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Virome remodeling rewires epigenetic and metabolic pathways linked to infection-associated colorectal cancer risk★

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ABSTRACT

Background: Colorectal cancer (CRC) incidence is rising in sub-Saharan Africa, coinciding with the high prevalence of immune-modulating infections such as HIV and helminths. The gut virome, a critical yet understudied component of the microbiome, may influence oncogenic processes through epigenetic and metabolic alterations. However, the interplay between gut viral communities, HIV-helminth co-infection, and CRC risk remains poorly characterized in African populations. This study aimed to investigate gut virome-associated epigenetic and metabolic signatures linked to CRC susceptibility among South African adults, with a focus on HIV and helminth co-infection dynamics.

Methods: Untargeted shotgun metagenomic sequencing was performed on stool DNA samples from 62 adults stratified into five groups: uninfected controls (n = 10), HIV-only (n = 14), helminth-only (n = 15), HIV-helminth co-infected (n = 13), and CRC-confirmed patients (n = 10). Bioinformatic analyses were used to identify differentially abundant viral genes and to functionally annotate epigenetic and metabolic pathways associated with infection status and CRC occurrence.

Results and discussion: Adenine-specific DNA methylase (COG2189) emerged as one of the most significantly enriched epigenetic markers across all infected and CRC groups, CRC (7.0 ± 1.26, q = 2.98e-06), helminth-only (7.1 ± 1.16, q = 1.30e-07), HIV-only (6.2 ± 1.21, q = 1.28e-05), and co-infected 6.5 ± 1.21, q = 6.11e-06), suggesting a shared viral epigenomic mechanism potentially contributing to tumorigenesis. Additionally, diverse metabolism-related genes were differentially abundant, particularly those linked to butyrate metabolism, oxidative stress response, and polyamine biosynthesis, metabolic pathways known to influence tumor initiation,

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immune evasion, and disease progression. These findings indicate that the gut virome may play an intermediary role in modulating host epigenetic and metabolic landscapes in infection-driven CRC risk.
Conclusion: This study identifies novel gut virome-associated epigenetic and metabolic functional signatures that may serve as early, non-invasive biomarkers of CRC susceptibility in HIV- and helminth-endemic populations. Integrating such molecular indicators into cancer surveillance and prevention frameworks could enhance early detection strategies and precision cancer care in underrepresented, high-infection burden African regions.

1. Introduction

Colorectal cancer (CRC) can take years to develop. In the initial stages, benign growth (polyps) on the epithelial layer lining the colon or the rectum can gradually become malignant and continue to grow without restriction. This is accompanied by mutational burden facilitated by genetic instability and DNA damage, which are the primary drivers of CRC development (Deschoolmeester et al., 2010). Over 20% of CRC patients usually present with an advanced stage of the disease due to a lack of early diagnosis, which is complicated by asymptomatic disease. Helminth infection may contribute to cancer development by inducing CRC-related mutations (Brindley et al., 2015; Scholte et al., 2018). Epigenetic regulation, particularly DNA methylation, is essential for maintaining genomic stability, directing cell fate decisions, and modulating immune responses (Liotti et al., 2022; Fritz et al., 2022). Studies suggest that people living with HIV (PLWHIV) display accelerated epigenetic aging, as evidenced by increased DNA methylation age, when compared to HIV-negative individuals. This epigenetic age acceleration may underlie their increased susceptibility to age-associated comorbidities and oncogenesis (Nelson et al., 2017). Moreover, epigenetic modifications induced by HIV infection have been increasingly recognized, with DNA methylation emerging as both a promising biomarker and a potential epi-therapeutic target (Arumugam et al., 2021).

Infection-driven CRC is characterized by epigenomic perturbations that play a key role in reshaping the intestinal microenvironment toward malignancy through chronic inflammation and microbial exposure. Aberrant DNA methylation, including both classical 5-methylcytosine (5 mC) and emerging markers such as N6-methyladenine (6 mA), has been implicated in oncogenesis and may serve as both a biomarker and a mechanistic link between infection and tumorigenesis (Eijk et al., 2012). The gut virome, comprising both bacteriophages and eukaryotic viruses, has been increasingly recognized as a potent modulator of host epigenetics. Viral elements can encode or influence methylation machinery, thereby altering host gene expression and immune responses (Nishijima et al., 2022; Yang et al., 2021). These interactions are dynamic and context-dependent, shaped by host genetics, co-infections, environmental exposures, and microbial community structure (Zuo et al., 2020).

On their own, evidence shows that helminth infections can induce epigenetic changes that help the parasite evade host immunity (Bohnacker et al., 2024; Banihashemian and Mirmajlessi, 2025), while simultaneously rewiring host metabolism in ways that sustain an immunosuppressive microenvironment and enable long-term parasite persistence (Kokova et al., 2021). In parallel, HIV induces metabolic reprogramming that can persist despite effective antiretroviral therapy, locking the immune system into a dysregulated state (Deme et al., 2022). Cancer cells, notorious for hijacking metabolic pathways to fuel growth and evade immune attack, may exploit these infection-driven shifts. The convergence of helminth- and HIV-mediated metabolic alterations may therefore create a permissive metabolic landscape that could accelerate oncogenesis in co-infected individuals.

Building on these insights, this study focused on characterizing methylation-associated genes within the gut virome, specifically COGs related to DNA methyltransferases, across distinct clinical groups: HIV-only, helminth-only, HIV-helminth co-infected, and CRC-confirmed groups. We hypothesized that chronic infection leads to shared and unique epigenetic and metabolic disruptions that have the potential to contribute to CRC risk. Our findings highlight how viral and bacterial components may collaborate to reshape the host epigenetic landscape, promoting genomic instability and immune evasion. Beyond cancer susceptibility, variations in gut virome composition and associated methylation pathways have been linked to a broad range of conditions, including obesity, diabetes, and autoimmune disorders (Yang et al., 2021; Cochetel et al., 2025).

2. Study methods

2.1. Study design and population

This cohort of study patients consisted of 62 South African adults across five clinical groups: uninfected controls (n = 10), HIV-only (n = 14), helminth-only (n = 15), and HIV-helminth co-infected (n = 13). Full details of the recruitment and study demographics are published elsewhere (Mpaka-Mbatha et al., 2023). Stool samples from CRC-confirmed patients (n = 10) were collected 24 h preoperatively, before bowel preparation and antibiotics were administered by a qualified surgeon from the Department of Surgery, University of KwaZulu-Natal, South Africa.

2.2. DNA extraction

Stool-derived genomic DNA was extracted from 200 mg of stool sample using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, CA, USA; Cat. No. D4300) according to the manufacturer's instructions. Mechanical lysis was performed using the Omni Bead Ruptor 4 prior to extraction to ensure sample homogeneity. DNA purification was completed using Zymo-Spin™ columns and humic removal columns. Quantification was performed with the Qubit™ 4 Fluorometer using the Qubit™ dsDNA HS Assay Kit (Thermo Fischer Scientific, Waltham, MA, USA).

2.3. Next generation sequencing

Libraries were constructed using the Illumina® DNA Prep Kit (Illumina Inc., San Diego, CA, USA, Cat. No. 20060060) according to the manufacturer's instructions. A limited-cycle PCR was conducted to amplify the library while incorporating sample-specific dual indexes. Magnetic bead-based purification was then used to eliminate excess reagents and perform size selection of the DNA fragments. Sequencing was carried out on the Illumina NextSeq 2000 platform with 2 × 150 bp paired-end reads. Quality control was performed using TrimGalore, and metagenomic analysis was conducted using the SqueezeMeta pipeline (v1.6.2) in coassembly mode:

SqueezeMeta.pl -m coassembly -p PROJECT -s Samplelist. txt -f TRIMMED -t 32.

Open reading frames (ORFs) were predicted with Prodigal, and functional annotation was performed via DIAMOND-based searches against the COG database. Furthermore, taxonomic classification of contigs was performed by SqueezeMeta, utilising a combination of ORF queries using DIAMOND, marker genes, and the authors' published script to combine results. Contigs identified as viral in origin by SqueezeMeta were extracted for downstream analyses:

viruses = subsetTax(PROJECT, 'superkingdom', tax = 'Viruses', rescale_copy_number = F).

Downstream analysis focused on COG functional categories, with differentially abundant functions identified using the R (v4.4.2) package SQMtools (v1.6.2) (Puente-Sánchez et al., 2020) and DESeq2 (Love et al., 2014). After defining sample groups COG abundances were extracted as follows:

rownames(metadata) = colnames(viruses$functions$COG$abund).

A DESeq2 object was formed as follows:

dds = DESeqDataSetFromMatrix(countData = viruses$functions$COG $abund, colData = metadata, design = ~ condition).

Rows summing to less than 10 were removed. Differential abundance comparisons were performed with contrasts and repeated for all comparisons, e.g.:

results = results (dds, contrast = c("condition","Controls","CRC"))

Statistical significance was determined using adjusted p-values with Benjamini-Hochberg correction with a threshold of 0.05.

3. Results

3.1. Putative viral sequences identified in stool metagenomes

From the assembled metagenomic contigs, a subset showed high similarity to known viral genomes (BLAST identity >90%, alignment >1 kb), including sequences related to BK023550.1, BK036789.1, and BK047185.1. These contigs are referred to as the viral elements throughout the study, reflecting inferred viral content from total metagenomic data. Their functional potential was predicted based on sequence homology, providing insight into virus-associated metabolic/epigenetic and host-interaction pathways within the gut ecosystem (Supplementary Table 1). The differential expression patterns of abundant genes are visualized in Fig. 1, which presents a clustered heatmap of significantly modulated COG metabolic pathways across the infection groups. The heatmap highlights distinct metabolic signatures, illustrating the divergent microbial responses associated with HIV and helminth infections. Together, these findings suggest that HIV and helminth infections induce distinct viral elements metabolic adaptations, with HIV infections favouring lipid metabolic activity, while helminth infections are associated with broader suppression of viral elements metabolic pathways.

3.2. Functional classification of differentially abundant functions

3.2.1. Differentially abundant functions annotated by COG categories

A majority of the differentially abundant functions were commonly detected across all infected groups and CRC cases when compared to HIV and helminth uninfected healthy controls. Notably, several genes with uncharacterized functions, such as ENOG410ZGRA, showed distinct patterns between infection groups. ENOG410ZGRA was underrepresented in the HIV-only group (-19.9 ± 4.53, $q = 0.00036$) but enriched in the HIV-helminth co-infected group (19.2 ± 4.57, $q = 0.00086$), suggesting infection-specific regulatory dynamics. Similarly, ENOG4112065 was significantly abundant in both the HIV-helminth co-infection group (24.6 ± 3.57, $q = 8.94 \times 10^{-10}$) and CRC samples (23.9 ± 3.74, $q = 1.86 \times 10^{-8}$). Interestingly, ENOG41122JG was consistently enriched in the helminth-only (18.7 ± 4.25, $q = 0.00041$) and CRC

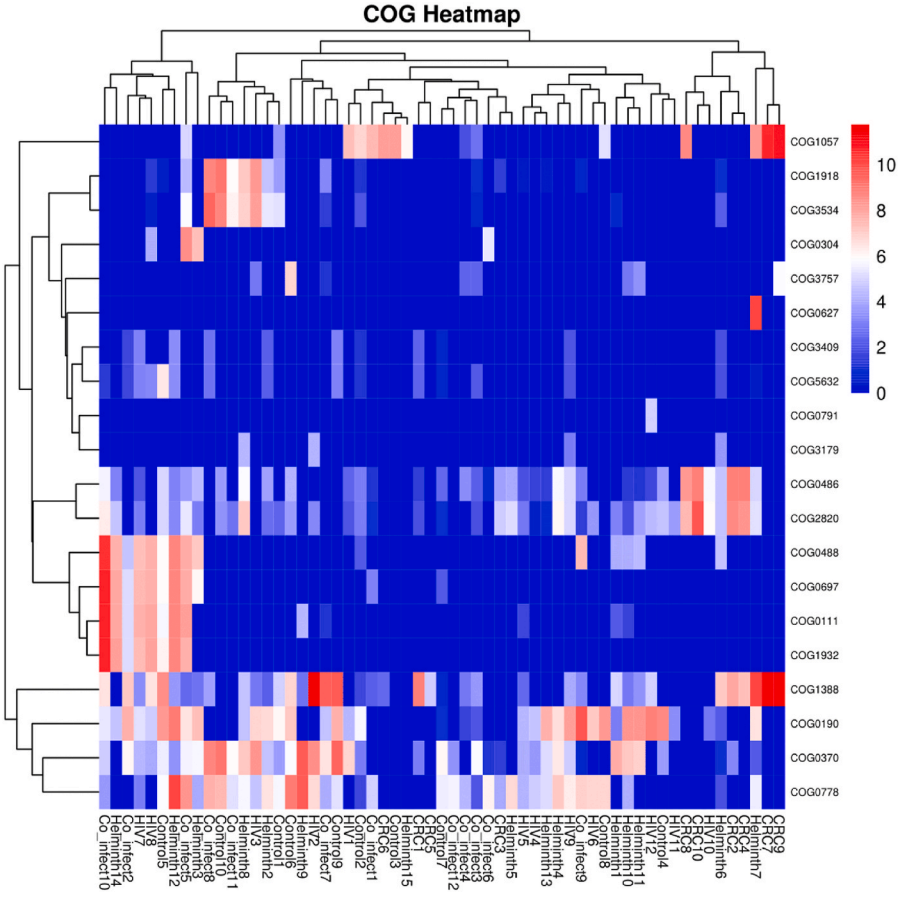


Fig. 1. Differential expression heatmap of functionally annotated microbial genes (COGs) across infection groups. The heatmap displays the normalized expression levels of selected COGs significantly altered between helminth-only, HIV-only, HIV-helminth co-infected, HIV and helminth uninfected controls, and CRC-confirmed groups. Hierarchical clustering reveals distinct metabolic profiles associated with each group. Color intensity represents Z-score normalized abundance values (red = high expression; blue = low expression). Rows represent viral elements; columns represent infection groups. Only statistically significant COGs (adjusted $p < 0.05$) are shown.

groups (18.5 ± 4.65, q = 0.0018), suggesting possible functional overlap or shared immunometabolic disruptions across parasitic infection and CRC states (Fig. 2), as further detailed in Table 1.

