

A Multi-omics PTM Atlas Reveals Key Insights into Metabolic Reprogramming in Colorectal Cancer

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Abstract

Background/Aim: Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, with limited effective targeted therapies. Metabolic reprogramming is a hallmark of cancer, and post-translational modifications (PTMs), such as phosphorylation, ubiquitination, and malonylation, play critical roles in regulating metabolic pathways. However, their contribution to metabolic reprogramming in CRC remains unclear.

Materials and Methods: Phosphorylation, ubiquitination, and malonylation were analyzed in paired CRC and adjacent normal tissues using high-resolution mass spectrometry. Differential PTM patterns were analyzed, followed by identification of key regulatory enzymes and modification sites. Functional enrichment, protein-protein interaction (PPI) networks, and multi-omics integration were used to explore PTMs' role in CRC metabolism.

Results: We identified 59 differential phosphorylation sites, 263 ubiquitination sites, and 64 malonylation sites in CRC tissues compared with normal tissues, affecting key metabolic enzymes such as IDH1, LDHA, PDHA1, and GAPDH. Altered ubiquitination of IDH1 and LDHA may be associated with changes in protein stability and activity.

continued

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Phosphorylation of PDHA1 correlated with its modified levels, potentially promoting glycolytic preference in CRC, while increased malonylation of GAPDH may influence its enzymatic activity and glycolytic flux. Protein interaction and pathway analyses further revealed a PTM-regulated metabolic network, suggesting a potential role of PTMs in CRC metabolic reprogramming.

Conclusion: This study suggests that PTMs may contribute to metabolic reprogramming in CRC by modulating key metabolic enzymes, including IDH1, LDHA, PDHA1, and GAPDH. These modifications may influence glycolysis and energy metabolism, highlighting PTM-regulated pathways as potential therapeutic targets. The integrated PTM atlas offers insights into the metabolic landscape of CRC.

Keywords: Colorectal cancer, phosphorylation, ubiquitination, malonylation, metabolism, PTM.

Introduction

Colorectal cancer (CRC) is one of the most common and deadly cancers worldwide. According to global cancer data, it is estimated that there were 1.9 million new cases and 900,000 deaths from CRC in 2020, with both mortality and incidence rates rising progressively (1). Despite significant advances in early screening techniques and therapeutic methods, poor clinical outcomes are still observed in CRC patients, particularly in cases of metastasis, recurrence, and resistance to therapy (2). Although treatment strategies exist, there are currently no precise therapies that target specific molecules, which makes CRC difficult to treat.

Metabolic reprogramming is widely accepted as a fundamental hallmark of cancer. Tumour cells alter energy production and macromolecule biosynthesis to meet the demands of rapid growth and proliferation. Recent studies have increasingly focused on metabolic processes in the development, growth, and metastasis of CRC (3). Cellular energy production, fatty acid synthesis, and respiration show strong correlations with the growth, migration, and survival of cancer cells (4).

However, a critical knowledge gap remains, despite significant progress in our understanding of metabolic reprogramming in CRC. This gap concerns our understanding of how post-translational modifications (PTMs) alter metabolic processes systemically. PTMs regulate protein function and cellular signaling. Yet they are often overlooked

in CRC research, particularly with regard to their direct impact on metabolic pathways and their role in the hallmarks of cancer. Although research has identified changes in metabolic pathways in CRC, most studies have focused on gene expression and specific proteins. Despite their importance in metabolic regulation and oncogenesis development, the role of PTMs, particularly phosphorylation, ubiquitination and malonylation, has not been sufficiently reviewed (5). Studies have demonstrated that PTMs, in the form of phosphorylation, ubiquitination and malonylation, have the capacity to modulate protein interactions and consequently play a role in the metabolic reprogramming of cells. The way in which the function of pyruvate kinase M2 (PKM2) and the mitochondrial channel protein VDAC3 is enhanced by malonylation is understood. The movement of the mitochondria is facilitated and the survival of tumour cells without sufficient nutrients is increased by this process (6). Furthermore, the stability of important transcription factors like c-Myc is controlled by ubiquitination, which in turn regulates the glycolytic pathway and promotes CRC cellular proliferation and migration (7). Tyrosine phosphorylation significantly reduces the catalytic activity of PKM2, leading to the accumulation of glycolytic intermediates and favoring the biosynthesis of tumour cellular building blocks. This sustains the Warburg effect (aerobic glycolysis) (8). Recent studies have shown that certain transporters and regulatory proteins play a role in tumor progression and metabolic stress adaptation, which is crucial for understanding metabolic regulation in CRC (9).

