, transferrin receptor protein 1 (TFR1), adenosylhomocysteinase (SAHH), and immunoglobulin heavy variable 3–7 (HV307) being significantly differentially expressed in tissue and plasma samples of CRC. They were also reported to be promising biomarkers for CRC and adenomatous polyps screening (Tang et al., 2021b).

It is important to note that proteomics analysis also helped to detect non-invasively diagnostic biomarkers in the body fluid such as serum and urine. For example, Tpm3 was identified as a potential serological biomarker for the early non-invasive detection of CRC (Thorsen et al., 2019). Another study identified 3 urinary biomarkers (CORO1C, ARPC5, and RAD23B) for CRC diagnosis in addition to 4 urinary biomarkers (CORO1C, RAD23B, GSPT5, and NDN) for CRC metastatic risk stratification. This signature allowed the differentiation of the non-metastatic group from the CRC with lymph node metastasis and CRC with distant metastasis. In these 3 CRC groups, the levels of 5 reported urinary proteins increased with disease progression reaching the highest levels in distant metastasis. The performance of the signatures was assessed and compared with that of FIT or serum CEA adopted for the early detection and evaluation of metastasis in CRC, respectively. Combination of the produced diagnostic signature with FIT resulted in a significantly increased sensitivity in comparison with FIT alone. In addition, the produced metastatic signature properly predicted more than 50% of serum CEA-negative metastatic patients (Sun et al., 2022a).

In addition to its potential diagnostic applications, proteomic analysis can also aid in predicting cancer recurrence. Proteomic analysis of tissue-derived small extracellular vesicles enabled the identification of protein panels that may be associated with CRC relapse. Protein profiling of small extracellular vesicles isolated from CRC tissues and paired adjacent tissues was compared between relapsed and non-relapsed patient groups. Bioinformatics analysis revealed that relapsed patients exhibited relatively poorer immune responses and an activated chronic inflammatory state compared with non-relapsed patients. Notably, the adjacent tissues of relapsed patients showed significant expression of 4 proteins HLA-DPA1, S100P, NUP205, and PCNA which could be explored as potential biomarkers for recurrence risk assessment during postoperative follow-ups (Ji et al., 2021) (Table 3).

## METABOLOMICS IN CRC

At the metabolomics level, the detailed analysis and quantification of metabolites related to a specific phenotype has the potential to understand the involved mechanism (Chen et al., 2022a) and to improve precision medicine application (Clish, 2015). As mentioned early, metabolome is the collection of metabolites (fatty acids, amino acids, lipids, vitamins, and carbohydrates) present in a biological system such as cell, tissue, and organs; it is dynamic and varies constantly depending on the state of the cell. Metabolomics is the study of metabolome using bioinformatics tools. The whole process goes through many steps: first, the metabolites are isolated and characterized using mass spectrometry or nuclear magnetic resonance techniques (Beisken et al., 2015); then, the raw data obtained is analyzed by software like MZmine3 (Schmid et al., 2023) or XCMS (Smith et al., 2006) to quantify the metabolites. Then, a quality control step is required to separate the low- and high-quality data (Mattoli et al., 2023). After that, a standard statistical analysis like ANOVA should be performed to detect the significant change in specific metabolites (Reshetova et al., 2014), which are finally linked to their biological function or

**Table 3.** Potential proteomic biomarkers with promising implications for CRC screening, diagnosis, metastatic risk stratification, and recurrence risk assessment

| Category | Potential proteomic biomarkers | References |
|---|---|---|
| Anatomical location-associated protein expression | FGF-21 (colon)<br>PPY (rectum) | Harlid et al. (2021) |
| Age-associated protein expression | NHP2 (old patients) | Gong et al. (2021) |
| Biomarkers for screening and diagnosis | SLBP<br>TFR1<br>SAHH<br>HV307 | Zhu et al. (2023)<br>Tang et al. (2021b) |
| | Tpm3<br>CORO1C<br>ARPC5<br>RAD23B | Thorsen et al. (2019)<br>Sun et al. (2022a) |
| Biomarkers for metastatic risk stratification | CORO1C<br>RAD23B<br>GSPT5<br>NDN | Sun et al. (2022a) |
| Biomarkers for recurrence risk assessment | HLA-DPA1<br>S100P<br>NUP205<br>PCNA | Ji et al. (2021) |

CRC, colorectal cancer; SAHH, adenosylhomocysteinase; TFR1, transferrin receptor protein 1; HV307, heavy variable 3-7.

pathway (Chong et al., 2018) using platform software such as PaintOmics3 (Hernandez-de-Diego et al., 2018).

Throughout history, the diagnosis of complex metabolic disorders was possible using a limited number of metabolites. Currently, omics approaches led to the discovery of a large number of metabolites allowing a better understanding of metabolic disorder with more accurate therapy. In 1934, phenylketonuria was described as a newborn error of metabolism and discovered by the excretion of phenylpyruvic acid in the urine (Følling, 1994). Later on, blood analysis for phenylalanine was done to detect the presence of phenylketonuria since the metabolism of phenylalanine was blocked. In our days, the omics approaches identify around 118 metabolites that could explain the main causes of the disease, an elevated concentration of phenylalanine, and a low level of tyrosine (Blasco et al., 2017).

Data generated from omics analysis can perfectly serve the identification of metabolic biomarkers for the diagnosis of mCRC. Moreover, the analysis and comparison of the urine, saliva, and serum from CRC patients and healthy people could help in the detection of altered metabolites related to CRC. To date, more than 32 studies have been conducted using urine samples for metabolomics analysis in CRC patients. A meta-analysis done in 2021, revealed that CRC can be determined by the evaluation of the metabolome in the urine samples. The analysis covered 28 studies for CRC and adenomas. In addition to the metabolome, the volatilome, which is the volatile part of the metabolome, was also addressed (Mezmale et al., 2023). Consequently, 244 compounds were found and proposed as associated with CRC; up to 11 compounds were hypothesized to be particular biomarkers of CRC in the urine samples: hippuric acid, pyruvic acid, tartaric acid, and hydroquinone were the downregulated metabolites, along with the volatiles 1,1,6-trimethyl-1,2-dihydronaphthalene, butyraldehyde, and ether. The upregulated metabolites were N1, N12-diacetylspermine, 3-hydroxybutyric acid, L-histidinol, and L-dopa (Mallafre-Muro et al., 2021). A multicenter study published in 2019 defined the upregulation of kynurenine as a specific biomarker of CRC (Deng et al., 2019). Another meta-analysis study done in 2018, including 41 studies, identified nucleosides as promising biomarkers for CRC, since their concentrations in CRC patients were higher than the controls (Erben et al., 2018). More recently, using nuclear magnetic resonance technology, Brezmes et al. (2022) identified 2 downregulated metabolites, creatinine and hippuric acid, in the urine of patients with CRC and advanced adenomas compared with control (Brezmes et al., 2022). Furthermore, p-hydroxyhippurate, N1, N12-diacetylspermine, hippurate, and glutamate were believed to be specific biomarkers capable of distinguishing CRC patients from healthy controls (Zhang et al., 2023).