3.2.1.1. Specific infection-associated metabolic reprogramming as a potential driver of CRC risk. Comparative analysis between infected groups revealed that COG1932, encoding phosphoserine aminotransferase (PSAT1), an enzyme central to amino acid and coenzyme metabolism, was consistently under-represented in HIV-helminth co-infection (−15.3 ± 4.80, q = 0.02044) and HIV-only comparisons (−19.0 ± 4.79, q = 0.00139) but enriched in helminth only group (21.1 ± 4.60, q = 0.00024), Fig. 2. This pattern suggests a helminth-specific metabolic modulation, potentially associated with immune evasion strategies or nutrient competition within the host gut microenvironment, and highlights broader disruptions in host-microbial metabolic crosstalk in helminth-dominated infections. Interestingly, PSAT1 activity appears enriched in helminth-only infections relative to HIV-associated groups, indicating that helminth infection may stimulate serine biosynthesis, possibly through anti-inflammatory pathways that modulate host metabolic demand. Supporting this, previous studies have reported multiple serine proteases in parasitic helminths, implicated in parasite development, nutrient acquisition, tissue and cell invasion, anticoagulation, and immune evasion. The presence of these enzymes provides a mechanistic basis for the observed microbial PSAT1-like upregulation, suggesting that helminth-associated metabolic adaptations enhance serine biosynthesis and one-carbon metabolism (Yang et al., 2015; Cummings et al., 2022).

The phosphoglycerate dehydrogenase (COG0111) involved in amino acid and coenzyme metabolism was under-represented in HIV-helminth co-infection (−15.7 ± 3.64, q = 0.00052), HIV only (−19.4 ± 3.62, q = 4.66e-06), and CRC confirmed (−16.9 ± 3.78, q = 0.000317) cases. Similarly, 5,10-methylene-tetrahydrofolate dehydrogenase, involved in coenzyme metabolism (COG0190), was modulated in HIV-helminth co-infection (−5.7 ± 1.29, q = 0.00040), HIV only (−6.8 ± 1.29, q = 6.56e-06), helminth only (−6.0 ± 1.25, q = 9.34e-05), and CRC confirmed (−5.5 ± 1.34, q = 0.00122) cases. The predicted GTPase with regulatory functions (COG0486) was consistently modulated in both helminth-only (3.5 ± 1.33, q = 0.0244) and helminth-HIV coinfected individuals (5.0 ± 1.20, q = 0.00092). Other genes dysregulated in the HIV-only group included exoribonuclease R (COG0557) (4.5 ± 1.11, q = 0.01030), which was also detected in helminth-only (−4.4 ± 1.06, q = 0.00010) and CRC-confirmed (−3.9 ± 1.56, q = 0.01055) samples. There was a common under-representation of permeases of the drug/metabolite transporter superfamily (COG0697) involved in amino acid and carbohydrate metabolism in HIV-helminth co-infection (−15.5 ± 4.43, q = 0.00964) and HIV infected individuals (−18.9 ± 4.41, q = 0.00046).

In contrast, comparison of all infected groups to the CRC-confirmed cohort revealed enriched COG0304 in both HIV-only and HIV-helminth co-infected samples. COG0304 encodes 3-oxoacyl-(acyl-carrier-protein) synthase, a key enzyme in fatty acid elongation essential for microbial membrane integrity and secondary metabolite production. This enrichment may reflect infection-driven adaptations of the viral elements in the context of chronic immune activation, particularly in HIV infection, where lipid metabolism is critical for immune regulation and microbial persistence. These findings collectively highlight how different infections distinctly modulate host metabolic circuits,

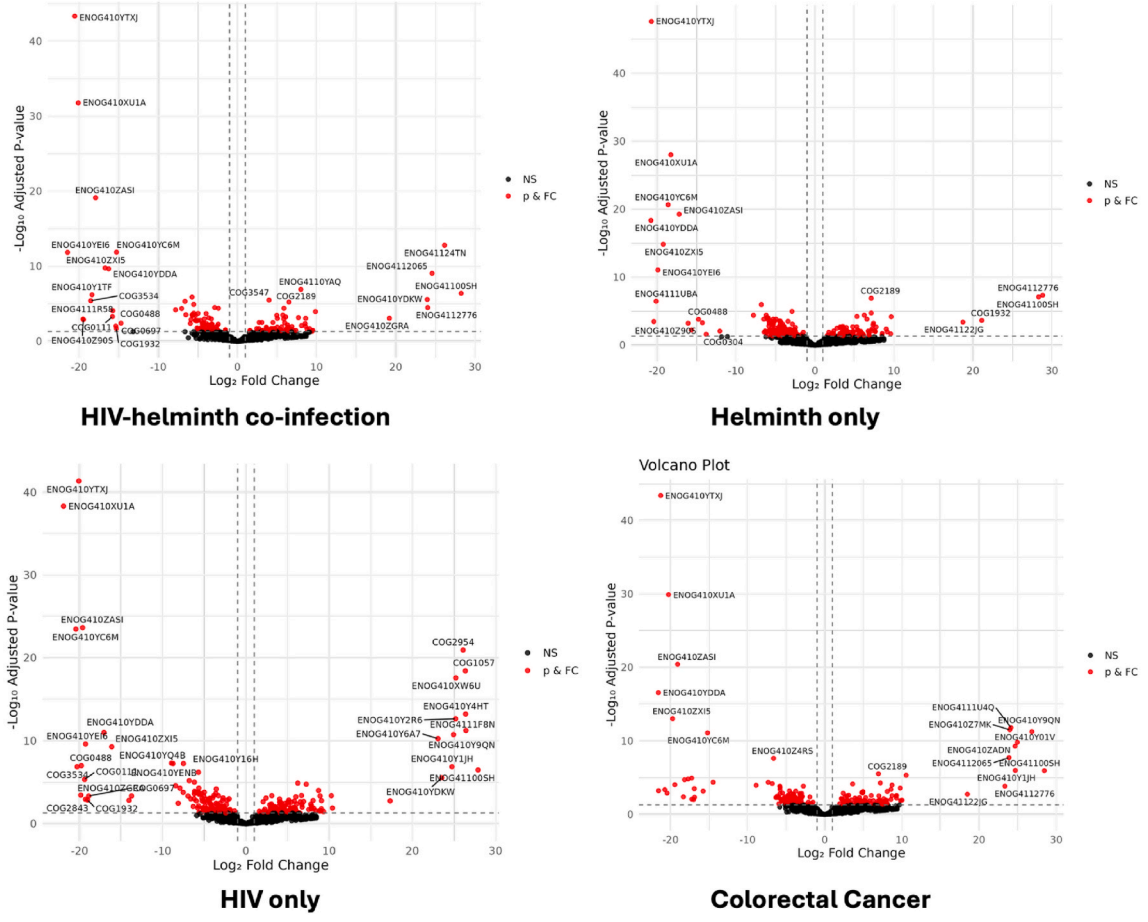


Fig. 2. Volcano Plot depicting differentially abundant functions between uninfected healthy controls, all the infected groups, and the CRC positive controls. The viral elements analysis was functionally categorized using clusters of orthologous groups (COG) pathways. The volcano plot displays gene distribution based on $\log_2$ fold change and adjusted p-values, indicating significantly enriched or down-abundant genes across conditions.

Table 1
Metabolic pathways observed in infected groups directly linked to cancer.

Gene Name	Functional Category	Cancer Association	References
Phosphoglycerate dehydrogenase	Amino acid/ Coenzyme metabolism	PHGDH is often amplified in cancers such as breast cancer and melanoma, contributing to serine biosynthesis and supporting rapid cell proliferation.	(Liu et al., 2013; Kuzuoglu-Ozturk, 2023)
5,10-methylene-THF dehydrogenase (MTHFD2)	Coenzyme metabolism	MTHFD2 is consistently enriched in various cancers, promoting tumor growth and metastasis, making it a potential therapeutic target.	(Pardo-Lorente and Sdelci, 2024; Ramos et al., 2024)
ABC transporter ATPase components	General function prediction	ABC transporters are linked to cancer hallmarks, including proliferation, metastasis, and drug resistance, by facilitating the efflux of chemotherapeutic agents.	Muriithi et al. (2020)
Nitroreductase (NTR)	Energy production and conversion	NTRs are overexpressed in various cancers, including colorectal, breast, and liver cancers, and are being explored for targeted cancer therapies.	Shang et al. (2024)
N-acetylmuramoyl-L-alanine amidase	Cell envelope biogenesis	This enzyme has been studied for its potential to induce cytotoxicity in cancer cells when combined with other agents, suggesting a role in cancer therapy.	Fuentes-Baile et al. (2020)
Phosphoserine aminotransferase (PSAT1)	Amino acid/ Coenzyme metabolism	PSAT1 is overexpressed in several cancers, including colorectal and breast cancer, supporting tumor cell proliferation and survival.	Yang et al. (2023)
Uridine phosphorylase (UPP1)	Nucleotide transport and metabolism	UPP1 supports metastasis in cancers such as breast cancer and is considered a potential prognostic factor in oral squamous cell carcinoma.	(Yang et al., 2023; Nwosu et al., 2023)

potentially mitigating or amplifying CRC risk. While metabolic adaptations can support viral persistence and immune evasion, they may also create a microenvironment conducive to neoplastic transformation, including enhanced lymphocyte infiltration, immune exhaustion, and redox imbalance (Ren et al., 2022). Significant abundance of Adenine-specific DNA methylase (COG2189) was shared by CRC (7.0 ± 1.26, q = 2.98e-06), helminth-only (7.1 ± 1.16, q = 1.30e-07), and co-infected groups (6.5 ± 1.21, q = 6.11e-06). This enzyme catalyzes the methylation of adenine residues (N6-methyladenine, 6 mA), a modification increasingly recognized for its regulatory role in microbial gene expression and genome stability. The consistent enrichment across helminth-only, HIV-helminth co-infected, and CRC groups suggests a shared adaptive or stress response within the gut microenvironment, potentially driven by chronic inflammation or altered immune signaling. Adenine methylation serves as a mechanism for phase variation, DNA repair, and protection against restriction enzymes needed for survival under immune pressure. Its overrepresentation in the infected groups may therefore indicate enhanced microbial resilience and persistence in response to host immunity. Similarly, in CRC, elevated 6 mA methylation has been linked to shifts in microbial virulence and to epigenetic crosstalk that can alter host gene regulation and chromatin dynamics (Chen et al., 2023). The parallel enrichment of COG2189 in both infection and cancer states points to a possible convergence of microbial adaptation and tumor-promoting conditions. It is plausible that infection-induced microbial methylation may reprogram the gut epigenetic landscape, creating an environment that favors both microbial persistence and malignant transformation. This observation indicates a potential mechanistic bridge between chronic infection and CRC risk, mediated through epigenetic modulation at the viral elements-host interface.

Furthermore, Table 1 outlines the COG viral elements-associated pathways that show potential links to cancer-related processes. These pathways were identified through functional profiling of viral genes enriched in the CRC group and compared to other infection states. The table highlights pathways associated with genome maintenance and metabolic processes. Of note, among these are genes involved in DNA repair, protein metabolism, and host immune evasion, potentially highlighting the role of the viral elements in modulating tumorigenic pathways.

When comparing all infected groups to the CRC group, both the HIV-helminth co-infection and HIV-only groups had the uncharacterized, ENG410ZZ4I (4.6 ± 1.13, q = 0.0058) and (5.1 ± 1.13, q = 0.00042), respectively. COG0304 (3-oxoacyl-(acyl-carrier-protein) synthase) (31.5 ± 4.78, q = 1.60e-08 and 31.3 ± 4.78, q = 1.23e-08, respectively) in common. Additionally, ENOG4112065 was shared between the HIV-only group (−18.1 ± 3.61, q = 4.19e-05) and the helminth-only group (−18.1 ± 3.44, q = 2.73e-05), Fig. 3A. Of interest, when comparing infected groups against each other, there were genes that were also shared amongst groups. For instance, COG1932 was also downregulated in Helminth only vs HIV only (−40.2 ± 4.33, q = 9.76e-18) and HIV-helminth co-infection (−36.4 ± 4.34, q = 1.96e-14), Fig. 3B.