Additionally, other key regulatory factors have been found to influence apoptosis and angiogenesis in colorectal cancer, further linking metabolic reprogramming with cancer cell survival and metastasis (10). Therefore, it is of significant interest to understand the combination of PTMs involving phosphorylation, ubiquitination and malonylation in CRC, and to investigate their characteristic changes, particularly with regard to metabolic reprogramming.

This study methodically analyzed the pivotal functions of phosphorylation, ubiquitination and malonylation PTMs in CRC using multi-omics approaches. The aim is to construct a comprehensive atlas of PTM-driven metabolic regulation in order to reveal how these modifications govern metabolic reprogramming. It will also demonstrate their contribution to CRC progression. The main objectives of this research are: (1) profiling the expression levels of these three PTMs and identifying differential patterns in CRC; (2) constructing a PTM-regulated metabolic network to elucidate their role in CRC metabolic reprogramming; (3) identifying key regulatory factors and modification sites to inform the development of targeted therapies. Revealing the PTM-driven metabolic regulatory mechanisms in CRC will deepen our understanding of the molecular mechanisms underlying the disease and offer novel theoretical insights and targeted therapeutic strategies for metabolic diseases and cancer treatment.

Materials and Methods

Data sources. The phosphoproteomic data (CRC $n=8$, NC $n=8$) (PXD021314), ubiquitinated proteomic data (CRC $n=8$, NC $n=8$) (PXD028504), and malonylated proteomic data (CRC $n=8$, NC $n=8$) (PXD021318) used in this study were derived from a CRC cohort established in our laboratory (11-13). Importantly, phosphoproteomic, ubiquitinomics, and malonylome analyses were performed on the same set of paired CRC and adjacent normal tissues ($n=8$ pairs). PTMs level and protein abundance were both quantified among the samples of the same batch which ensured the comparability of the PTM data. A quantitative PTM proteomic method quantifies each PTM site with the

corresponding protein abundance to measure the absolute occupancy of each global PTM site. These data were obtained through affinity enrichment followed by high-resolution mass spectrometry. The detailed experimental workflow is outlined below.

The malonylated proteomes were analyzed using the 4D-label-free proteomics method. Tissue samples were lysed, quantified, and digested before enrichment of modified peptides using an anti-malonylated lysine antibody-based immunoaffinity enrichment technique. The enriched products were separated using nano-scale high-performance liquid chromatography and analyzed by four-dimensional (retention time, m/z , charge state, ion mobility) coupled mass spectrometry on the Bruker timsTOF Pro platform to enhance detection sensitivity and depth for modified peptides. The raw mass spectrometry data were processed and identified using MaxQuant software (Max Planck Institute of Biochemistry, Martinsried, Germany), with the Uniprot human protein database, setting malonylated lysine (+86.0004 Da) as a variable modification and controlling the false discovery rate (FDR) at <1%. The high-confidence modified sites identified were subsequently used for quantitative and bioinformatic analysis.

Patient information. Patients with primary CRC who underwent surgical resection at Shenzhen People's Hospital and had not received prior radiotherapy or chemotherapy were eligible for inclusion in this study. Patients with hereditary CRC were excluded. Each case was classified by gastroenterologists based on pathological analysis. Clinical, pathological, and treatment information were obtained from the patients' electronic medical records. All participants provided informed consent after being fully informed about the study. This study was approved by the Ethics Committee of Shenzhen People's Hospital (approval number: LL-KY-2019213).