Besides these discoveries, many studies showed the potential of polyamines to screen CRC. Both N1,N12-acetylspermidine (Hiramatsu et al., 2005; Kawakita et al., 2011) and N1,N8-acetylspermidine have been reported as possible biomarkers for CRC detection (Nakajima et al., 2018; Umemori et al., 2010). These 2 susceptible biomarkers were also detected in saliva samples and proved their ability to differentiate CRC patients from normal controls. However, N1,N8–diacetylspermidine along with N1-acetylspermine showed higher discriminability than N1,N12–diacetylspermine (Kuwabara et al., 2022). In line with these results, another study was done in 2021, for polyamines identified N1-acetylspermine as a promising biomarker for CRC (Igarashi et al., 2021).

Gas-chromatography/mass-spectrometry-based serum metabolome analysis confirmed an upregulation in cystamine, kynurenine, 2-hydroxybutyrate, and aspartic acid in CRC patients with 85% specificity and sensitivity (Nishiumi et al., 2012). Benzoic acid was also reported for its potential as a novel biomarker for CRC diagnosis (Uchiyama et al., 2017). Qiu et al identified pyruvic acid, lactic acid, tyrosine, uridine, and tryptophan as altered metabolites in patients with CRC and could help in CRC screening (Qiu et al., 2009). Long et al. (2017) showed that compared with controls, polyps and CRC cases displayed lower levels of xanthine and hypoxanthine and higher levels of D-mannose. Zhang et al. (2021b) identified 2 metabolic pathways that may be activated in CRC tissues (the vitamin B metabolic pathway and the alanine, aspartate, and glutamate metabolic pathways), and 4 possible metabolic biomarkers (formylanthranilic acid, hexadecanedioic acid, 2-pyrocatechuic acid, and 4-dodecylbenzenesulfonic acid) that have been found may be used to distinguish CRC patients from healthy controls. Furthermore, 2 proteins, plasma kallikrein and coagulation factor XIII A chain, as well as 3 metabolites, hydroquinone, leucenol, and sphingomyelin, were identified as possible biomarkers for advanced CRC (Rao et al., 2021). Gut microbiome-associated metabolites in serum (GMSM) was also investigated and showed alteration

of 8 GMSM between patients with CRC or adenoma and healthy subjects, suggesting its potential in CRC and adenoma detection (Chen et al., 2022b). Serum metabolomic profiles have been also used to differentiate between locoregional CRC, liver-only metastases, and mCRC. The profile changed significantly with metastasis; sera from patients with extrahepatic metastases showed higher levels of isoleucine and 2-oxoglutarate, whereas liver-only metastases showed higher levels of fumarate and methionine (Farshidfar et al., 2012).

Metabolomics power is not limited to the identification of biomarkers; it can predict the response to neoadjuvant chemotherapy in patients with CRC. Due to the heterogeneity of CRC disease, neoadjuvant chemotherapy may only be beneficial for a limited number of CRC patients. So, a precise prediction of neoadjuvant chemotherapy response in CRC patients would be extremely helpful in designing personalized medicine which could improve their overall survival and clinical outcomes. Among 57 CRC patients treated with neoadjuvant chemotherapy, only 27 were diagnosed as responsive. Plasma metabolomics was carried out to find possible biomarkers that can predict neoadjuvant chemotherapy response for CRC. Responsive patients displayed significant differences in the concentrations of 9 metabolites compared with non-responsive ones and were shown to be strongly correlated with neoadjuvant chemotherapy sensitivity of CRC (dioleoyl lecithin, anthranilic acid, leucine, glucose, s CE(18:2(9Z,12Z)), SM(d16:1/18:1), PC(18:1(9Z)e/2:0) and PC(16:1/22:6), and 11-keto-beta-boswellic acid) (Yang et al., 2018) (Table 4).

## EPIGENOMICS IN CRC

Epigenomics is the part of omics that studies the alterations of gene activity by chemical modifications (ie, methylation and acetylation), enzymes (ie, DNA methyltransferase), or some forms of RNA (ie, microRNA). Such modifications can alter the histone proteins or the DNA without interfering with the DNA sequence and regulate the expression/activity of the gene (Weinhold, 2006). One of the most interesting applications of epigenomics is the identification of differentially methylated promoters as biomarkers for the diagnosis of CRC. When it comes to epigenomic indicators, DNA methylation is the most researched epigenetic marker in CRC. Methylation microarrays,

**Table 4.** Potential metabolomic biomarkers for CRC diagnosis

| Study | Potential metabolomic biomarkers | Reference |
|---|---|---|
| Meta-analysis | Hippuric acid, pyruvic acid, tartaric acid, and hydroquinone | Mallafre-Muro et al. (2021) |
| | N1, N12-diacetylspermine, 3-hydroxybutyric acid, L-histidinol, and L-dopa | |
| | 1,1,6-trimethyl-1,2-dihydronaphthalene, butyraldehyde, and ether | |
| Multicenter study | Kynurenine | Deng et al. (2019) |
| Meta-analysis | Nucleosides | Erben et al. (2018) |
| | Creatinine, hippuric acid | Brezmes et al. (2022) |
| | p-Hydroxyhippurate, N1, N12-diacetylspermine, hippurate, and glutamate | Zhang et al. (2023) |
| Polyamine studies (various) | N1,N12-acetylspermidine, N1,N8-acetylspermidine | Hiramatsu et al. (2005) |
| | N1,N8-diacetylspermidine, N1-acetylspermine | Nakajima et al. (2018) |
| Polyamine study | N1-acetylspermine | Kuwabara et al. (2022) |
| GC/MS serum metabolomics | Cystamine, kynurenine, 2-hydroxybutyrate, and aspartic acid | Nishiumi et al. (2012) |
| | Benzoic acid | Uchiyama et al. (2017) |
| | Pyruvic acid, lactic acid, tyrosine, uridine, and tryptophan | Qiu et al. (2009) |
| | Xanthine, hypoxanthine | Long et al. (2017) |
| | D-Mannose | |
| | Formylanthranilic acid, hexadecanedioic acid, 2-pyrocatechuic acid, and 4-dodecylbenzenesulfonic acid | Zhang et al. (2021b) |
| | Vitamin B pathway, alanine/aspartate/glutamate metabolic pathways | |
| Plasma protein study | Plasma kallikrein, coagulation factor XIII A chain | Rao et al. (2021) |
| | Hydroquinone, leucenol, and sphingomyelin | |
| Gut microbiome-associated metabolites | Eight altered GMSM | Chen et al. (2022b) |
| Serum metabolomics (metastasis) | Isoleucine, 2-oxoglutarate (extrahepatic metastases) | Farshidfar et al. (2012) |
| | Fumarate, methionine (liver-only metastases) | |
| Neoadjuvant chemotherapy response study | Dioleoyl lecithin, anthranilic acid, leucine, glucose, s CE (18:2(9Z,12Z)), SM(d16:1/18:1), PC(18:1(9Z)e/2:0), PC(16:1/22:6), and 11-keto-beta-boswellic acid | Yang et al. (2018) |

GMSM, gut microbiome-associated metabolites in serum; MS, mass spectrometry.

reduced bisulfite sequencing, and whole-genome bisulfite sequencing are among the approaches used to find methylation markers; PCR is primarily the technique used to validate candidate biomarkers (Gallardo-Gómez et al., 2022).