Gut viral elements-related differentially abundant genes that were significantly expressed in infected groups are tabulated in Table 2. These findings indicate gut viral elements in HIV-helminth co-infection and their potential utility as a source of candidate biomarkers or mechanistic targets in infection-associated disease progression.

These shared alterations suggest convergent functional pressures, particularly affecting metabolic pathways. A central feature of the infection-altered gut viral signature landscape observed in this study was the structured reprogramming of viral elements' metabolic gene expression, reflecting dynamic shifts in the core biosynthetic and energy-producing pathways (Fig. 4).

Furthermore, differential pathway enrichment analysis was performed to quantify coordinated metabolic activity across infection and CRC groups using gene set variation analysis (GSVA; Table 3). Overall, modest but biologically relevant alterations were observed in one-carbon metabolism, amino acid pathways, and transporter activity. In infection states relative to controls, the one-carbon metabolism by folate pathway showed mild down-abundance ($\log_2$FC = −0.23; adj. P = 0.23), while glycine, threonine, and serine metabolism displayed a trend toward enrichment ($\log_2$FC = +0.15; adj. P = 0.35). ATP transporter activity remained largely unchanged ($\log_2$FC = −0.03; adj. P = 0.83), suggesting limited energy transport alteration during infection. Although none of these pathways reached false discovery rate (FDR) significance, their consistent directionality points to a subtle remodeling of one-carbon and amino acid metabolism under infection conditions.

In the helminth-infected group, similar low-amplitude but coherent alterations were observed. The glycine, threonine, and serine metabolism pathway showed modest enrichment ($\log_2$FC = +0.19; adj. P = 0.38), implying slight upregulation of one-carbon substrate utilization and amino acid turnover during helminth infection. This pathway contributes to folate-mediated methylation and nucleotide synthesis, processes that may reflect adaptive host metabolic adjustments to chronic infection. In contrast, ATP transporter activity ($\log_2$FC = −0.14; adj. P = 0.38) and one-carbon metabolism by folate ($\log_2$FC = −0.05; adj. P = 0.65) were under-represented, consistent with reduced energy transport and partial suppression of folate-dependent reactions. While these differences were not statistically significant, the pattern suggests a shift toward an energy-conserving phenotype and altered amino acid flux.

When CRC samples were compared with HIV-helminth co-infected samples, a distinct metabolic bias emerged. One-carbon metabolism by folate was enriched ($\log_2$FC = +0.30; adj. P = 0.054), supporting the notion of enhanced folate-dependent methylation and nucleotide biosynthesis, hallmarks of tumorigenic metabolic reprogramming. Conversely, ATP transporter ($\log_2$FC = −0.23; adj. P = 0.11) and glycine, serine, and threonine metabolism ($\log_2$FC = −0.09; adj. P =

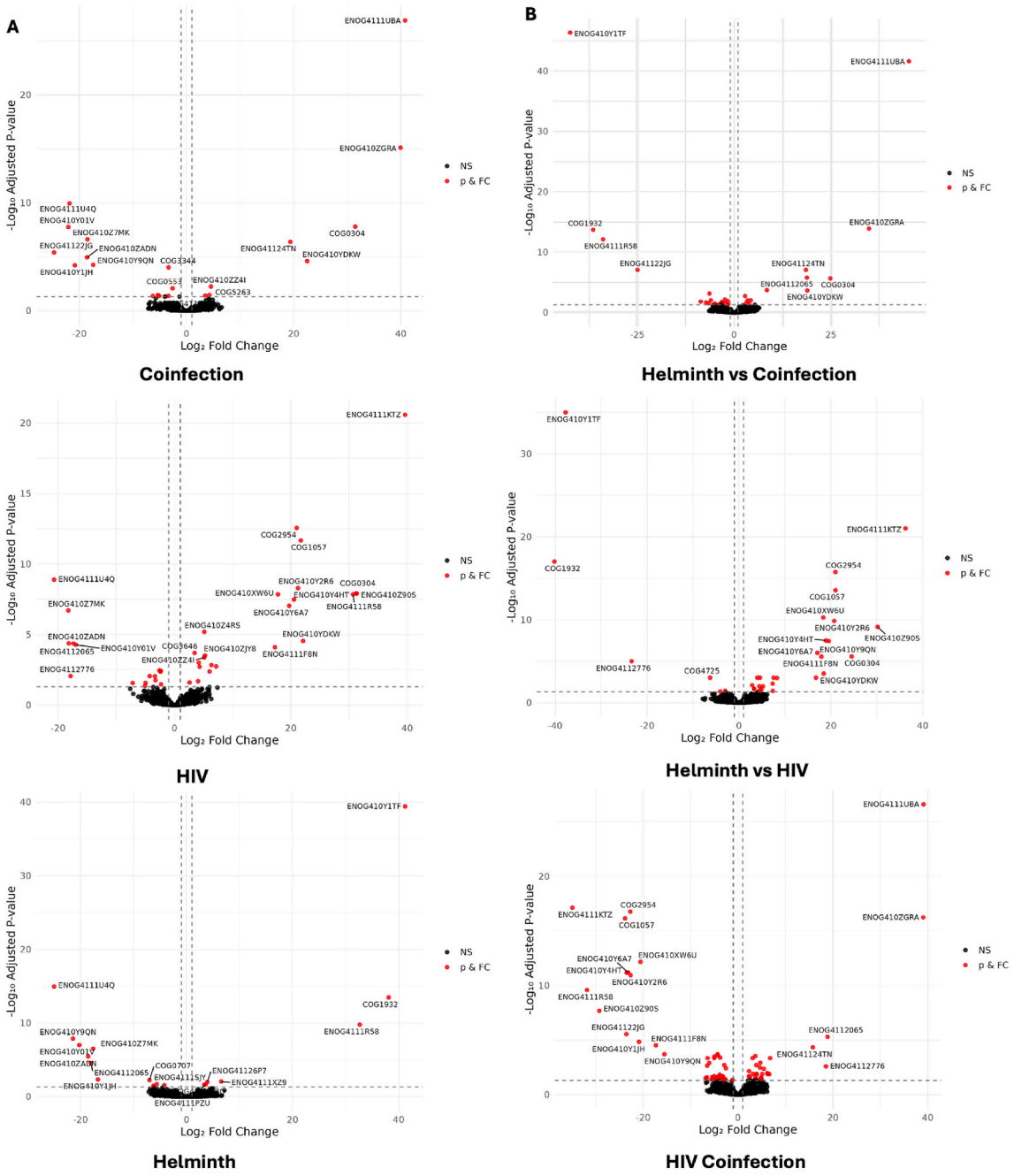


Fig. 3. Differentially abundant microbial functional categories across infection and CRC groups. (A) Volcano plots showing differentially abundant protein function categories between CRC-positive controls and all infected groups combined, identifying broad functional and evolutionary relationships associated with CRC. (B) Volcano plots comparing all infected groups (HIV-only, helminth-only, and HIV–helminth co-infected) against each other to reveal infection-specific functional signatures. Functional annotation and clustering were performed using Clusters of Orthologous Groups (COG) classification. Each point represents a distinct COG function, plotted by $\log_2$ fold change (x-axis) and $-\log_{10}$ adjusted p-value (y-axis). Red points denote significantly differentially abundant functions (FDR <0.05). Functions on the right indicate enrichment, while those on the left indicate reduced abundance relative to the reference group. Together, the analyses highlight distinct microbial functional remodeling associated with infection states and CRC pathogenesis.

0.49) were under-represented, implying reduced energy and amino acid homeostasis. Together, these findings suggest that HIV-helminth co-infection may foster a pro-tumorigenic metabolic environment characterized by increased one-carbon flux and suppressed energy balance.

Comparisons between CRC and helminth-only groups revealed comparable but subtler shifts. One-carbon metabolism by folate was modestly elevated in CRC ($\log_2$FC = +0.13; adj. P = 0.33), suggesting gradual metabolic reorientation toward nucleotide synthesis and methylation support. In contrast, glycine, serine, and threonine metabolism ($\log_2$FC = −0.12; adj. P = 0.33) and ATP transporter activity ($\log_2$FC = −0.12; adj. P = 0.33) were under-represented, reflecting reduced amino acid turnover and energy transport. Although not statistically significant, these coordinated shifts align with emerging evidence of metabolic reprogramming during CRC development, particularly in helminth-exposed individuals.

Finally, CRC versus HIV comparisons showed more pronounced divergence. Glycine, serine, and threonine metabolism was significantly under-represented ($\log_2$FC = −0.3282; adj. P = 0.032), consistent with reduced one-carbon substrate cycling and potential depletion of methyl donors required for DNA methylation. Down-abundance of ATP

Table 2
Significantly expressed metabolic genes in infected groups.

COG ID	Gene Name/Annotation	Functional Category	Group(s)	mean ± 95% CI	q-Value
0111	Phosphoglycerate dehydrogenase	Amino acid/Coenzyme metabolism	HIV only, Helminth, Co-infection	−19.4 ± 3.62 −12.0 ± 3.50 −15.8 ± 3.64	4.66e-06 0.00897 0.00052
0190	5,10-methylene-THF dehydrogenase	Coenzyme metabolism	HIV only, Helminth, Co-infection	−6.8 ± 1.29 −6.1 ± 1.25 −5.7 ± 1.29	6.56e-06 9.34e-05 0.00040
2189	Adenine-specific DNA methylase	Mediates N6-methyladenine modification known to regulate microbial gene expression and virulence.	HIV only, Helminth, Co-infection	6.2 ± 1.21 7.1 ± 1.16 6.5 ± 1.21	1.28e-05 1.30e-07 6.11e-06
0304	3-oxoacyl-(acyl-carrier-protein) synthase	Secondary metabolites biosynthesis, transport and catabolism/Lipid metabolism	HIV	31.2 ± 4.78	1.23e-08
0370	Fe^{2+} transport system protein B	Inorganic ion transport/metabolism	HIV only, Helminth	−6.0 ± 1.32 −5.4 ± 1.27	0.00017 0.00089
0486	Predicted GTPase	General function prediction	HIV only, Co-infection	4.24 ± 1.19 5.0 ± 1.20	0.005137 0.00091
0488	ATPase components of ABC transporters with duplicated ATPase domains	General function prediction	HIV only, Helminth	−19.8 ± 3.27 −14.7 ± 3.15	9.89e-08 0.00016
0697	Permeases of the drug/metabolite transporter (DMT) superfamily	General function prediction only/Amino acid transport and metabolism/Carbohydrate transport and metabolism	HIV Co-infection	−15.5 ± 4.43 −18.9 ± 4.42	0.00964 0.00046
0778	Niroreductase	Energy production and conversion	HIV, Helminth	−5.7 ± 1.27 −5.2 ± 1.23	0.00027 0.00074
1057	Nicotinic acid mononucleotide adenylyltransferase	Coenzyme metabolism	HIV	26.4 ± 2.80	2.16e-12
1388	FOG: LysM repeat	Cell envelope biogenesis, outer membrane	HIV, Co-infection	7.2 ± 1.42 6.1 ± 1.42	1.73e-05 0.00058
1932	Phosphoserine aminotransferase	Amino acid transport and metabolism/Coenzyme metabolism	HIV Helminth Co-infection	−19.0 ± 4.79 21.1 ± 4.60 −15.5 ± 4.80	0.00139 0.00024 0.02044
2820	Uridine phosphorylase	Nucleotide transport and metabolism	Co-infection	5.0 ± 1.12	0.00035
3534	Alpha-L-arabinofuranosidase	Carbohydrate transport and metabolism	HIV, Helminth, Co-infection	−20.3 ± 3.39 −14.2 ± 3.27 −18.5 ± 3.39	1.36e-07 0.00052 4.05e-06
3969	Predicted phosphoadenosine phosphosulfate sulfotransferase	General function prediction only	Helminth, Co-infection	6.2 ± 1.23 5.7 ± 1.29	4.02e-05 0.00033
ENOG410XU3I	No name	Function unknown	HIV, Helminth	−6.2 ± 1.53 −5.1 ± 1.47	0.00096 0.00821
ENOG410XQ2T	type iii restriction	Defense mechanisms	HIV, Helminth	6.0 ± 1.42 7.1 ± 1.37	0.00058 1.92e-05
ENOG410XNW6	Phage protein	Function unknown	HIV	6.7 ± 1.53	0.00039
ENOG410XRH6	Integrase	Replication, recombination and repair	Co-infection	−6.5 ± −4.49	0.00030
ENOG410XUQ2	YjcQ protein	Function Unknown	HIV	−5.3 ± 1.29	0.00086
ENOG410ZGRA	No name	Function unknown	HIV, Co-infection	−19.9 ± 4.53 19.2 ± 4.57	0.00036 0.00086
ENOG410Y1TF	No name	Function unknown	HIV, Co-infection	−13.8 ± 3.20	0.00045 6.39e-07

(*continued on next page*)

Table 2 (*continued*)