Sample collection, tissue handling, protein extraction, and digestion. Tumor tissues (CRC) and adjacent normal tissues (NC) were collected from the same patient during

surgery. Tumor tissues were obtained within 1 h post-surgery, while normal tissues were harvested from a colon segment 5 cm away from the tumor. Both tumor and normal tissues were immediately placed in liquid nitrogen for at least 3 h before being stored at -80°C for long-term preservation. Protein extraction was performed by homogenizing tissue samples in lysis buffer containing 8 M urea and 1% protease inhibitor cocktail (Merck Millipore, Darmstadt, Germany, 156535140). The mixture was sonicated on ice, followed by centrifugation at $12,000 \times g$ for 10 min at 4°C to remove cellular debris. The resulting supernatant was collected, and the protein concentration was determined using a BCA kit (Beyotime, Shanghai, PR China), according to the manufacturer's instructions. The protein sample was then incubated with 5 mM dithiothreitol at 56°C for 30 min for reduction. Afterwards, 11 mM iodoacetamide was added, and the sample was incubated at room temperature in the dark for 15 min. For digestion, proteins were treated with trypsin at a 1:50 ratio for the first digestion step, which lasted for 12 h, followed by a second digestion with trypsin at a 1:100 ratio for an additional 4 h. The resulting peptides were used for further analysis.

LC-MS analysis. LC-MS/MS analyses were conducted using a Bruker timsTOF Pro (Bruker Daltonics, Billerica, MA, USA) mass spectrometer in parallel accumulation-serial fragmentation (PASEF) mode (14). Tryptic peptides were dissolved in 0.1% formic acid (solvent A) and loaded directly onto a homemade reversed-phase analytical column (15-cm length, 75- μ m i.d. for proteomic analyses and 25-cm length, 100- μ m i.d. for phosphoproteomic, ubiquitin proteomics, and malonylation proteomics analyses). The proteomic analysis was carried out with a solvent gradient starting from 6% solvent B (98% acetonitrile with 0.1% formic acid) for 70 min, increasing to 24% over the first 70 min, followed by a further increase to 35% over 14 min, and then to 80% over 3 min. The gradient was maintained at 80% for an additional 3 min. For the phosphoproteomic analysis, the gradient started at 2% solvent B, which gradually increased over 50 min to

22%, then to 35% over 2 min, and finally to 90% over 3 min, and maintained at 90% for 5 min. For ubiquitin proteomic analysis, the gradient started at 6% solvent B, increasing to 22% over 44 min, then to 30% over 12 min, and finally to 80% over 2 min. The gradient was maintained at 80% for 2 min. For malonylation proteomic analysis, the gradient started at 6% solvent B, increasing to 24% over 42 min, then to 32% over 14 min, and finally to 80% over 2 min. The gradient was maintained at 90% for 2 min (15). All steps were performed using a constant flow rate of 450 nl/min on a nanoElute UPLC system (Bruker Daltonics). The peptides were processed using the timsTOF Pro mass spectrometer with an applied electrospray voltage of 1.6 kV. The mass spectrometer measured both precursors and fragment ions across the range of 100 to 1700 m/z, using the TOF detector. Dynamic exclusion was set to 30 seconds, and the collision energy was fixed at 30 eV for MS/MS scanning.

MS/MS data were analyzed using the MaxQuant software package (v1.6.6.0). The reference database used was Homo_sapiens_9606_SP_20191115 (20,380 sequences). A reverse library was incorporated to calculate the false positive rate, and common contaminant libraries were added to minimize contamination effects. Trypsin/P was used for enzyme digestion, with a missed cleavage limit of 2.

Protein functional enrichment analysis. Differentially expressed proteins and their corresponding genes were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the clusterProfiler package (version 4.10) in R (version 4.3.2). For pathway enrichment, the enricher function was used with a custom background, and a *q*-Value < 0.05 (after Benjamini-Hochberg correction) was set as the threshold for significance (16).

Protein-protein interaction network construction and submodule analysis. A protein-protein interaction (PPI) network of differentially expressed proteins was constructed using STRING v11.5, with a minimum

interaction score of 0.700, to filter for high-confidence human protein interaction data. The protein data were imported into Cytoscape 3.10.1 software, where the MCODE plugin was used to mine network modules (17, 18).

Hub protein identification. Key proteins (hub proteins) within the PPI network and submodules were calculated using the CytoHubba plugin in Cytoscape (18). Hub proteins were identified by ranking them according to five centrality algorithms (Degree, MCC, Betweenness, EPC, and Closeness) and selecting the top 10% of proteins. Further cross-comparison of phosphorylation, ubiquitination, and malonylation modification sites allowed the identification of core proteins that are modified by multiple PTMs.