Several research efforts that have identified biomarkers for DNA methylation in the blood have focused on particular ethnic groups but also on small numbers of individuals with varying characteristics. Choosing a biomarker should take into account the fact that a study has demonstrated that the global leukocyte DNA methylation in peripheral blood can vary by gender and race/ethnicity (Zhang et al., 2011). The majority of research for HPP1 and HLTF genes methylation was conducted in Germany (Philipp et al., 2012, 2014), among the same ethnic populations; these findings should be confirmed between alternative ethnic groups. DNA methylation seen in CRC tissues has been demonstrated to vary throughout populations, according to studies (Fraser et al., 2012; Xia et al., 2014). Compared with Iranian, African-American CRC patients showed higher methylation for promotors of the GPNMB, ICAM5, and CHD5 genes even though both populations displayed hypermethylation for SYNE1, MMP2, APC2, GPNMB, EVL, PTPRD, and STARD8 genes (Mokarram et al., 2009). Such studies highlight the importance of using different ethnicity when looking for biomarkers.

A paired tissue/serum study revealed a correlation between the hypermethylation status of RUNX3 and SFRP1 genes and the absence of their mRNA expression (Pasha et al., 2019). A recent study confirmed that as the disease progressed, the methylation levels of RUNX3 in peripheral blood mononuclear cells progressively increased (Mosallaei et al., 2023). Regardless of the stage of the tumor, methylation of the NEUROG1 gene was commonly seen in the serum of patients with CRC (Herbst et al., 2011). MMEPD2 was claimed to be hypermethylated in CRC and it might be used as a biomarker for early detection (Gu et al., 2019). Using a methylation-sensitive restriction enzyme-coupled qPCR system, Pulverer et al. (2021) were able to identify 6 hypermethylated genes (WT1, PENK, SPARC, GDNF, TMEFF2, and DCC). The stool DNA ColoDefence test demonstrated power in the detection of methylated biomarkers such as SEPT9 and SDC2 (Zhao et al., 2020). In line with this, Sobanski et al. (2021) observed that compared with control, in CRC, SEPT9 methylation occurs at a higher frequency (92%) in addition to a high methylation rate for ALX4 (85.1%). Other studies have proposed that the combination of both SPET9 and ALX4 genes is probably a more useful biomarker (He et al., 2010; Tanzer et al., 2010). According to a Korean study based on methylation data from high-throughput microarrays, CpG island methylator phenotype-high tumors were strongly associated with MLH1 hypermethylation (Joe et al., 2023).

Beyond DNA methylation, nucleosome positioning, and histone modifications are also key epigenetic mechanisms altered in CRC (Walker et al., 2022). CRC tumors display disrupted patterns in histone modifications, such as methylation of Lysine 9 on histone H3 (H3K9me) (Liu et al., 2013) and acetylation of Lysine 27 on histone H3 (H3K27ac) (Karczmarski et al., 2014). These modifications alter chromatin structure and gene expression, particularly around transcription start sites, driving tumorigenesis (Hesson et al., 2014).

In CRC patients, circulating nucleosomes in plasma exhibit distinct signatures of histone post-translational modifications, including upregulation of H3K9 and H3K27 methylation, H3 acetylation, and citrullination of histone H2A1R3 (Van den Ackerveken et al., 2021). Additionally, high concentrations of circulating nucleosomes in CRC patients correlate with disease progression, suboptimal therapeutic responses, and diminished survival rates (Fahmueller et al., 2012). Furthermore, nucleosome occupancy is correlated with cell free DNA fragmentation (Ivanov et al., 2015), which is considered an early epigenetic biomarker for CRC (Cristiano et al., 2019) and useful in predicting immunochemotherapy response (Rodriguez-Casanova et al., 2021) (Table 5).

## MULTIOMICS APPROACHES IN CRC

Single omics approach only reveals a limited view of the complex and heterogeneous biology of the tumor. In contrast, integration of multiple omics offers greater potential to reveal a holistic view of cancer biology. In CRC research, there is a growing trend toward the adoption of integrated multiomics analysis to improve our insights into tumor behavior across several biological layers.

As spatial transcriptomics were not extensively discussed in the previous “Transcriptomics in CRC” section, this section will place greater emphasis on the findings derived from its integration with single-cell transcriptomics. Then, the discussion shifts to other multiomics approaches that offer complementary insights into cellular and molecular processes in CRC.

**Table 5.** Potential epigenomic biomarkers for CRC diagnosis

| Category | Promising epigenetic biomarkers | References |
|---|---|---|
| DNA methylation | HPP1, HLTF<br>C9orf50, KCNQ5, and CLIP4<br>CpG methylation | Philipp et al. (2012, 2014)<br>Jensen et al. (2019)<br>Li et al. (2022) |
| Hydroxymethylation | 5-hydroxymethylcytosine (5hmC) | Gao et al. (2019) |
| Histone modifications | H3K27me3 (trimethylation of Lysine 27 on histone H3)<br>H2AK119Ub, H3K9Ac, H3K27Ac, H3K9Ac, and H4K20me3 | Gezer et al. (2015)<br>Rahier et al. (2017) |
| Nucleosome | Nucleosome concentration<br>DNA fragmentation | Fahmueller et al. (2012)<br>Cristiano et al. (2019) |

### Integration of Spatial Transcriptomics With Single-Cell Transcriptomics in CRC

Spatial transcriptomics allows to map gene expression to specific locations within a tissue section but cannot provide deep transcriptomic data on the single-cell level. On the other hand, single-cell RNA sequencing uncovers the transcriptome of individual cells and identifies distinct cell subpopulations within an organ. However, the tissue dissociation required to isolate single cells disrupts the spatial context, eliminating information about their localization within the tissue being studied (Longo et al., 2021). To circumvent these limitations, spatial transcriptomics were frequently integrated with single-cell transcriptomics in CRC research to map transcriptionally profiled single cells within their spatial context.

This integrative approach provides insights into the complex spatial organization, gene expression patterns across distinct tissue regions, cellular interactions, and the intricate interplay between tumor cells and their immune and stromal counterparts in CRC.

Through such integrative approaches, combining spatial and single-cell transcriptomics data from CRC tissues allowed to map the inferred cell types and associated gene expression patterns to their spatial locations within CRC tissues. The results showed that these tissues are composed of 4 regions namely tumor, stroma, immune infiltration, and colon epithelium. The tumor and stromal regions were in close proximity, and robust intercellular interactions were observed between them. Additionally, a high expression of TMSB4X and VIM was detected in the tumor and stromal regions, respectively. Moreover, the ligand-receptor pair C5AR1 and RPS19 was inferred to mediate the crosstalk between stromal and tumor regions (Xiao et al., 2024).

Moreover, analysis of primary CRC and normal colorectal tissue at the single-cell level uncovered the malignant crosstalk between CRC cells and stromal cells, particularly at the invasive front of CRC. At this site, CRC cells secrete human leukocyte antigen G, inducing the transformation of macrophages into secreted phosphoprotein 1 (SPP1)+ macrophages. These SPP1+ macrophages secrete cytokines and chemokines to recruit immunosuppressive cells, thereby reducing cytotoxicity through immune checkpoint (Ozato et al., 2023).