COG ID	Gene Name/Annotation	Functional Category	Group(s)	mean ± 95% CI	q-Value
				−18.4 ± 3.15	
ENOG410Y1ZG	No name	Function unknown	HIV	−4.0 ± 0.96	0.00078
ENOG410YB2E	No name	Function unknown	Co-infection	9.9 ± 2.08	0.00012
ENOG410YDR6	No name	Function unknown	HIV, Helminth	−6.2 ± 1.39	0.00024
				−5.8 ± 1.33	0.00055
ENOG410YK3D	No Name	Function Unknown	HIV, Co-infection	−6.2 ± 1.19	9.71e-06
				−5.5 ± 1.19	0.00018
ENOG410YPT3	No name	Function Unknown	HIV, Helminth, Co-infection	−6.17 ± 1.22	0.00022
				−5.7 ± 1.27	4.18e-05
ENOG410YUBV	No name	Function Unknown	HIV	−7.0 ± 1.62	0.00029
ENOG410Z7Y1	No name	Function unknown	HIV	10.3 ± 2.37	0.00046
ENOG410ZHCN	No name	Functional Unknown	HIV, Helminth, Co-infection	−4.4 ± 1.06	0.00042
				−3.5 ± 1.01	0.00086
				−3.9 ± 1.06	0.00897
ENOG4111FT5	fibronectin type III domain protein	Function Unknown	HIV, Helminth, Co-infection	8.9 ± 4.30	0.00433
				9.7 ± 1.96	0.00044
				8.6 ± 2.06	6.83e-05
ENOG4111PAV	Phage-related protein	Function unknown	HIV, Helminth	3.1 ± 0.72	0.00089
				2.4 ± 0.68	0.00042
ENOG4111R20	No name	Function unknown	HIV	−6.2 ± 1.33	0.00851
ENOG4111W9D	No name	Function Unknown	Co-infection	−4.0 ± 0.86	0.00013
ENOG4111WWI	No name	Function unknown	HIV	−2.7 ± 0.66	0.00021
ENOG4112065	No name	Function unknown	Co-infection	24.6 ± 3.57	0.00078
ENOG4112923	Caudovirus prohead protease	Function unknown	HIV, Helminth, Co-infection	8.7 ± 1.85	8.94e-10
				7.4 ± 1.72	9.77e-05
				7.7 ± 1.81	0.00061
ENOG411297B	Dj domain protein	Posttranslational modification, protein turnover, chaperones	Helminth, Co-infection	−5.3 ± 1.19	0.00071
				−5.6 ± 1.24	0.00040
ENOG4111XQJ	No name	Function unknown	HIV	−5.7 ± 1.24	0.00030
					0.00016

Footnote: Where more than one p-value is given, they represent statistical significance for each group individually.

transporter and one-carbon metabolism by folate pathways further supports suppressed energy metabolism and folate turnover. Together, these data highlight HIV-associated metabolic modulation that could influence CRC pathogenesis through disturbed cellular energetics and epigenetic instability. Although statistical significance was not reached for most pathways, the coherent directionality across multiple comparisons may be an indication of biologically meaningful remodeling. These trends warrant validation in a larger cohort, where increased statistical power could confirm the subtle metabolic reprogramming observed here.

3.3. Evidence of viral DNA methylation machinery

Investigation of viral elements-associated epigenetic modulation revealed significant alterations in DNA methylation-related genes in individuals with HIV or helminth infections only, confirmed CRC, and HIV and helminth uninfected controls, Fig. 5. Most notably, COG2189, which encodes an adenine-specific DNA methylase, was consistently enriched in the CRC-confirmed (adj. $p = 2.98e-06$), helminth-only (adj. $p = 1.30e-07$), and HIV-helminth co-infected (adj. $p = 6.11e-06$) groups. This epigenetic pattern was further supported by the enrichment of COG0286, which encodes a Type I restriction modification (RM) system methyltransferase. Its selective expression in the HIV-helminth co-infected group (adj. $p = 0.0238$) indicates a potential microbial mechanism of host-epigenetic interference. Additionally, COG0190, encoding 5,10-methylene-tetrahydrofolate dehydrogenase (MTHFD2), was significantly enriched in HIV-only, helminth-only, and co-infected groups (adj. $p < 0.0005$).

4. Discussion

The interplay between chronic infections, particularly HIV and helminth infections, and host epigenetic reprogramming presents a critical axis for understanding CRC susceptibility. This study aimed to explore how chronic infections may influence CRC risk by examining viral elements-associated epigenetic and metabolic changes in HIV- and helminth-infected individuals. Our stratified metagenomic analysis identified distinct methylation/metabolism-associated signatures across

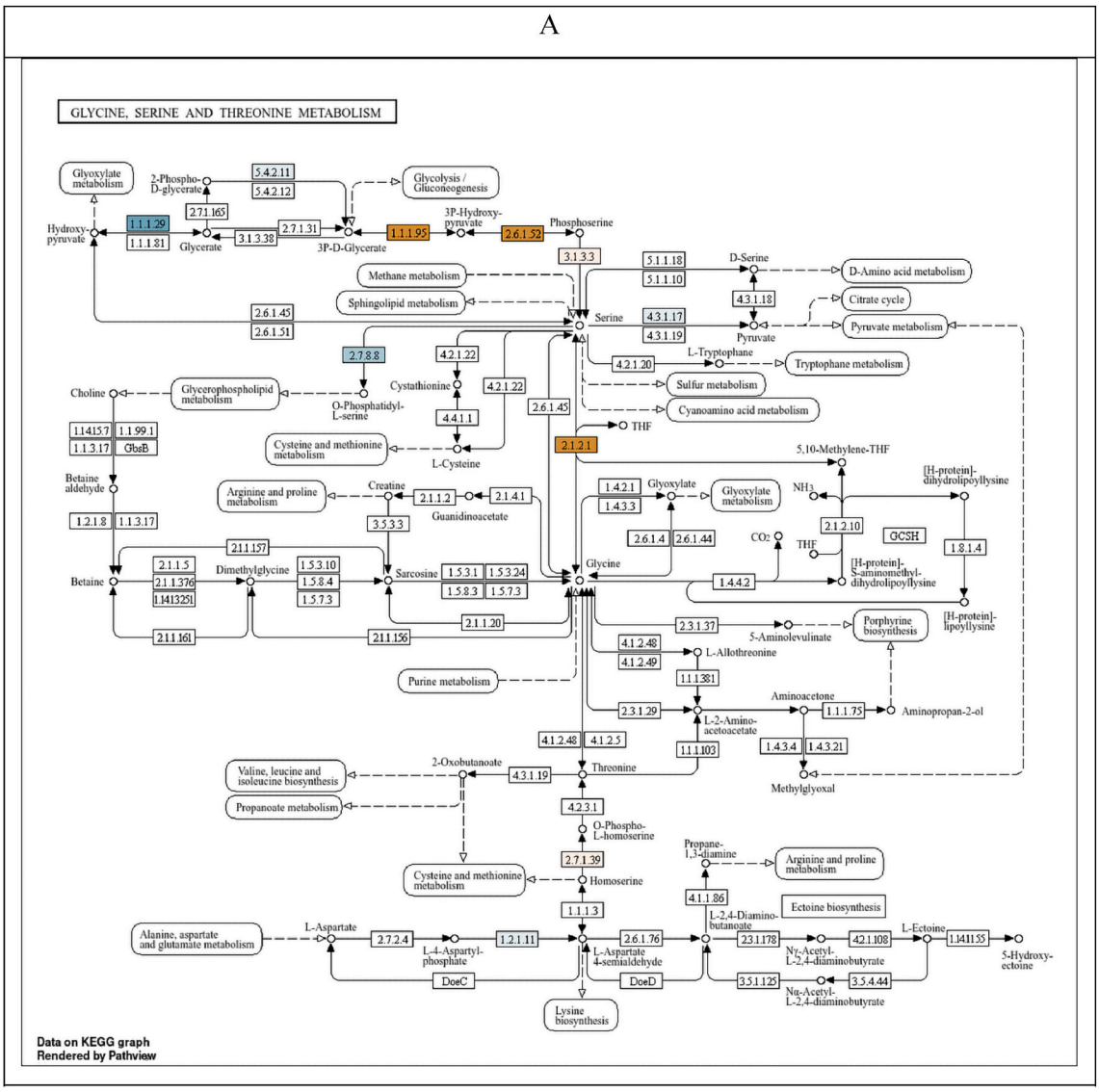
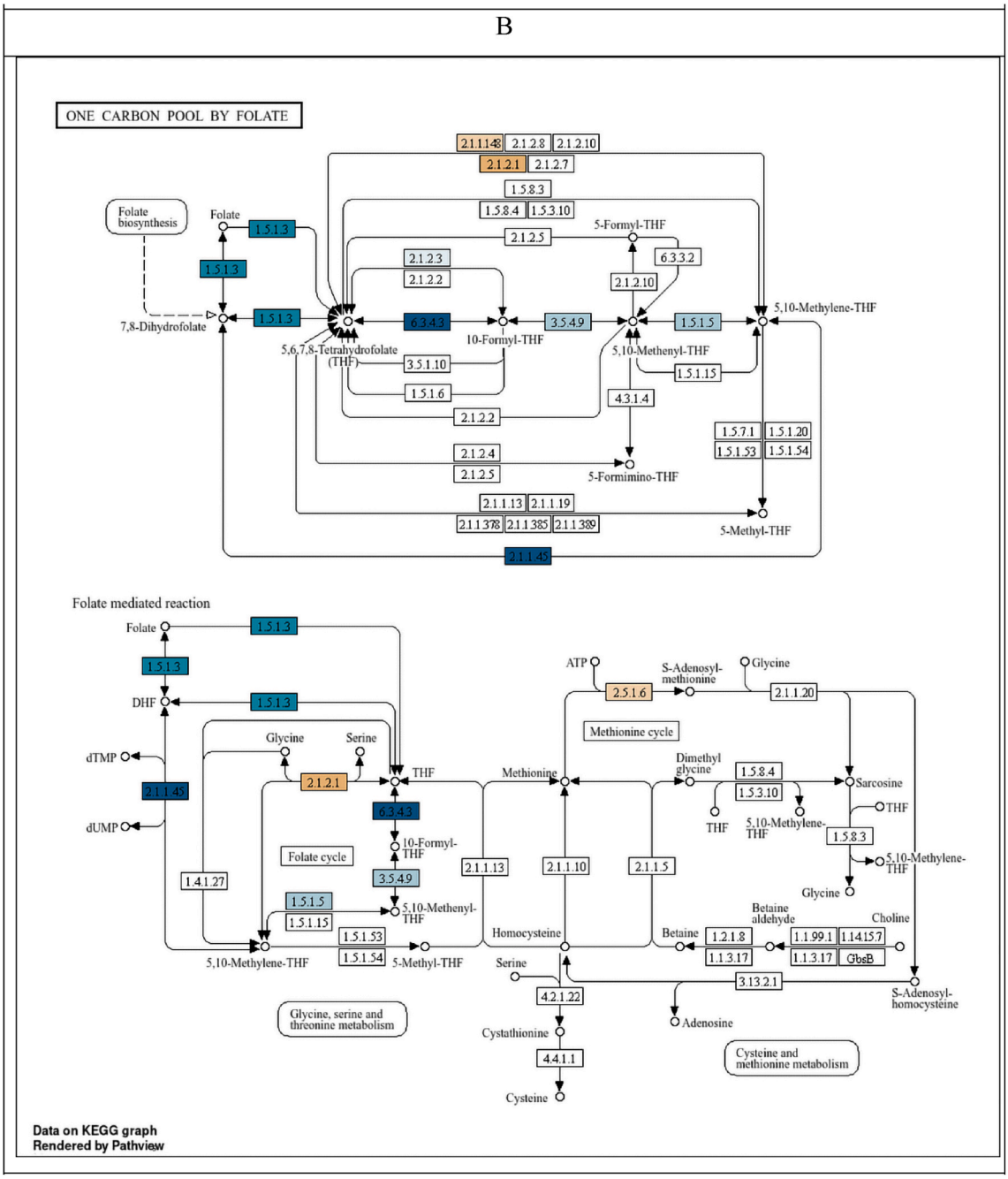


Fig. 4. Infection-induced metabolic reprogramming reveals coordinated activation of amino acid, folate, heme, and transporter pathways. (A) Serine-glycine metabolism: Infection and co-infection states show enhanced flux through the serine-glycine biosynthetic arm linking glycolytic intermediates to one-carbon units. Key enzymes, including PHGDH, PSAT1, and SHMT1/2, are enriched, increasing serine-glycine interconversion and supplying substrates for nucleotide and methyl-donor synthesis. (B) One-carbon pool by folate: Elevated activity of MTHFD2 and associated enzymes sustains the folate-mediated one-carbon cycle, generating 5,10-methylene-THF and S-adenosylmethionine (SAM). (C) Porphyrin and heme metabolism: Upregulation of ALAS1, UROD, and FECH indicates increased iron turnover and altered heme homeostasis. (D) ABC transporters: Induction of multiple ABC transporter families enhances trafficking of iron complexes, peptides, and xenobiotics.

the HIV-only, helminth-only, CRC confirmed, and HIV-helminth co-infected groups, with each exhibiting unique patterns of viral elements enzyme expression implicated in methylation dynamics. Both metabolic reprogramming and epigenetic modifications are established cancer hallmarks, enabling transformed cells to support proliferation, evade immune detection, and persist in hostile microenvironments (Xu et al., 2023). Shotgun metagenomic sequencing of stool DNA revealed a shared "onco-viral elements" between CRC patients and HIV-helminth co-infected individuals. The HIV-helminth co-infection group exhibited the most extensive methylation-related reprogramming. Both 5,10-methylene-tetrahydrofolate dehydrogenase (COG0190) and Adenine-specific DNA methylase (COG2189) were significantly enriched, along with COG0286, which encodes a Type I restriction-modification system methyltransferase. The co-expression of microbial and host-mimetic methyltransferases suggests epigenetic convergence through viral, bacterial, or horizontal gene transfer (Miyashita et al., 2002).