Protein domain analysis. The amino acid sequences and three-dimensional structures of the target proteins were retrieved from the AlphaFold database (<https://www.alphafold.ebi.ac.uk/>) (19). To analyze the spatial distribution of the modification sites, the protein structures were visualized and mapped using PyMOL 3.1.3.1 molecular visualization software (20).

PTM site localization and annotation. To accurately localize the PTM sites, phosphorylation, ubiquitination, and malonylation sites were compared with the PhosphoSitePlus® 2024-02 database. Motif analysis of phosphorylation sites was performed using Motif-X (occurrence ≥ 20 , $p < 1 \times 10^{-6}$). Ubiquitination chain types were predicted using GPS-Ubi 2.0, and malonylation sites were predicted using GPS-Mal 2.0. Protein structure localization was visualized in three dimensions using PyMOL, with models obtained from AlphaFold (21, 22).

Statistical analysis. Statistical comparisons between groups were performed using independent *t*-tests. Statistical significance was defined as follows: $p < 0.05$, $^*p < 0.01$, $^{**}p < 0.001$, and $^{***}p < 0.0001$ (23, 24). When p -value < 0.05 , a fold change greater than 1.5 is considered the threshold for significant upregulation, while a fold change less than 1/1.5 is considered the threshold for

significant downregulation. Statistical comparisons between paired CRC and adjacent normal tissues were performed using paired *t*-tests.

Pathway network analysis. To comprehensively elucidate the potential regulatory networks of metabolic processes in CRC, this study first integrated data on significantly differentially modified proteins from CRC tissues. By combining functional annotations from the GO and KEGG databases and reviewing related literature from PubMed, the functions of these proteins were determined. Their roles in key biological processes, particularly glycolysis, lipid metabolism, energy regulation, cell proliferation, and migration, were then classified and screened. Based on existing literature on PPIs and their roles in metabolic pathways, we have constructed a potential metabolic regulatory pathway network. The aim of this network is to reveal how differentially modified proteins in CRC participate in metabolic reprogramming and tumour progression regulation. It provides strong support for understanding how PTMs fine-tune metabolic processes and their role in cancer cell metabolic adaptation, laying the theoretical foundation for future targeted therapeutic research in CRC.

Results

Differential analysis of phosphorylation, ubiquitination, and malonylation modifications in metabolism-related proteins in CRC. PTMs such as phosphorylation, ubiquitination and malonylation are vital for cellular function and cancer spread. In order to investigate the dysregulation of metabolism-related PTMs in CRC, we analyzed the expression patterns of phosphorylation, ubiquitination and malonylation in tumour tissues and matched adjacent normal tissues. We identified 59 differential phosphorylation sites, 263 differential ubiquitination sites, and 64 differential malonylation sites (Figure 1A). Of these, the highest number of differentially modified proteins were found in ubiquitination, followed by phosphorylation and malonylation. Furthermore, the number of proteins that