Furthermore, this integrative approach elucidated dynamic cellular and spatial changes in the immune microenvironment between primary tumors and metastatic liver sites. Notably, immunosuppressive cells, such as MRC1+ CCL18+ macrophages and SPP1+ macrophages, were found this time to be enriched in treatment-naive liver metastases. Further analysis showed that neoadjuvant chemotherapy can modulate the immune microenvironment differently in responsive and non-responsive liver metastases. Responsive tumors showed reduced immunosuppressive cells, such as SPP1+ macrophages, and increased cytotoxic T cells, while nonresponsive tumors exhibited elevated levels of immunosuppressive macrophages (SPP1+ and MRC1+ CCL18+) and decreased cytotoxic immune cells. Therefore, combining immunotherapy with neoadjuvant chemotherapy could be a possible therapeutic strategy to treat the non-responsive patients (Wu et al., 2022).

Additionally, this integrative approach revealed the presence of a stem-like cell subtype in primary CRC, characterized by the overexpression of ASCL2 and PTPRO, and responsible for conferring CRC a metastatic phenotype. Interestingly, the transcriptomic profiles of these stem-like cells revealed their heterogeneity and organ-specific metastatic potential. Specifically, stem-like cells with high expression of delta-like ligand 4 and MAF bZIP transcription factor A were enriched in primary CRC and ovarian metastases, indicating their potential association with ovarian metastasis. Conversely, stem-like cells exhibiting a gene expression pattern similar to that of cholangiocytes were found in primary CRC and liver metastases, suggesting their potential association with liver metastasis (Li et al., 2024).

Collectively, such insights are pivotal for understanding the complex biology of CRC and its metastases, as well as their intricate interactions with the stromal and immune microenvironment.

### Exploration of Other Multiomics Approaches in CRC

Other multiomics approaches have enabled the identification of regulatory networks and the development of models to predict survival and therapeutic drug response, which could not have been achieved using single omics.

The integrative analysis of RNA sequencing, miRNA sequencing, and DNA methylation data from a cohort of colon adenocarcinoma patients enabled the development of a deep learning-based survival prediction model, which effectively distinguished 2 subgroups with significantly different survival rates. At the molecular level, miR-133b and its 7 target genes (GNB4, PTPRZ1, RUNX1T1, EPHA7, GPM6A, BICC1, and ADAMTS5) were differentially expressed between these 2 subgroups. The 8 genes are known to be implicated in cellular oncogenic processes in addition to being associated with poor prognosis in various cancers (Lv et al., 2020).

Exploring a different dimension of molecular complexity, an integrated analysis of transcriptomic and metabolomic data from CRC tissues was conducted to construct metabolite and gene relationship networks. Interestingly, a strong correlation was found between phosphatidylcholine (30:0) and GART and PCNA genes, which was further verified by cellular experiments. Treatment of CRC cells with phosphatidylcholine (30:0) significantly increased cancer cell viability as well as the relative mRNA expression levels of PCNA and GART which makes phosphatidylcholine (30:0) a promising therapeutic target for CRC treatment (Zheng et al., 2024).

In a distinct approach emphasizing epigenetic regulation, integration of RNA sequencing with genome-wide patterns of DNA methylation and hydroxymethylation revealed 7 genes that were aberrantly regulated by DNA methylation in colorectal tumors (HIGD1A, AHCYL2, IL11RA, CHL1, SEMA6D, BIRC3, and HADHB). As for the HADHB gene, its hypermethylation was consistently found to be associated with the reduction of its transcription in CRC. Tumor functional analysis showed that HADHB overexpression reduced cancer cell migration and invasiveness, indicating that silencing HADHB may drive CRC oncogenesis and progression (Zhu et al., 2018).

Broadening the perspective to microbial profiles, an integrated metagenomic and metabolomic analysis was performed to elucidate the interactions among gut microbiome, metabolites, and

KEGG orthology (KO) genes for microbial enzymes in both early and late onset of CRC. Alterations in microbial KO genes and metabolites were suggested to be associated with changes in microbiota in CRC feces. The link between fecal short-chain fatty acid acetate, cancer-associated species *Bacteroides fragilis* and *Parabacteroides asaccharolytica*, butyrate-producing bacteria *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Roseburia intestinalis*, and acetate metabolism-related KO genes (*atoA* and *atoD*), underscores that enhanced acetate metabolism and acetyl-CoA synthesis are distinct characteristics of late-onset CRC. In contrast, early-onset CRC samples showed an enrichment of red meat-associated species *B. vulgatus* and *Parabacteroides spp. CT06*, which were linked to the accumulation of the choline metabolite 1-acyl-sn-glycero-3-phosphocholine and the downregulation of the choline metabolism-related KO gene *pldB*. Early-onset CRC samples also exhibited an enrichment of *Flavonifractor plautii* that had a positive correlation with cancer-associated metabolites, such as perfluorooctanesulfonic acid, linoleate, L-phenylalanine, and the KO gene *pldA*. Therefore, these results hold great potential to non-invasively detect and distinguish between early and late onset of CRC (Kong et al., 2023).

Shifting the focus to protein networks, Li et al. (2020) performed an integration of genomics, proteomics, and phosphoproteomics of CRC tissues. The results identified 3 CRC subtypes having distinct clinical prognosis and molecular signatures. In addition, although metastatic tissues displayed high similarities with primary tumors at the genetic level, proteomic and phosphoproteomic analysis of primary tumors distinguished metastatic and non-metastatic cases. The 3 proteomic subtypes were reported to be enriched for distinct kinases. The primary and metastatic tissues in the same proteomic subtype exhibited different activities for the same kinase as well as significant differences in the kinase-phosphosubstrate networks. These results led the authors to develop a machine learning model based on omics dataset and in vivo drug testing models to predict drug response. By taking advantage of the kinase-substrate correlations, it is possible to accurately select the most appropriate targeted therapy, especially for patients without druggable mutations (Li et al., 2020).