4.1. *Epigenetic reprogramming in infection-associated CRC risk*

Adenine-specific DNA methylase catalyzes the formation of N6-methyladenine (6 mA), a DNA modification increasingly recognized for its regulatory roles in eukaryotic systems, particularly in transcriptional activation, stem cell maintenance, and chromatin organization (Chen et al., 2017). Elevated 6 mA levels have been associated with genomic instability, proliferation, and oncogenic progression in multiple tumor types (Li et al., 2017), suggesting that its presence in infection may constitute a precursor to malignant transformation. Previous studies on tissue samples observed significantly higher levels of 5-methylcytosine (5 mC) compared to 6 mA. However, a substantial number of samples exhibited either upregulation or downregulation of both 5 mC and 6 mA in hepatocellular carcinoma (Zhang et al., 2022). Of interest, 6 mA has sparked interest in multiple studies investigating its role in parasitic infections (Lizarraga et al., 2020) and viral infections (Zhang



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et al., 2023). Fig. 6 presents a comparative overview of the regulatory functions of these methylation marks. It is important to note that an integrated framework of these methylation marks has been proposed for precision epigenome editing aimed at treating methylation-related diseases (Liu et al., 2025).

Although traditionally involved in bacterial defense, RM system methyltransferases have been implicated in the regulation of virulence, phase variation, and modulation of host transcriptional responses (Pflueger et al., 2018; Zemach et al., 2013). Their presence in the viral elements of HIV-helminth co-infected individuals may reflect epigenetic mimicry strategies used to evade host immune response. The Type I RM system methyltransferase makes up an important component of a multi-subunit antiviral defense complex. Type I RM systems comprise three proteins, HsdR, HsdS, and HsdM, that assemble into different configurations to control whether foreign DNA is cleaved or protected. In their pentameric arrangement, these complexes function as restriction endonucleases that recognise and cut unmethylated or improperly methylated DNA from invading bacteriophages (Fig. 7). This activity requires ATP, Mg^{2+}, and S-adenosyl-methionine (AdoMet) as cofactors (Zhang et al., 2017). The enrichment of Type I restriction modification methyltransferases (e.g., COG0286) in HIV-helminth co-infection may alter viral composition, reshape bacteriophage-host dynamics, and drive changes in viral gene exchange. Such shifts may destabilize viral elements' community structure, influence immune regulation, and sustain chronic inflammation processes that hold potential relevance for cancer risk in affected individuals.

4.1.1. *Biological implications of 6 mA methylation*

In the current study, the persistent enrichment of COG2189, which encodes an adenine-specific DNA methylase, in both the infection-only

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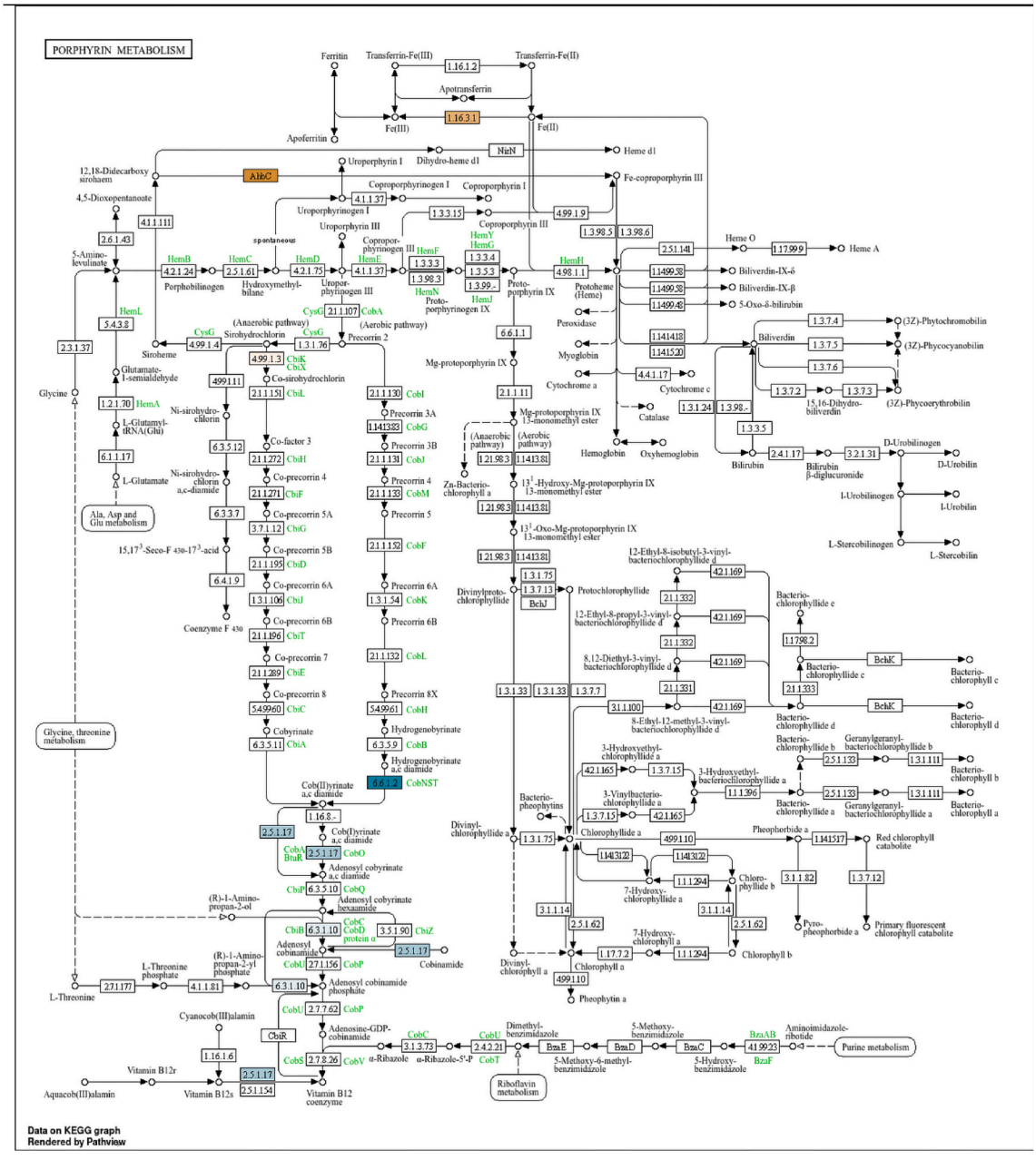

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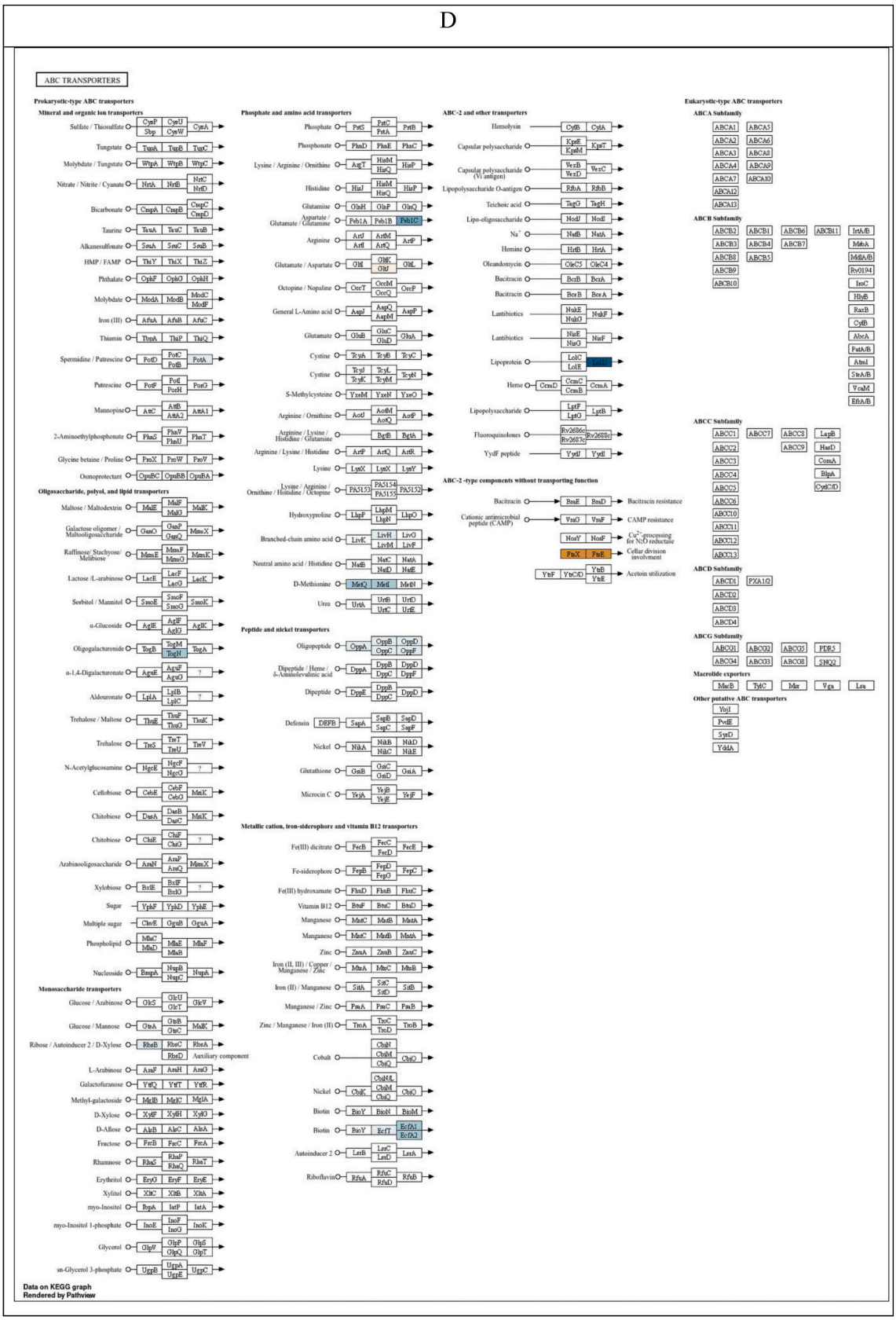
and CRC-confirmed groups highlights the potential of microbial 6 mA-modifying enzymes to contribute to host epigenetic reprogramming. This trend suggests a shared molecular axis between chronic infection and early oncogenic transformation, possibly mediated by viral elements-encoded methylation machinery. Once integrated or expressed within the host intestinal environment, these enzymes may disrupt normal methylation homeostasis and reinforce transcriptional programs that favour proliferation, immune suppression, or epithelial-to-mesenchymal transition (Xie et al., 2020). N6-methyladenine (6 mA) methylation represents a significant expansion of the classical DNA methylation paradigm, which is traditionally dominated by 5-methylcytosine (5 mC). While 5 mC is well established in gene silencing and heterochromatin formation, emerging research in eukaryotic systems has revealed that 6 mA serves distinct, often activating, roles in gene regulation. Notably, 6 mA is frequently enriched in heterochromatic regions, particularly within histone H3K9me3-marked domains, where it appears to counterbalance repression and support the selective transcriptional activation (Liu et al., 2025).

In developmental and disease contexts, 6 mA exhibits dynamic and context-specific regulation. Its enrichment in stem-like populations has been linked to the maintenance of tumor-initiating cell phenotypes, facilitating the expression of genes involved in self-renewal, metabolic adaptation, and resistance to apoptosis (Xiao et al., 2017). This epigenetic landscape not only supports sustained tumor growth but also contributes to immune evasion by modulating the expression of immune-related genes, including major histocompatibility complex (MHC) molecules and co-stimulatory ligands (Luan et al., 2024). The functional significance of 6 mA is further underscored by its regulation



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via demethylases, such as ALKBH1, which actively remove this mark. Aberrant expression of ALKBH1 has been reported in glioblastoma, gastric cancer, and other malignancies, where it contributes to epigenomic instability, therapeutic resistance, and cellular invasiveness (Koh et al., 2018). Its dysregulation reinforces the view that 6 mA is not merely a microbial vestige but a malleable epigenetic switch with

Table 3
Differential pathway activity across comparison groups.