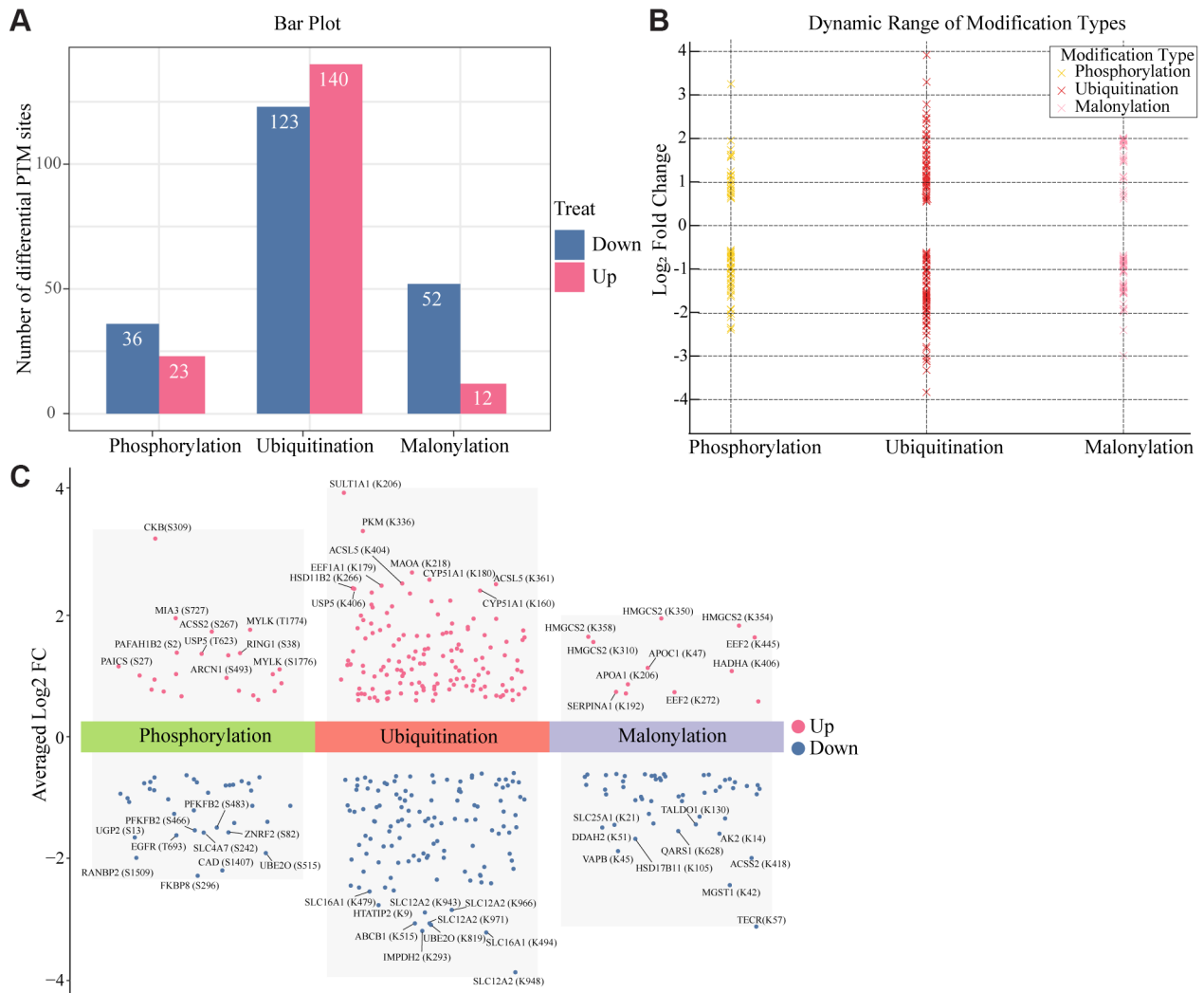


Figure 1. Continued

were downregulated by malonylation was significantly higher than the number that were upregulated. We further performed a quantitative analysis of the $\log_2$ fold changes (FC) of these PTMs. The results showed that the dynamic range of ubiquitination was the largest, with high variability. This suggests that the regulation of ubiquitination is highly heterogeneous (Figure 1B). Additionally, we calculated the average $\log_2$ FC for each modification type, which confirmed the significant suppression of malonylation, while ubiquitination displayed greater variability and phosphorylation showed moderate downregulation

(Figure 1C). It is particularly noteworthy that within the context of fatty acid metabolism, the malonylation modification of enzymes such as HMGCS2 was significantly augmented, underscoring the intimate interconnection between lipid metabolism reprogramming and the escalated metabolic demands of cancer. This reprogramming plays a crucial role in the progression of CRC (25).

To better understand the complexity of PTM regulation, we examined the extent of cross-modifications between proteins (Figure 1D). While most proteins were modified by just one type of PTM, we identified 17 proteins that were