## OMICS APPROACHES TO EXPLORING CMS, MSI, AND DRUG RESISTANCE IN CRC

### Omics-Driven Insights Into CMS of CRC

The consensus molecular subtypes (CMS) classification system provides a comprehensive framework for stratifying CRC based on the intrinsic biomolecular heterogeneity of the tumor. This approach, leveraging transcriptomics as its foundation, uses complementary data from other omics technologies, such as genomics and epigenomics, to define and characterize the distinct subtypes of CRC, thereby advancing the understanding of its biological and clinical diversity. Genomic analyses have revealed hypermutation in approximately 16% of CRCs (Cancer Genome Atlas, 2012), including mutations in well-known driver genes such as APC, TP53, SMAD4, PIK3CA, and KRAS, as well as novel mutations in ARID1A, SOX9, and FAM123B (Yaeger et al., 2018). APC mutations include splice alterations in intronic regions and large in-frame deletions in CTNNB (Yaeger et al., 2018). By comparing gene expression profiles of the tumor with its surrounding microenvironment, CRC can be classified into 4 CMS subtypes, each defined by specific molecular and clinical features. CMS1 is immunogenic, characterized by microsatellite instability (MSI), BRAF mutations, and a high mutational burden, leading to increased immune activation and potential responsiveness to immune-based therapies. CMS2 is an epithelial subtype marked by chromosomal instability and hyperactivation of WNT and MYC signaling pathways, frequently involving APC, KRAS, and TP53 mutations, contributing to tumorigenesis through genomic instability and cell proliferation. CMS3, while also epithelial, is distinguished by metabolic dysregulation, KRAS mutations, and lower chromosomal instability. CMS4, the mesenchymal subtype, is associated with TGF-β signaling, stromal invasion, angiogenesis, and immune suppression, resulting in aggressive disease profiles, increased fibrosis, and poor responses to standard treatments (Becht et al., 2016; Guinney et al., 2015). CMS-based classification highlights the heterogeneity of CRC, emphasizing the need for personalized therapeutic strategies. CMS4 tumors, with their poor prognosis (Piskol et al., 2019), higher metastatic potential, and resistance to standard chemotherapy regimens (Rodman, 2020), often require irinotecan-based therapies, which have demonstrated improved efficacy (de Boer et al., 2019). In contrast, CMS1 is associated with poorer survival in mCRC, despite immune activation (Lenz et al., 2019), while CMS2 exhibits the best prognosis, and CMS3 is characterized by favorable responses to adjuvant chemotherapy (Ten Hoorn et al., 2022). The integration of CMS classification into clinical practice has advanced the understanding of CRC and enabled more effective, tailored approaches to treatment (Ten Hoorn et al., 2022).

In parallel, early-onset CRC has been shown to exhibit distinct epigenomic, transcriptomic, proteomic, and metabolic characteristics when compared with late-onset CRC (Du et al., 2023), highlighting the potential therapeutic and prognostic significance of integrating multiomics data in CRC research. A recent multiomics study further linked the biological mechanisms underlying CMS to their clinical outcomes, identifying 2 distinct CRC subtypes with unique prognoses and molecular features. Within the TCGA-CRC cohort, these 2 subtypes were identified and validated using 3 external datasets. The CS1 subtype, associated with a poor prognosis, exhibited enhanced activity in tumor-related hallmark pathways and reduced activity in metabolic pathways. Furthermore, CS1 was predicted to have a lower response rate to immunotherapy and displayed a higher frequency of genomic alterations when compared with the CS2 subtype. A prognostic model incorporating 5 genes was developed, revealing that patients in the high-risk group exhibited characteristics similar to the CS1 subtype, while those in the low-risk group resembled the CS2 subtype. Notably, MID2, one of the genes included in the model, was found to be linked to the epithelial-mesenchymal transition pathway and specific DNA methylation patterns. Knockdown of MID2 in CRC cell lines resulted in a significant reduction in colony formation, migration, and invasion, suggesting its potential role in CRC progression (Ma et al., 2024).

## Omics-Driven Insights Into MSI and MSS in CRC

MSI, associated with CMS1, is characterized by mutations or alterations in microsatellite regions, which are short, repetitive DNA sequences. These mutations typically result from defects in the mismatch repair (MMR) system, leading to the accumulation of insertions or deletions during DNA replication. Approximately 15% of all CRCs exhibit MSI (De Smedt et al., 2015), making it an important biomarker in CRC. Moreover, MSI is one of the few biomarkers that has received regulatory approval for clinical use in CRC (Qiu et al., 2022).

Microsatellites can be classified into 3 types based on the level of instability: microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L), and microsatellite stable (MSS). MSI-H, characterized by 30% or more of the loci showing instability, is associated with a significant number of mutations in microsatellite regions, often due to defects in the MMR system, particularly in the MSH2 and MLH1 genes. This results in a high tumor mutational burden. MSI-H tumors may respond well to immunotherapies, such as immune checkpoint inhibitors, due to their high mutation burden. In contrast, MSI-L tumors exhibit lower instability and are often seen in tumors with partial MMR defects or sporadic cancers. MSS tumors remain stable, with no insertions or deletions, and typically have a functional MMR system. Both MSI-L and MSS tumors generally do not respond well to immunotherapies (Battaglin et al., 2018).

Clinically, MSI-H is detected using PCR or immunohistochemistry assays. PCR typically detects instability in 5-7 loci (Murphy et al., 2006), while next-generation sequencing enables the analysis of thousands of microsatellite loci, allowing for a more comprehensive determination of MSI status. This advancement enhances the classification of samples as either MSI-H or MSS, contributing to a better understanding of the molecular heterogeneity associated with MSI (Bonneville et al, 2019).

Hause et al. (2016) classified and characterized MSI in 18 types of cancer, including CRC. For CRC, they differentiated MSI-H from MSS samples. Of the 27 known recurrent mutational targets associated with CRC MSI, 25 exhibited significant instability in MSI-H samples compared with MSS samples. These loci are located in or near genes already known to have biological functions related to cancer (Hause et al., 2016).

Using next-generation sequencing, Salem et al. (2020) identified some mechanisms causing MSI in CRC. They demonstrated that tumors with alterations in MSH2/MSH6 coexpression were associated with a higher tumor mutational burden in MSI-H tumors compared with those with MLH1/PMS2 coexpression. Inactivation of MLH1/PMS2 appeared to be the dominant mechanism driving MSI in CRC (Salem et al., 2020).

Furthermore, combining omics data with immunohistochemistry assays can effectively predict MSI status. Integrating DNA methylation data with hematoxylin and eosin (H &E) images yielded the most accurate MSI prediction. While combining H&E images with all omics data improved performance compared with using H&E images alone, it was still less effective than when H&E images were combined with individual omics data (Qiu et al., 2022).

Finally, omics-based approaches play a crucial role in advancing immunotherapy and guiding therapeutic decision-making for CRC patients, particularly by elucidating the differential responses of distinct MS types to immune therapy. While MSI and MSS CRC $CD8^+$ T cells exhibit overlapping phenotypic characteristics, they demonstrate significant differences in TCR antigen specificities. Notably, TCR activation in MSI tumors may contribute to a preprimed state, enhancing tumor responsiveness to immunotherapy (Borràs et al., 2023).

## Unraveling Drug Resistance Mechanisms in CRC

While chemotherapy, molecularly targeted therapy, and immunotherapy have greatly enhanced patient survival, drug resistance remains a significant clinical challenge undermining the potential for durable tumor control in CRC (Wang et al., 2022a). To address this challenge, omics approaches were adopted to identify the diverse and complicated molecular mechanisms responsible for drug resistance at the genomic, transcriptomic, proteomic, and metabolomic levels and consequently switch to alternative therapeutic strategies that could potentially treat resistant CRCs.

### Chemotherapy

#### *5-Fluorouracil*

##### *Transcriptomics*

CRC cells have been shown to enter a reversible dormant state that can be responsible for chemotherapy resistance. Using an in vitro model of dormant cells, metabolomics data revealed a drastic decrease in the metabolite levels involved in glycolysis accompanied by an increase in the levels of metabolites involved in glutaminolysis, an indicator of energy metabolism reprogramming. The results also indicated that these cells rely on glutamine as an energy source. On the other hand, transcriptome data from 2 datasets (TCGA-COAD and GSE39582) helped identify 9 prognosis-related genes having the potential to induce or maintain a dormant state of CRC. Additional bioinformatic analysis of the correlations between expressions of these 9 dormancy-related genes and drug sensitivity showed that the level of expression of LMOD1, MAB21L2, and ASPN increased significantly with drug resistance suggesting them as key proteins that contribute to chemoresistance. Interestingly, these findings led to the selection of alternative treatment options to overcome resistance to 5-Fluorouracil (5-FU); Erlotinib, CB-839 (glutaminase inhibitor), and austocystin D were predicted to eliminate dormant CRC cells. Cell viability assays showed the inhibitory effect of erlotinib and CB-839 against dormant CRC cells (Xie et al., 2022).