Comparison	Pathway	log_2FC	adj.P.Val	Direction	Interpretation
Control vs Co-infected	One-carbon by folate metabolism	−0.23	0.23	Under-represented	Mild suppression during infection
Control vs Co-infected	Glycine, threonine, and serine metabolism	+0.15	0.35	Enriched	Slight amino acid activation
Control vs Co-infected	ATP transporter activity	−0.03	0.83	No change	Unaltered energy transport
CRC vs Co-infected	One-carbon metabolism by folate	0.304	0.054	Enriched	Increased folate-dependent methylation
CRC vs Co-infected	ATP transporter	−0.229	0.110	Under-represented	Reduced energy transport
CRC vs Co-infected	Glycine, serine and threonine metabolism	−0.087	0.493	Under-represented	Lower amino acid flux
CRC vs Helminth	One-carbon metabolism by folate	0.1311	0.3305	Enriched	Trend toward higher folate activity
CRC vs Helminth	Glycine, serine and threonine metabolism	−0.1219	0.3305	Under-represented	Reduced amino acid turnover
CRC vs Helminth	ATP transporter	−0.1182	0.3305	Under-represented	Lower energy metabolism
CRC vs HIV	Glycine, serine and threonine metabolism	−0.3282	0.0320	Under-represented	Significant suppression of one-carbon substrates
CRC vs HIV	ATP transporter	−0.2044	0.1642	Under-represented	Reduced energy-related transport
CRC vs HIV	One-carbon metabolism by folate	−0.0988	0.4377	Under-represented	Minimal folate pathway change

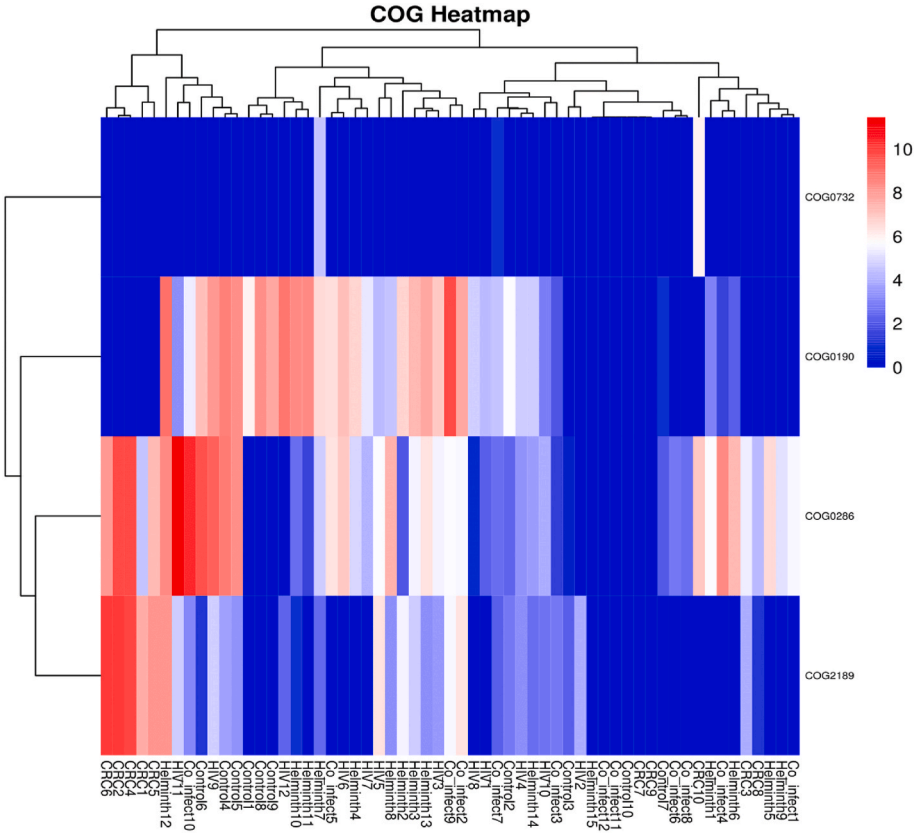

Fig. 5. Heatmap illustrating Virome-Derived Epigenetic Enzymes Differentially Abundant Across Clinical Groups. The heatmap illustrates the clustering of COG gene expression profiles across HIV and helminth uninfected controls, HIV only, Helminth only, HIV-helminth co-infected, and CRC confirmed groups. Rows represent specific COG categories (COG0732, COG0190, COG0286, COG2189), while columns corresponding to individual samples are indicated on the x-axis. The color scale represents the expression levels, with red indicating higher expression, blue indicating lower expression, and white representing intermediate levels. Hierarchical clustering dendrograms are shown for both samples (top) and COG categories (left), highlighting patterns of similarity in gene expression across samples and functional groups.

oncogenic potential in mammalian systems, including humans.

Moreover, the identification of infection-specific 6 mA methylation signals suggests that such epigenetic changes may occur before the emergence of genetic mutations, positioning them as early and potentially reversible events in tumorigenesis. From a translational perspective, this raises the possibility of exploiting microbial 6 mA methylation patterns as non-invasive biomarkers in populations with elevated infection and CRC risk. Collectively, these findings support a conceptual shift in our understanding of methylation dynamics, from a static host-centered model to a bidirectional host-microbe epigenetic interface. Viral signatures and their enzymatic repertoire may play a critical role in shaping the epigenome under chronic inflammatory conditions, with implications for both cancer initiation and progression. Future studies should explore the therapeutic targeting of microbial methyltransferases or the restoration of host 6 mA demethylation capacity as avenues for modulating this axis in CRC prevention and care.

4.1.2. Virulence-associated type I restriction modification systems

Supporting this, COG0286 (Type I restriction-modification methyltransferase) and COG0190 (Type II site-specific DNA methyltransferase) were also differentially abundant. These genes are known components of bacterial restriction-modification systems, which not only protect against phage infection but also modulate transcription and epigenetic landscape (Takahashi et al., 2025). Helminth infections were also associated with site-specific methylation patterns, with shared CpG sites detected across all individuals at both the nestling and fledged juvenile

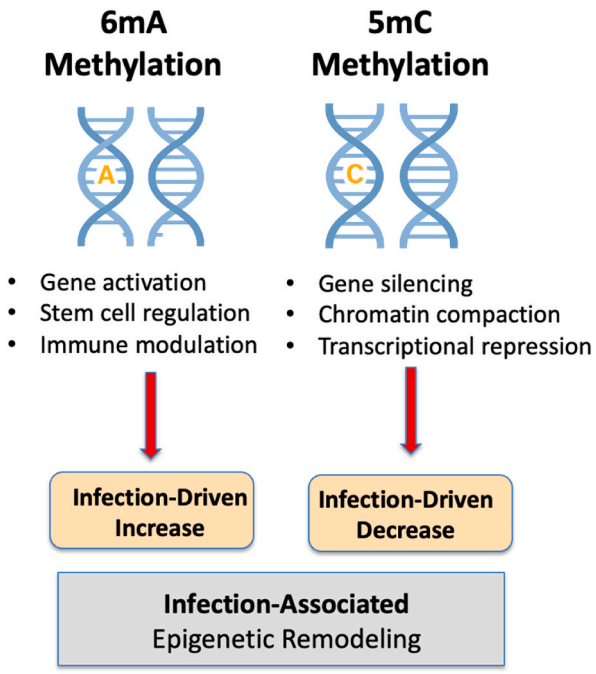

Fig. 6. Comparison between N6-methyladenine (6 mA) and 5-methylcytosine (5 mC) in the context of infection-associated epigenetic remodeling. While 6 mA is linked to gene activation, stem cell regulation, and immune modulation (with infection-driven increases), 5 mC is typically associated with gene silencing and chromatin compaction, and may be reduced in chronic infection contexts. Together, their imbalance reflects a shift toward a tumor-permissive epigenetic state.

stages. These CpG sites were located in gene promoters, transcription start sites, exons, first introns, other introns, or in intergenic regions (Lundregan et al., 2022). The expression of these methyltransferases suggests a functional role of the gut viral signature's epigenetic machinery in mediating infection-driven molecular changes that may promote colorectal oncogenesis.

4.1.3. *MTHFD2 and the metabolic-epigenetic interface in cancer*

In the HIV-only cohort, there was a selective enrichment of COG0190, which encodes 5,10-methylene-tetrahydrofolate dehydrogenase (MTHFD2), a central enzyme in the one-carbon metabolism pathway. MTHFD2 is instrumental in the biosynthesis of S-adenosylmethionine (SAM), a universal methyl donor required for DNA and histone methylation. Its abundance suggests an enhanced methylation potential within host cells, which could predispose the epigenome to aberrant hypermethylation of tumor suppressor loci (Corley et al., 2021; Oriol-Tordera et al., 2020). This mechanism is consistent with reports of epigenetic silencing of antiviral genes in HIV latency and the emergence of chronic inflammation-associated methylation signatures that affect immune pathways and cognitive function (Corley et al., 2016).

Key metabolic genes, COG1932 (phosphoserine aminotransferase), COG0111 (phosphoglycerate dehydrogenase), and COG0190 (5,10-methylene-tetrahydrofolate dehydrogenase), were consistently enriched across infected groups. These enzymes participate in the serine-glycine-one-carbon pathway, crucial for nucleotide biosynthesis and redox homeostasis, and are frequently co-opted during tumorigenesis (Nong et al., 2023; Jack et al., 2001). Additionally, COG0304, which encodes 3-oxoacyl-(acyl-carrier-protein) synthase involved in fatty acid synthesis, was abundant in HIV-infected individuals, supporting lipid biosynthesis, a key component of cancer metabolic rewiring. Interestingly, COG0486, a predicted regulatory GTPase, was also enriched, suggesting potential changes in microbial and viral signaling that may influence host cell cycle control or apoptosis. Infection-specific modulations were observed as well: in HIV-only participants, genes such as COG0557 (exoribonuclease R) and COG0697 (a drug/metabolite transporter family member) were uniquely dysregulated, possibly reflecting virus-induced adaptations in microbial stress response and nutrient transport (Manna et al., 2014). These functional changes reinforce the idea that infection-driven microbiome alterations may support a pro-tumorigenic microenvironment.

4.2. *Metabolic reprogramming in infection-associated CRC risk*

The differential abundance of viral elements involved in one-carbon metabolism (COG0190: MTHFD2-like), serine biosynthesis (COG0111: PHGDH-like; COG1932: PSAT1-like), fatty acid elongation (COG0304), and iron transport (COG0370) indicates a coordinated shift in viral

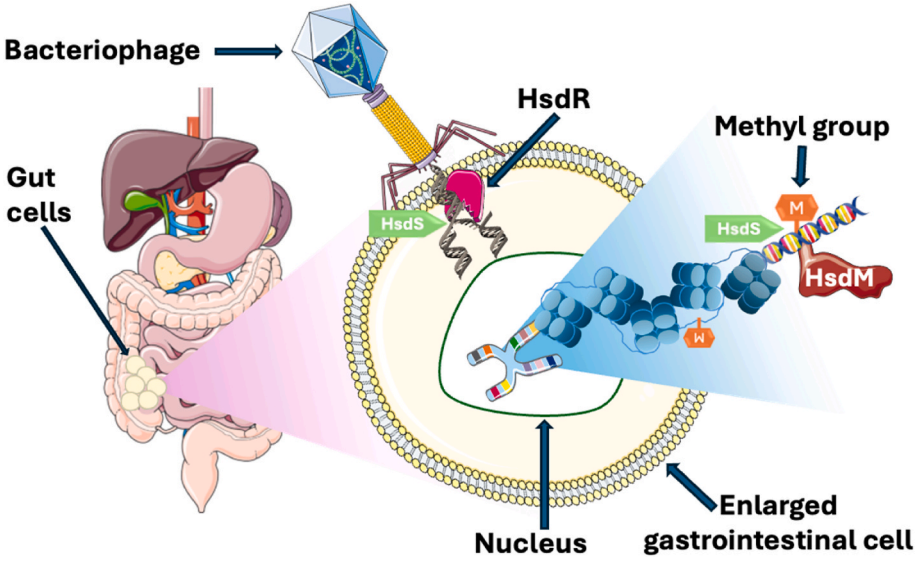

Fig. 7. Type I restriction modification (RM) enzyme function within the gut viral signatures and its potential significance in HIV-helminth co-infection. Within the gut virome, bacteriophages infect bacterial hosts, triggering defense mechanisms such as the Type I R-M system. The specificity subunit (HsdS) recognises defined DNA sequences, guiding the methyltransferase subunit (HsdM) to methylate host bacterial DNA and protect it from cleavage. Incoming phage DNA lacking the host-specific methylation pattern is targeted for restriction-mediated degradation (HsdR).

elements' function. These pathways are well-established components of colorectal tumor metabolism, facilitating nucleotide synthesis, redox balance, immune evasion, and cellular proliferation (Nong et al., 2023; Zhang et al., 2018). Importantly, these metabolic signatures were not limited to patients with CRC. They were consistently detected in HIV-only and co-infected individuals, indicating that chronic infection may precondition the gut microenvironment by establishing a metabolic state similar to that of neoplastic transformation. In contrast, helminth-only individuals exhibited selective underrepresentation of anabolic enzymes, such as PSAT1, suggesting a distinct metabolic suppression profile, indicating that infection type may dictate divergent microbial responses.