##### *Proteomics*

Proteo-transcriptomic analyses were performed to explore the proteins and transcripts altered in 3 5-FU-resistant CRC cell lines. Findings showed that proteins involved in RNA metabolism were largely affected by the induction of chemoresistance to 5-FU in CRC cells. The most significant effect found at the transcriptomic level in response to this chemoresistance was the reduction in transcripts of genes involved in transcriptional repression and corresponding to zinc finger proteins containing Krüppel-associated box domains (KRAB-ZFP). Almost 25% of the KRAB-ZFP family was found downregulated in 5-FU-resistant CRC cells among which ZNF649 is the common one between the 3 CRC cell

lines. However, its role has not been studied yet (Chauvin et al., 2022). In another report, exosomes derived from 5-FU-resistant CRC cells were reported to mediate resistance in the sensitive ones. To determine the key proteins involved in exosome-mediated 5-FU resistance, proteomics analysis was performed on exosomes derived from 5-FU-resistant cells followed by RNA sequencing of 5-FU-sensitive and -resistant CRC cells. After combining the results of both analyses, the authors looked for reported chemoresistance-associated proteins and selected STAT3 for further study. Findings revealed that p-STAT3 transfer via exosomes-mediated 5-FU resistance, and p-STAT3 inhibition can restore the sensitivity of CRC cells to 5-FU (Zhang et al., 2019).

#### *Metabolomics*

Metabolomic analysis on 5-FU-resistant and -sensitive CRC cells led to the identification of a set of significantly modulated metabolites that include key intermediates of nucleotide metabolism. 5-FU-resistant CRC cells were also shown to increase the flux toward the de novo purine synthesis, whereas pyrimidine biosynthesis was impaired. Purine biosynthesis was further reported to be supported by increased serine consumption by the resistant cells and compartmentalization of serine metabolism inside the mitochondria. Consequently, fueling purine biosynthesis resulted in improving resistant CRC cells' ability to prevent the accumulation of 5-FU-induced DNA damage. Therefore, 5-FU resistance could be reverted by interfering with serine availability or targeting its mitochondrial metabolism (Pranzini et al., 2022). Additionally, lipidomic analysis on 5-FU-resistant and -sensitive CRC cells revealed a novel mechanism of 5-FU resistance. The results showed that the most significantly modulated sphingolipid metabolism pathways in 5-FU-resistant cells were the upregulation of sphingomyelin and downregulation of ceramide. The enzyme acid sphingomyelinase that catalyzes the hydrolysis of sphingomyelin to ceramide was found to be downregulated in the 5-FU-resistant cells compared with the sensitive ones. Moreover, silencing the expression of this enzyme in the sensitive cells partly blocked the inhibitory effect of 5-FU. Therefore, modulation of sphingolipid metabolism was suggested as a potential strategy to overcome 5-FU resistance (Jung et al., 2020).

### ***Oxaliplatin***

#### *Genomics*

Excision repair sequencing was performed to identify repair events across the genome at single-nucleotide resolution in both sensitive and resistant CRC cell lines and test whether elevated repairing efficiency of the oxaliplatin-induced DNA damage in the resistant cells can account for the resistance to this therapeutic. Findings did not show significant differences in repair efficiencies between sensitive and oxaliplatin-resistant CRC cell lines. In contrast, the levels of DNA damage were found to be significantly lower in the resistant cells, suggesting that reduced damage formation rather than increased damage repair is responsible for oxaliplatin resistance in CRC (Vaughn et al., 2020).

#### *Transcriptomics*

RNA sequencing helped to identify a novel miRNA, named miR-46146, that was significantly upregulated in the exosomes generated by oxaliplatin-resistant CRC cells. Exosomal miR-46146 was shown to spread to sensitive cells, resulting in an increase in miR-46146 level in recipient cells and reduction of their sensitivity to oxaliplatin. Moreover, apoptosis-related protein PDCD10 was shown to be a direct functional target of miR-46146, indicating a functional role of exosomal miR-46146/PDCD10 signaling in promoting oxaliplatin resistance in CRC (Xu and Zhu, 2020). Another report also showed that miR-483-3p regulates oxaliplatin resistance by targeting FAM171B gene in human CRC cells. Small RNA sequencing and transcriptome sequencing followed by the joint analysis of both results showed that downregulation of miR-483-3p was accompanied by an upregulation of FAM171B gene in oxaliplatin-resistant CRC cells. In addition, overexpression of miR-483-3p in resistant CRC cells led to the reduction of the levels of FAM171B and increased cells' sensitivity to oxaliplatin. In contrast, inhibition of miR-483-3p in sensitive CRC cells resulted in an increase of the expression of FAM171B and promoted cells resistance to oxaliplatin (Liang et al., 2019c).

#### *Proteomics*

Proteomic analysis allowed the identification of 37 differently expressed proteins in oxaliplatin-resistant CRC cells compared with their sensitive counterparts. Among these proteins, western blot analysis showed that the expression of poly(C)-binding protein 1 (PCBP1) was 15.6-fold higher in resistant cells compared with that in sensitive CRC cells. Interestingly, this protein was found to be overexpressed in oxaliplatin-resistant patient colorectal tumor samples. Additionally, knockdown of PCBP1 in the CRC-resistant cells sensitized them to oxaliplatin, while overexpression of this protein in the sensitive cells promoted resistance to this therapeutic. Moreover, PCBP1 was suggested to mediate resistance to oxaliplatin through the activation of Akt signaling (Guo et al., 2017), which makes it a potential target to overcome resistance to oxaliplatin in CRC.

### ***Irinotecan***

#### *Transcriptomics*

RNA sequencing was adopted to identify determinants of sensitivity/resistance for irinotecan in CRC cell lines. The results showed a negative correlation between the expression of CDC20, TP73, CTNNAL1, FZD7, and CITED2 and irinotecan sensitivity, while a positive correlation was found between the expression of ABR, ARHGEF7, and RNMT and irinotecan sensitivity. Quantitative PCR data further confirmed the sequencing results. The expression of CDC20, CTNNAL1, FZD7, and CITED2 genes was elevated in resistant CRC cells, whereas the expression of ABR, ARHGEF7, and RNMT genes was high in the sensitive ones. The CITED2, CTNNAL1, and FZD7 genes are known to be implicated in cancer, and here they are suggested to serve potential biomarkers for irinotecan resistance (Li et al., 2014).

#### *Metabolomics*

Metabolomic analysis of CRC cell lines at their early stage of resistance to irinotecan showed significantly more elevated levels of unsaturated lipids and fatty acids compared with those found in the sensitive ones. This was accompanied by an

overexpression of SCD1 which is one of the most important desaturases catalyzing saturated fatty acids to mono-unsaturated fatty acids. Interestingly, inhibition of the activity of SCD1 led to a decrease of the levels of multiple unsaturated lipids as well as a reduction in the resistance level to irinotecan treatment. Mechanistically, SCD1 upregulates the expression of ALDH1A1 known to be implicated in the protection of cells from reactive oxygen species (ROS) and consequently reduces ROS levels which could possibly let CRC cells resist irinotecan therapeutic effect (Sun et al., 2022b).

## Targeted Therapy

### *Cetuximab*

#### *Genomics*

Whole-exome sequencing helped to discover 37 significantly mutated genes in KRAS wild-type cetuximab-resistant colorectal tumors. CYP4A11 was found to be the most frequently mutated gene, while BCAS1 and GOLGA6L1 were the second group of most frequently mutated genes. These genes could possibly play key roles in cetuximab resistance which needs further investigations (Jing et al., 2020). In another report, Xu et al. (2017) sequenced ctDNA taken from mCRC patient plasma samples before and during treatment with cetuximab. Interestingly, they adopted an innovative strategy named quartile-based selection strategy to identify mutations that began at very low allelic fractions but increased in frequency during the course of treatment, regardless of the existing mutations in colorectal tumor tissues or in the known "hot-spots." This resulted in the identification of 5 novel mutations in exon 19 of PIK3CA associated with acquired cetuximab resistance. Importantly, patients harboring these mutations displayed an enhanced reduction in progression-free survival compared with those without these mutations. These findings suggest the combination of a PIK3CA inhibitor with an anti-EGFR antibody as a potential therapeutic strategy for overcoming this resistance (Xu et al., 2017). In addition, targeted capture deep sequencing of ctDNA obtained from 17 mCRC patients with resistance to cetuximab led to the identification of an average of 44 mutations in each patient, including a total of 743 mutations impacting 166 different genes. Surprisingly, up to 9 different mutations associated with cetuximab resistance were detected in one of the patients. Interestingly, findings revealed that several mechanisms of resistance coexisted in the same patient, and these mutations may impact several constituents of mTOR/PI3K/AKT and RAS/RAF/ERK/MEK pathways, reflecting the necessity of the adoption of combination therapy rather than monotherapy to overcome this resistance (Ye et al., 2020).

#### *Transcriptomics*

Using whole-exome sequencing and RNA sequencing, the long non-coding RNA MIR100HG and 2 embedded microRNAs, miR-100 and miR-125b, were found to be the most upregulated non-coding RNA in a 3D model of CRC resistant to cetuximab, while no known genetic events associated with cetuximab resistance were detected. Importantly, miR-100 and miR-125b were shown to activate Wnt/β-catenin signaling as a compensatory pathway allowing CRC cells to survive under EGFR inhibition. Therefore, these non-coding RNA can serve as potential predictive biomarkers for cetuximab resistance which was further demonstrated to be overcome by Wnt inhibition (Lu et al., 2017). Moreover, LINC00973 is another long RNA that was reported to be the most significantly upregulated lncRNA in a generated cetuximab-resistant CRC cell line. Of note, these cells showed elevated glucose consumption and lactate secretion, indicating a possible contribution of glucose metabolism to cetuximab resistance. Remarkably, LINC00973 inhibition significantly increased CRC cells sensitivity to cetuximab and also reduced glucose consumption and lactate secretion, showing a possible relationship between cetuximab resistance, LINC00973, and glucose metabolism in CRC (Jing et al., 2019).

#### *Proteomics*

Proteomics was adopted to explore possible adaptations in signaling pathways that can contribute to resistance to cetuximab in KRAS/NRAS/BRAF$^{V600}$ wild-type CRC. Findings revealed that the levels of phospho-RPS6 and phospho-PRAS40 that are both related to PI3K pathway were decreased in cetuximab-sensitive but not in cetuximab-resistant cell lines in response to this drug. In addition, the level of phospho-AKTSer473 was elevated in 3 out of 4 CRC samples obtained from patients with clinical resistance to cetuximab and treated *ex vivo* with this therapeutic. Moreover, combination of PI3K pathway inhibitors with cetuximab yielded a more pronounced inhibitory effect on resistant CRC cells compared with cetuximab alone, suggesting that activation of the PI3K pathway as a mechanism of cetuximab resistance (Georgiou et al., 2021). A different mechanism of resistance to cetuximab in CRC was also identified, namely the macrophage migration inhibitory factor (MIF)-induced MAPK and AKT activation. Proteomic analysis showed that MIF was the most upregulated protein in cetuximab-resistant CRC cells. Silencing the MIF gene in resistant cells restored cell sensitivity to cetuximab, whereas treating sensitive colon cancer cells with exogenous MIF led to the development of resistance to this drug. The combination of cetuximab with the MIF inhibitor led to an enhanced inhibitory effect in CRC-resistant cell lines mediated by the inhibition of key downstream effectors of the MAPK and AKT signaling pathways (Russo et al., 2019). An additional advantage offered by proteomics is the investigation of kinome reprogramming responsible for the mediation of resistance to cetuximab in CRC. In a recent study, Torlot et al reported considerable reprogramming of both proteome and kinome in CRC cell lines resistant to cetuximab and harboring activating KRAS alterations. Ephrin type-A receptor 2, known to be a driver of cellular motility and migration, was shown to be among the most strongly overexpressed kinases in these cell lines. Interestingly, targeting ephrin type-A receptor 2 signaling effectively reduced the enhanced migration in resistant cells, making it a potential actionable target in CRC cell lines with acquired cetuximab resistance (Torlot et al., 2023).

### *Palbociclib*

#### *Proteomics, Metabolomics, and Glycoproteomics*

Proteomic analysis revealed the upregulation of metabolism-related pathways in palbociclib-resistant CRC cells and metabolomics analysis identified an increase in N-glycan

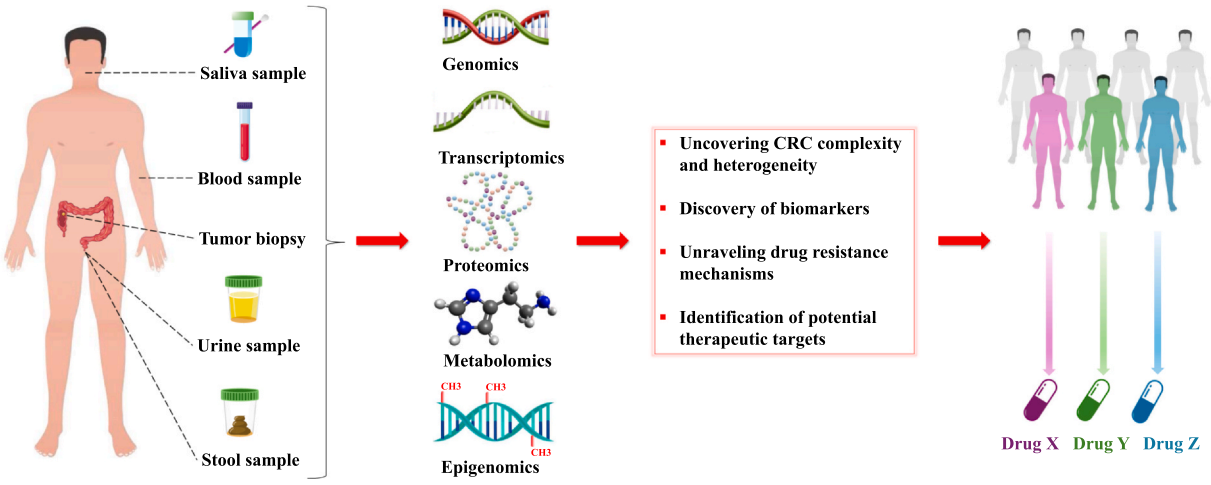


**Fig. 1.** Schematic overview of the use of colorectal cancer biopsy, saliva, blood, urine, and stool samples to obtain omics data crucial for the promotion of personalized treatment in colorectal cancer therapy.