The functional convergence of these infection-specific shifts with known CRC hallmarks suggests that chronic infections may induce or mimic early metabolic remodeling in the absence of overt neoplasia. This is consistent with the finding that metabolic reprogramming in cancer often begins at the adenoma stage, driven by transcriptional regulators such as MYC (Satoh et al., 2017). In this context, infection-triggered microbial shifts in methyl donor production and redox metabolism may act synergistically with inflammation to shape a tumor-permissive microenvironment. Furthermore, studies on the role of the microbiome in CRC have highlighted how bacteria, such as *Fusobacterium nucleatum*, promote tumor progression through metabolic and immune signaling (Kostic et al., 2013). The patterns observed in this study align with these models, suggesting the idea that microbial metabolism may serve as both a marker and mechanism of CRC risk in chronically infected populations. Finally, the broader potential implications of these findings are supported by the identification of distinct metabolic signatures in colorectal tumors with prognostic relevance (Shang et al., 2021). The detection of similar signatures in the infection-only groups suggests that gut microbial metabolism may function as a biosensor of early disease risk and a target for preventive intervention.

4.2.1. *One-carbon metabolism and methyl donor flux*

One-carbon metabolism emerged as a potential critical axis of metabolic reprogramming across all infection groups in this study, primarily via the enrichment of COG0190, which encodes MTHFD2. This enzyme functions at the core of the folate cycle, catalyzing the interconversion of methylene-THF to formyl-THF while generating NADPH, an essential reducing equivalent for oxidative stress buffering and biosynthetic activity. In cancer biology, MTHFD2 overexpression is strongly associated with enhanced de novo nucleotide synthesis, epigenetic flexibility, and redox homeostasis, enabling tumor cells to survive under proliferative and inflammatory pressures (Zhang and Wang, 2024; Maddocks et al., 2016). Its infection-specific enrichment in this dataset, notably in HIV-only, helminth-only, and co-infected individuals, may mirror a pre-neoplastic shift in microbial metabolism that converges mechanistically with pathways commonly dysregulated in CRC.

Elevated SAM production by MTHFD2, in tandem with infection-induced inflammation, may foster methylation drift, a precursor to epigenetic instability, gene silencing, and oncogene activation, which are key hallmarks of tumorigenesis (Mentch and Locasale, 2015; Mentch et al., 2015; Ser et al., 2016; Zhang et al., 2021). The relevance of this viral elements metabolic remodeling is further emphasized by the parallels with viral infections, such as SARS-CoV-2, which hijacks host one-carbon metabolism to support viral replication (Qiu et al., 2014). This suggests that the abundance of MTHFD2 is not exclusive to tumor contexts but reflects a broader mechanism of infection-induced metabolic exploitation in host cells. Collectively, the infection-induced abundance of MTHFD2 suggests a microbial contribution to the host epigenetic and metabolic vulnerability. In this context, microbial one-carbon metabolism may serve as both a mechanistic link and an early biosensor for CRC risk in high-exposure populations.

4.2.2. *Amino acid biosynthesis: serine-glycine axis and tumor metabolism*

The relationship between amino acid biosynthesis, particularly the serine-glycine axis, and tumor metabolism has gained prominence in understanding infection-associated CRC risk. This axis is central to the serine-glycine-one-carbon (SGOC) network, which links glycolytic intermediates to the synthesis of amino acids, nucleotides, glutathione, and methyl donors. These metabolic processes are essential for redox homeostasis, epigenetic maintenance, and rapid cell proliferation, which are hallmarks of cancer progression (Wang et al., 2019; Ananieva, 2015). In this study, the abundance of COG0111 (phosphoglycerate dehydrogenase, PHGDH-like) and COG1932 (phosphoserine aminotransferase, PSAT1-like) was observed in HIV-infected and co-infected individuals, indicating potential activation of the serine biosynthesis pathway. PHGDH catalyzes the oxidation of 3-phosphoglycerate to 3-phosphohydroxypyruvate, whereas PSAT1 converts this intermediate into phosphoserine, a precursor of serine and subsequently glycine. This pathway contributes to the folate cycle, glutathione maintenance, and production of SAM, a universal methyl donor for DNA and histone methylation. Additionally, microbial homologs of methylenetetrahydrofolate dehydrogenase (MTHFD2, COG0190) contribute to the mitochondrial folate-dependent one-carbon metabolism pathway. This enzyme plays a crucial role in producing SAM (Valle-Casuso et al., 2019) (Fig. 8). Elevated levels of MTHFD2 have been widely observed in various cancers, where it contributes to tumor growth, maintenance of stem cell-like properties, and resistance to chemotherapy. Due to its role in driving tumor progression and association with poor clinical outcomes, MTHFD2 represents a promising target for anticancer therapies. Our findings suggest a mechanistic link between infection-induced viral elements and metabolic reprogramming.

Thus, activation of the serine-glycine biosynthesis pathway in helminth individuals may reflect a multi-layered metabolic adaptation that simultaneously supports persistent infection and primes the host for oncogenic transformation. Its suppression in helminth-only cases further underscores the infection-specific metabolic trajectories. Thus, the SGOC axis serves as both a mechanistic bridge between infection and CRC risk and a potential intervention point for early cancer prevention in high-risk populations. The SGOC axis emerges as a promising potential therapeutic target. Modulating PHGDH or PSAT1 activity could impair tumor-supportive metabolic fluxes while restoring immune functionality. As Wang et al. (2019) suggested, targeting enzymes in this pathway could simultaneously limit infection-driven immune exhaustion and tumor proliferation.

4.2.3. *Lipid biosynthesis and membrane remodeling*

A distinct feature of the infection-altered metabolic landscape in this study was the enrichment of COG0304, which encodes 3-oxoacyl-ACP synthase, particularly in HIV-positive individuals. This enzyme is a key catalyst in the fatty acid elongation cycle, driving de novo lipid biosynthesis, which fuels membrane remodeling, vesicle formation, and proliferation. In both viral infections and cancer, fatty acid synthesis is frequently reprogrammed to accommodate the increased membrane turnover and signaling needs of rapidly replicating cells (He et al., 2018; Lorizate et al., 2013; Waheed and Freed, 2010). In the context of HIV infection, this metabolic shift suggests that the host lipid machinery is repurposed to support viral replication while sustaining inflammatory and regenerative responses within the gut microenvironment. Elevated lipid biosynthesis not only provides the structural lipids necessary for viral envelope assembly but also contributes to bioenergetic flexibility and immune modulation in the host (Chan et al., 2010; Heaton and Randall, 2011). Such changes may synergize with broader tumorigenic processes, as metabolic autonomy through lipid production is a hallmark of both infections and neoplastic transformation.

These findings are consistent with evidence from other viral systems. For instance, Hepatitis C Virus (HCV), coronaviruses, and herpesviruses have been shown to hijack host fatty acid biosynthesis pathways to create replication organelles or evade immune detection (Syed et al.,

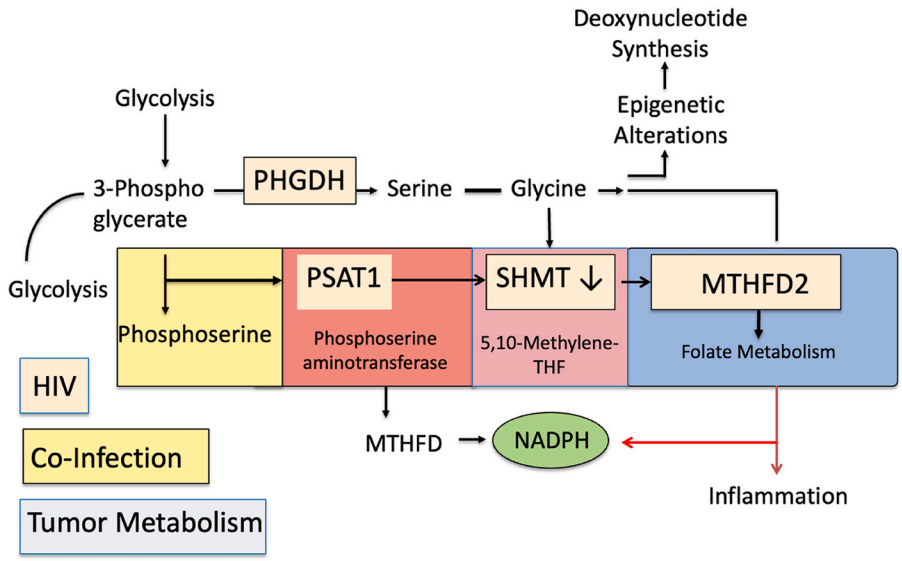

Fig. 8. The serine-glycine-one-carbon (SGOC) metabolic axis linking glycolytic intermediates to folate cycle activation, NADPH production, and epigenetic modulation. Enriched enzymes in HIV and co-infection contexts (PHGDH, PSAT1, MTHFD2) contribute to increased methyl donor synthesis and redox buffering, mirroring tumor metabolism and enhancing CRC risk.

2010; Song et al., 2024). This convergence suggests a conserved viral strategy that mimics cancer-like metabolic remodeling to maximize replication efficiency. Viruses and tumors share dependencies on elevated lipid turnover, redox buffering, and membrane-bound signaling platforms (Butler et al., 2020; Bartolacci et al., 2021). Moreover, microbial interactions may have amplified this metabolic phenotype. Altered gut viral elements configurations in HIV-positive individuals may stimulate compensatory lipid biosynthesis as part of a broader host-microbe adaptation to inflammation and oxidative stress. While these shifts are initially protective, they may inadvertently prime epithelial tissues for malignant progression by promoting cell survival and immune escape mechanisms. In summary, the abundance of lipid biosynthesis genes in HIV-infected individuals may reflect a metabolic interface between chronic viral infection and tumorigenesis, where HIV-driven reprogramming of host lipid pathways parallels oncogenic metabolic processes. This metabolic convergence suggests that inhibition of fatty acid synthesis may simultaneously impede HIV replication and mitigate infection-associated carcinogenic risk.

4.2.4. *Ion transport, redox balance, and proliferation signals*

Dysregulation of iron metabolism is increasingly recognized as a critical factor in the pathological processes associated with HIV infection and may contribute to the mechanisms underlying colorectal carcinogenesis. Evidence suggests that an increase in microbial iron transport genes, such as COG0370, occurs in individuals infected with HIV and helminths, indicating that these pathogens or their associated dysbiosis exploit host iron to support microbial survival. This activity may inadvertently generate a pro-oxidative environment in the gastrointestinal tract. Iron, a redox-active metal, facilitates Fenton chemistry, producing hydroxyl radicals that promote DNA damage, lipid peroxidation, and inflammation, all of which are closely linked to tumor initiation and genomic instability (Shankaran et al., 2017; Shytaj et al., 2020).

Furthermore, the upregulation of COG0486, a predicted regulatory GTPase, in samples from HIV and helminth infected individuals suggests alterations in host-microbe signaling networks that affect cell proliferation and migration. GTPases are central to cell cycle progression, cytoskeletal remodeling, and vesicle transport, and have been increasingly implicated in cancer cell invasion and chemoresistance (Gonçalves et al., 2019; Kerins and Ooi, 2018). Although the exact function of the microbial GTPase identified here remains unknown, its consistent upregulation implies a microbial strategy of mimicking or modulating host signaling to promote epithelial transformation. Additionally, research has shown that HIV-infected cells exhibit increased iron uptake and oxidative stress, partly due to reduced glutathione (GSH) levels, which weakens their antioxidant defenses and renders them more susceptible to damage and transformation (Bhaskar et al., 2015). Dysregulation of hepcidin, the central regulator of iron homeostasis, further exacerbates this vulnerability by disrupting systemic iron distribution, contributing to iron overload or anaemia, depending on the context (Cunha et al., 2015; Huibers et al., 2020). These findings align with the microbial gene expression shifts observed in this study and indicate a multi-level disturbance of redox balance.

In summary, the coordinated upregulation of microbial genes involved in iron acquisition and signal transduction during chronic infection, particularly in the setting of HIV or co-infection, may reshape the mucosal microenvironment in favour of proliferation, inflammation, and DNA instability. These mechanisms represent converging pathways that can potentiate colorectal cancer development, emphasizing the need to consider iron metabolism and oxidative stress as key mediators of infection-associated carcinogenesis.

4.2.5. *Convergence on tumor-promoting metabolic states*

The provided data highlight that infection-associated shifts in microbial metabolism, especially under HIV-helminth co-infection, may remodel metabolic states in ways that mirror early tumorigenesis. This is reflected in the observed upregulation of microbial genes supporting key biosynthetic processes, including SAM synthesis, amino acid and fatty acid metabolism, and iron transport. These coordinated metabolic changes suggest a structured reprogramming of the gut microbiome that may precede and facilitate the neoplastic transformation. Chronic infections, particularly those involving HIV, disrupt microbial community composition and function, leading to a dysbiotic gut environment that supports oncogenic signaling through specific metabolic routes. For example, the upregulation of COG0370, which encodes an Fe^{2+} transport system protein, was observed in the HIV- and helminth-infected groups. Iron is a key redox-active metal that, when dysregulated, promotes oxidative stress through the generation of hydroxyl radicals, damaging DNA, and promoting inflammation. Such oxidative and inflammatory environments can promote genomic instability and contribute to tumorigenesis.