biosynthesis. Glycoproteomics further demonstrated an elevation in global N-glycosylation in these resistant cells. Notably, the integration of proteomic and glycoproteomic data revealed that nearly half of the glycoproteins identified through glycoproteomics were not detected in the proteomic analysis, highlighting the unique insights offered by glycoproteomic profiling. Importantly, resistance to palbociclib was shown to be reversed by glycosylation inhibition, indicating a critical role of glycosylation in driving drug resistance (Xue et al., 2023).

### *Immunotherapy*

#### *Transcriptomics*

Single-cell RNA sequencing was adopted to analyze colorectal tumor tissues from patients sensitive and resistant to immunotherapy, particularly anti-PD-1 treatment (Tislelizumab). In the sensitive group, the proportions of CD8+ T-cell subsets were significantly higher compared with the resistant group. Further bioinformatic analysis identified IL-1β and MMP9 as the genes most strongly correlated with anti-PD-1 resistance. Supporting these findings, in vivo experiments demonstrated that IL-1β promotes the infiltration of myeloid-derived suppressor cells (MDSCs), which suppress the accumulation of CD8+ T cells, thereby exacerbating anti-PD-1 resistance. Notably, MDSCs are a heterogeneous population of bone marrow-derived cells known to mediate CD8+ T-cell inactivation. Therefore, IL-1β antagonists can possibly overcome resistance to PD-1 inhibitors (Wu et al., 2023).

Collectively, most omics-based studies have extensively uncovered the mechanisms of resistance to chemotherapy and targeted therapies, particularly those targeting EGFR. However, comparatively less emphasis has been placed on exploring resistance to anti-HER2 and anti-VEGF targeted therapies, as well as immunotherapies, in CRC. This gap in research underscores the urgent need for further investigation into these resistance pathways to enhance therapeutic outcomes.

## ROLE OF OMICS IN ADVANCING PERSONALIZED MEDICINE IN CRC

Omics technologies unraveled the complex, heterogeneous, and highly dynamic nature of CRC at the genomic, transcriptomic, proteomic, metabolomic, and epigenomics levels. They also allowed to decipher the diverse mechanisms of adaptation and resistance by which CRC cells escape treatment at these multiple omics' levels. This could possibly explain the reason for the failure of the standard therapeutics to effectively achieve durable tumor control in all the treated patients. The ample evidence reported here highlights the significant importance of the comprehensive understanding of CRC landscape provided by omics technologies at the time of diagnosis and throughout the disease course to select appropriate cancer therapeutic modalities. It also emphasizes the great potential of omics technologies to tailor treatment to the individual CRC patient based on the evolving genetic and molecular profile of his tumor paving the way for the personalization of therapy for CRC patients. Additionally, omics technologies can accelerate the discovery of screening, predictive, diagnostic, and prognostic biomarkers using CRC tissue, saliva, serum, urine, and stool samples from CRC patients. Although they are still in their explorative stage and need further clinical validation, these biomarkers identified by omics technologies could possibly help in the early non-invasive detection of CRC, mapping out the prognosis of the CRC, prediction of the overall survival of CRC patients and clinical outcome, monitoring treatment effectiveness, detection of residual disease and cancer recurrence, differentiation of non-metastatic CRC patients from the metastatic ones, stratification of metastatic risk, and most importantly identification of potential therapeutic targets. Therefore, the discovery of these biomarkers plays an instrumental role in promoting the implementation of personalized medicine. This is because each colorectal tumor adopts distinct cellular/molecular mechanisms to grow and resist standard anticancer treatment and undergoes oncogenic alterations that vary according to the age of the patient and the anatomical location of the tumor and its metastases (Fig. 1).

## LIMITATIONS IN OMICS RESEARCH IN CRC

A common limitation faced in single and multiomics studies in CRC research is the reliance on small sample sizes. This could affect the statistical power and the accuracy of the generalization of the conclusions. In addition, the majority of these discoveries lack validation using external cohorts. To ensure their robustness, reproducibility, and clinical relevance, these findings should be verified with independent datasets from external cohorts. Ideally, validation samples should be sourced from different institutions, populations, and laboratories to establish the broad applicability and reliability of the derived data. Besides, some of these studies lack functional and mechanistic investigations to determine whether the identified molecules play a causal role in colorectal carcinogenesis or to clarify the causal versus associative relationships among different molecules within regulatory networks. On the other hand, as highlighted by Ullah et al. (2022) and Zafari et al. (2023), the absence of standardized research platforms and bioinformatics methods for processing large-scale omics datasets poses an additional significant challenge.

## CONCLUSIONS

In recent years, monumental efforts using omics technologies have been devoted to understanding the complex heterogeneous nature of CRC and its potential interaction with the stromal and immune microenvironment, discovering CRC biomarkers, developing predictive models, identifying regulatory networks, stratifying CRC patients, and exploring drug resistance mechanisms.

The major recent preclinical advancements of omics technologies in CRC research emphasized here show that omics technologies hold great potential to tackle the current limitations encountered in CRC management. They also show that omics technologies represent a powerful approach to attain the ultimate goal which is an earlier non-invasive cancer diagnosis accompanied by enhanced cancer prognosis, and more tailored therapies for individual patients. Therefore, the adoption of omics offers a valuable opportunity to revolutionize the management of CRC and curb the increasing trends of CRC prevalence and mortality.

However, these omics-derived findings necessitate further validation before their effective translation into clinical practice. Additionally, multiomics integration is still in its early phase, confronted with challenges such as data complexity, the need for large-scale validation cohorts, and the standardization of research platforms and bioinformatics methodologies. Nevertheless, ongoing advancements in computational tools and data integration techniques offer considerable promise for refining multiomics strategies, ultimately leading to a potential transformative shift in CRC management.

## AUTHOR CONTRIBUTIONS

Z.F., M.H., and M.F. reviewed the literature and drafted the paper; H.G.M. initiated the idea and revised the paper. All authors have read and approved the final paper.

## CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

**Gali-Muhtasib Hala:** Writing – review and editing, Conceptualization. **Fatfat Maamoun:** Writing – original draft, Investigation. **Hussein Marwa:** Writing – original draft, Investigation. **Fatfat Zaynab:** Writing – original draft, Investigation.

## DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## ORCID

Maamoun Hussein https://orcid.org/0000-0001-9642-6708

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