A broader understanding of these shifts is captured by the concept of functional redundancy, whereby different microbial communities,

regardless of their species composition, converge functionally by expressing similar metabolic capabilities (Glymenaki et al., 2017; Villéger et al., 2019). This functional convergence implies that chronic infections can lead to distinct microbial assemblages that activate shared tumor-promoting metabolic networks, particularly those involved in redox balance and proliferation signals. The concurrent upregulation of genes such as MTHFD2 and PHGDH in this context underscores the tight link between microbial metabolism and host cellular pathways (Aarnoutse et al., 2019; Gopalakrishnan et al., 2018). This crosstalk may enhance the biosynthetic and oxidative capacity of the local microenvironment, priming tissues for neoplastic transformation even before pathological changes become histologically visible. Moreover, these infection-altered microbial gene signatures have implications beyond pathogenesis, as they offer opportunities for early detection and therapeutic modulation. Recent findings suggest that modifying the gut microbiota can influence host responses to cancer therapy (Villéger et al., 2019; Oh et al., 2021). Such interventions may be particularly beneficial for high-risk individuals exposed to chronic infections, where microbial iron transport and redox regulation act as upstream modulators of tumorigenesis. In conclusion, dysregulation of ion transport and redox-related microbial genes in individuals co-infected with HIV and helminths reveals a mechanism by which infection-induced metabolic reprogramming contributes to early events in colorectal cancer development. These findings emphasize the therapeutic value of targeting microbial metabolic functions, such as iron acquisition and oxidative stress modulation, as part of a personalized strategy for CRC prevention and intervention.

4.2.6. Differential pathway activity

Aggregate pathway analysis revealed distinct metabolic signatures associated with CRC in the context of HIV and helminth co-infection. The upregulation of the one-carbon metabolism by the folate pathway in CRC relative to the co-infection group (logFC = 0.30, adj. p = 0.054) suggests enhanced folate-dependent methylation and nucleotide biosynthesis that could facilitate tumor proliferation. This observation aligns with the known role of folate metabolism in sustaining rapid cell division and maintaining DNA methylation patterns that favour oncogenic transcriptional programs. In contrast, the downregulation of ATP transporter and glycine, serine, and threonine metabolism pathways implies suppressed mitochondrial activity and amino acid flux, potentially reflecting metabolic trade-offs during tumor adaptation in immune-altered environments. Together, these findings suggest that disruption of folate and energy metabolism may represent a critical mechanistic link between HIV-helminth co-infection and heightened CRC susceptibility through dysregulated methylation and metabolic reprogramming. When compared with helminth infection alone, CRC samples showed a consistent, though nonsignificant, elevation in folate-mediated one-carbon metabolism, reinforcing the notion that even mild shifts in this pathway may signal early tumorigenic events under chronic inflammatory or parasitic conditions. Meanwhile, the CRC versus HIV comparison revealed a significant suppression of the glycine, serine, and threonine metabolism pathway (adj. p = 0.032), suggesting that HIV infection may alter amino acid utilization and one-carbon substrate availability. Such alterations could compromise metabolic flexibility, thereby influencing tumor energetics and methylation balance in a manner distinct from helminth-driven effects.

Collectively, these results point toward an intricate metabolic interplay at the intersection of infection and tumorigenesis. They highlight the need for deeper investigation into how chronic infectious exposures modulate methylation-linked metabolism and thereby shape cancer risk in African populations, where HIV-helminth co-infection remains prevalent.

The GSVA-based pathway quantification revealed context-dependent but consistent modulation of folate-linked and amino acid pathways across infection and cancer states. The upregulation of one-carbon metabolism in CRC, contrasted with its suppression in infection, suggests adaptive reactivation of methylation and nucleotide synthesis pathways to support tumor growth. Concurrent downregulation of amino acid and ATP transporter activity indicates disrupted energy balance, likely compounding the metabolic stress induced by chronic infection. While most pathways did not reach FDR significance, these coordinated shifts provide a mechanistic framework linking infection-driven immune alterations to CRC-associated metabolic reprogramming.

Overall, this study's findings suggest that microviral elements' metabolism and epigenetics represent central axes through which chronic infections may modulate the gut environment, potentially reprogramming it to promote colorectal oncogenesis. Although causality cannot be established from this cross-sectional metagenomic study, the convergence of immunosuppressive profiles, virome network restructuring, and metabolic and epigenetic reprogramming supports a model in which chronic infection disrupts host-microbiome equilibrium in ways that increase cancer susceptibility. These insights are particularly important for African populations, where HIV-helminth co-infections are endemic and virome-related cancer research remains underexplored. Future longitudinal and mechanistic studies are essential to unravel the causal pathways linking chronic infection, virome perturbation, and CRC risk.

4.3. Clinical and translational relevance

The identification of infection-associated microbial methylation enzymes, particularly COG2189 (adenine-specific DNA methylase) and COG0286 (Type I restriction-modification methyltransferase), has substantial implications for early detection of CRC and targeted intervention. These enzymes, consistently enriched in the helminth-only, co-infected, and CRC-confirmed groups, appear to reflect a state of heightened methylation permissiveness in the gut epithelium. Their microbial origin and infection-specific enrichment suggest that they may serve as early, non-invasive biomarkers of epigenetic dysregulation and cancer risk. Stool-based metagenomic detection of methylation-related enzymes presents a practical and scalable diagnostic approach, particularly suited to low-resource regions where access to conventional screening modalities, such as colonoscopy and histopathology, remains limited (Emlet et al., 2020; Li et al., 2022). In sub-Saharan Africa, where both the infectious disease burden and CRC incidence are rising, such tools could enable earlier detection and stratified surveillance among high-risk populations (Luo et al., 2022; Sun et al., 2023).

Importantly, these microbial methyltransferases not only serve as potential biomarkers but may also act as therapeutic targets for CRC. For instance, COG2189 and its associated 6 mA activity have been linked to transcriptional activation in repressive chromatin regions, tumor stemness, and immune evasion. Similarly, the co-expression of COG0286 in co-infection states underscores the role of virome-encoded epigenetic machinery in shaping host gene expression. Targeted inhibition of these microbial methylases or modulation of downstream host pathways disrupted by 6 mA accumulation could restore epigenetic equilibrium and mitigate carcinogenic potential (Chen et al., 2022; Qin et al., 2018). Restoration of host epigenetic surveillance may also involve the reactivation or therapeutic enhancement of demethylating enzymes, such as ALKBH1, whose dysregulation in cancer models promotes 6 mA persistence and epigenomic instability (Chen et al., 2022). Integrating these microbial targets with host methylation regulators could form the basis for multi-modal epigenetic therapies.

Moreover, by coupling metagenomic signatures with host genomic and transcriptomic data, precision oncology frameworks can be developed to better characterize infection-associated CRC subtypes (Li et al., 2022). This approach supports a paradigm shift from static host-centric models to dynamic host-microbe interactions, in which chronic infection and microbial metabolism synergize to rewire the epigenome and promote malignancy (Emlet et al., 2020; Luo et al., 2023). In conclusion, the integration of viral elements associated methylation markers into

clinical practice offers an innovative strategy for detecting and disrupting infection-driven carcinogenesis. As sequencing technologies become more accessible and epidemiological surveillance expands, these findings can inform cost-effective screening programs, guide molecularly targeted therapies, and improve CRC outcomes in regions facing the overlapping burdens of infection and cancer (Zuo et al., 2022; Borin et al., 2023).

5. Challenges and limitations

All stool samples were collected before surgical intervention, bowel preparation, or antibiotic administration, minimizing external perturbations of the gut microbiota. Nonetheless, detailed perioperative metadata (e.g., diet, medications, and clinical parameters) were not systematically recorded, which restricted deeper contextual analysis. Although excluding or adjusting for perioperative conditions would have strengthened the analysis, this was not feasible due to the limited sample size and lack of standardized metadata. Nonetheless, because all CRC samples were obtained under comparable preoperative conditions, intra-group variability is likely minimal. The relatively small sample size limited the statistical power to detect subtle microbial-epigenetic associations; however, consistent patterns observed across groups highlight biologically meaningful trends that warrant further exploration. Future studies should increase cohort size, include longitudinal sampling, and apply integrated multi-omics to validate causal pathways linking infection-driven microbiome remodeling, host methylation changes, and CRC susceptibility. Standardized and well-documented sample collection protocols are also noted for future studies with a larger cohort to validate these findings. Despite these limitations, this study provides the first evidence in a Sub-Saharan African context linking HIV-helminth co-infection to gut microbial and epigenetic signatures resembling those observed in CRC.

6. Conclusion

In a setting burdened by overlapping infectious diseases and an escalating CRC burden, our integrative multi-omic approach illuminates how chronic HIV and helminth co-infections reprogram the molecular landscape toward oncogenesis. This framework enabled a comprehensive interrogation of metabolic and epigenetic dynamics in a South African cohort uniquely positioned at the intersection of infection and cancer vulnerability. These poorly characterized viral elements suggest a cryptic reservoir of oncogenic potential, supporting the emerging concept of an "onco-viral elements" in African populations, where dysbiotic viral communities may drive early carcinogenic processes. Our findings reveal a potential novel convergence of viral reprogramming, viral elements metabolism, and epigenetic dysregulation that together may foster a permissive microenvironment for CRC initiation. This study not only advances our understanding of infection-associated carcinogenesis but also emphasizes the critical need to integrate infection history and viral elements ecology into CRC risk stratification, particularly in resource-limited regions facing syndemic disease pressures. Our findings also suggest that chronic infections may enhance the host's methylation potential not only through the direct expression of viral methyltransferases but also via metabolic upregulation of the methyl donor biosynthesis machinery (Fig. 9). Together, these observations underscore the potential for both direct and indirect epigenetic modulation by the gut virome in the risk of infection-associated CRC. The integration of viral elements methyltransferase expression (e.g., COG2189 and COG0286) with metabolic reprogramming (COG0190) reveals a multi-pronged mechanism through which chronic infections may prime the host intestinal epigenome toward a pro-oncogenic state. These trends align with the broader metagenomic findings of this study, which illustrate the distinct enrichment of methylation-related genes across the infected and CRC-positive groups. Such insights are particularly important in regions such as sub-Saharan Africa, where overlapping infectious burdens are high and molecular predictors of cancer susceptibility remain poorly characterized. The convergence of microbial signaling, metabolic plasticity, and host epigenetic dysregulation strengthens the hypothesis that chronic infection is a modifiable risk factor for CRC via methylation-driven genomic instability.

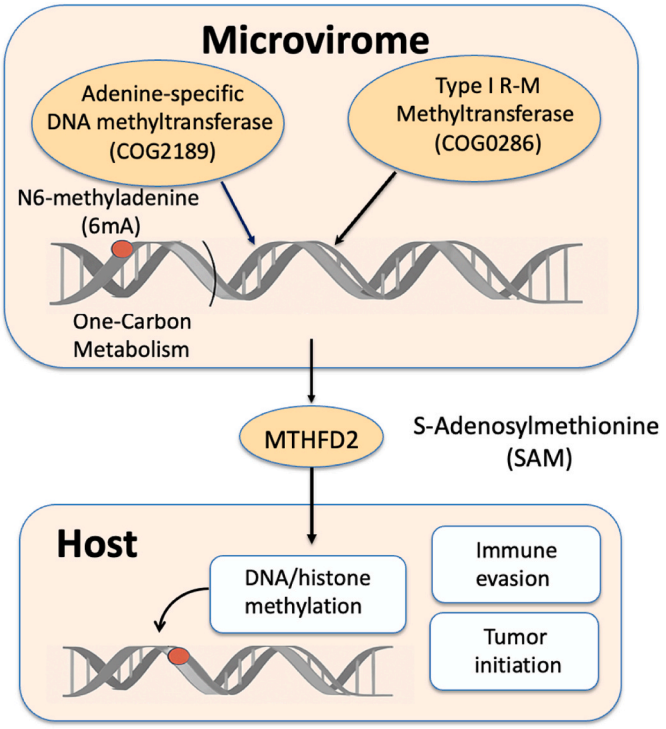

Fig. 9. Intersecting Viral elements Regulatory pathways. Viral elements-derived epigenetic enzymes (COG2189, COG0286) and one-carbon metabolism (MTHFD2) collaboratively drive host DNA and histone methylation via S-adenosylmethionine (SAM) production. These infection-associated mechanisms promote immune evasion and tumor initiation, establishing a methylation-permissive microenvironment in the gut epithelium.

CRediT authorship contribution statement

Botle Precious Damane: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Jonathan Featherston:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Shakeel Kader:** Writing – review & editing, Data curation. **Mohammed Alaouna:** Writing – review & editing, Visualization. **Pragalathan Naidoo:** Writing – review & editing, Validation, Supervision. **Zodwa Dlamini:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Zilungile Lynette Mkhize-Kwitshana:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Data availability statement

Data generated from this study can be accessed from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate. The study was conducted in accordance with the Declaration of Helsinki and approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (BREC/00005458/2023) on May 18, 2023. Informed

consent was obtained from all subjects involved in the study.

Clinical trial number

Not applicable.

Consent to publish declaration

All authors have read and agreed to the published version of the manuscript.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Botle Precious Damane, Zodwa Dlamini and Zilungile Mkhize-Kwitshana reports financial support was provided by South African Medical Research Council and National Research Foundation. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amolm.2026.100103.

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