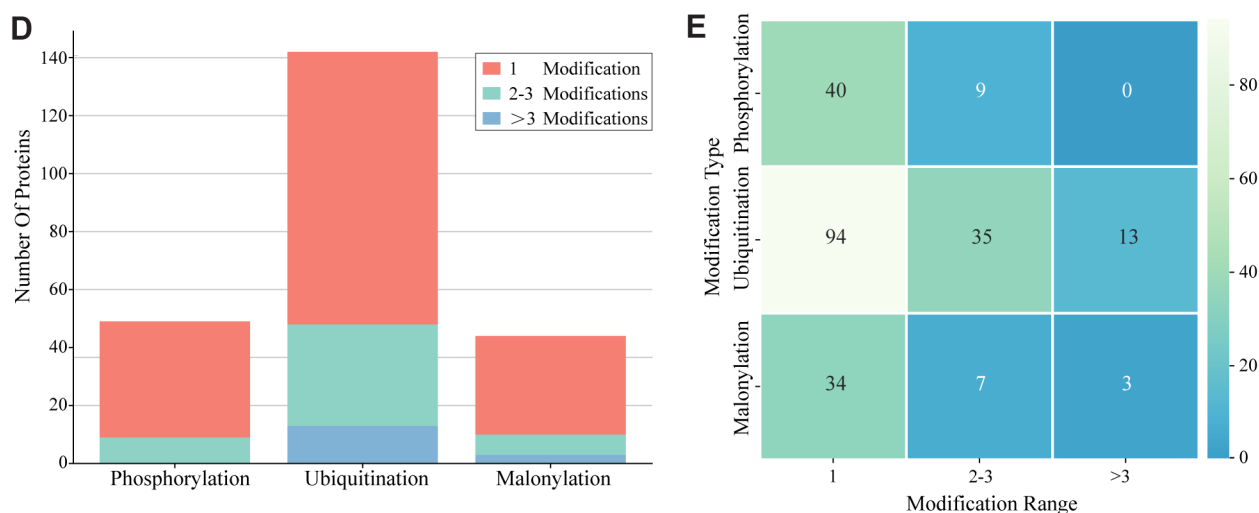


Figure 1. Global differential landscape of phosphorylation, ubiquitination, and malonylation modifications in metabolic proteins in CRC. (A) Bar plot showing the number of upregulated and downregulated differential PTM sites for each PTM type in CRC tissues compared to normal tissues. (B) Dot plot showing the dynamic range of $\log_2 FC$ for phosphorylation, ubiquitination, and malonylation modifications. (C) Average $\log_2$ fold change ($\log_2 FC$) for each modification type across all modified proteins, with upregulated modifications shown in positive values and downregulated modifications in negative values. (D) Stacked bar chart depicting the distribution of proteins with different numbers of modification sites for each PTM. (E) Interaction heatmap displaying the overlap of the three PTM types in proteins, with color intensity corresponding to the number of overlapping. Up- and downregulated PTM sites were defined as fold change greater than 1.5 indicating upregulation and fold change less than 1/1.5 indicating downregulation. A p -value < 0.05 was considered statistically significant. CRC, Colorectal cancer; PTM, post-translational modification; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function.

modified by two different types of PTMs. This suggests that there may be crosstalk between the different modification layers. Additionally, a modification density analysis (Figure 1E) revealed that ubiquitinated proteins were frequently modified at multiple sites, with 35 proteins containing 2 to 3 modification sites and 13 proteins containing more than three. This suggests that ubiquitination plays a complex role in controlling protein stability and turnover, which is important in many biological processes. In contrast, phosphorylation and malonylation were generally confined to fewer sites.

In summary, these findings reveal a unique PTM landscape in CRC that affects key metabolic regulators and establishes the foundation for cancer-specific metabolic reprogramming. Together, phosphorylation, ubiquitination and malonylation modulate CRC metabolic reprogramming across various metabolic pathways. They play critical roles in tumour metabolic adaptation, progression and therapeutic resistance through their interactions.

Functional enrichment and site-specific regulation of metabolic enzymes by PTMs in CRC. In order to explore the relationship between metabolic regulation and PTMs in CRC, we performed an intersection analysis of metabolic genes and proteins that are modified by phosphorylation, ubiquitination and malonylation. The Venn diagram (Figure 2A) shows that, among 2,912 genes related to metabolism, 425 were phosphorylated, 569 were ubiquitinated, and 123 were modified by malonylation. Importantly, we found five proteins that were changed by all three types of PTM: ACLY, ALDOA, GPI, PKM and TALDO1. As central nodes in the metabolic network, these proteins are involved in key processes such as glycolysis, the pentose phosphate pathway and the generation of acetyl-CoA. This indicates that CRC cells utilize a multi-layered PTM network to regulate metabolic flux, enzyme activity and protein stability.

These findings show that while each PTM contributes to metabolic regulation, specific metabolic proteins are

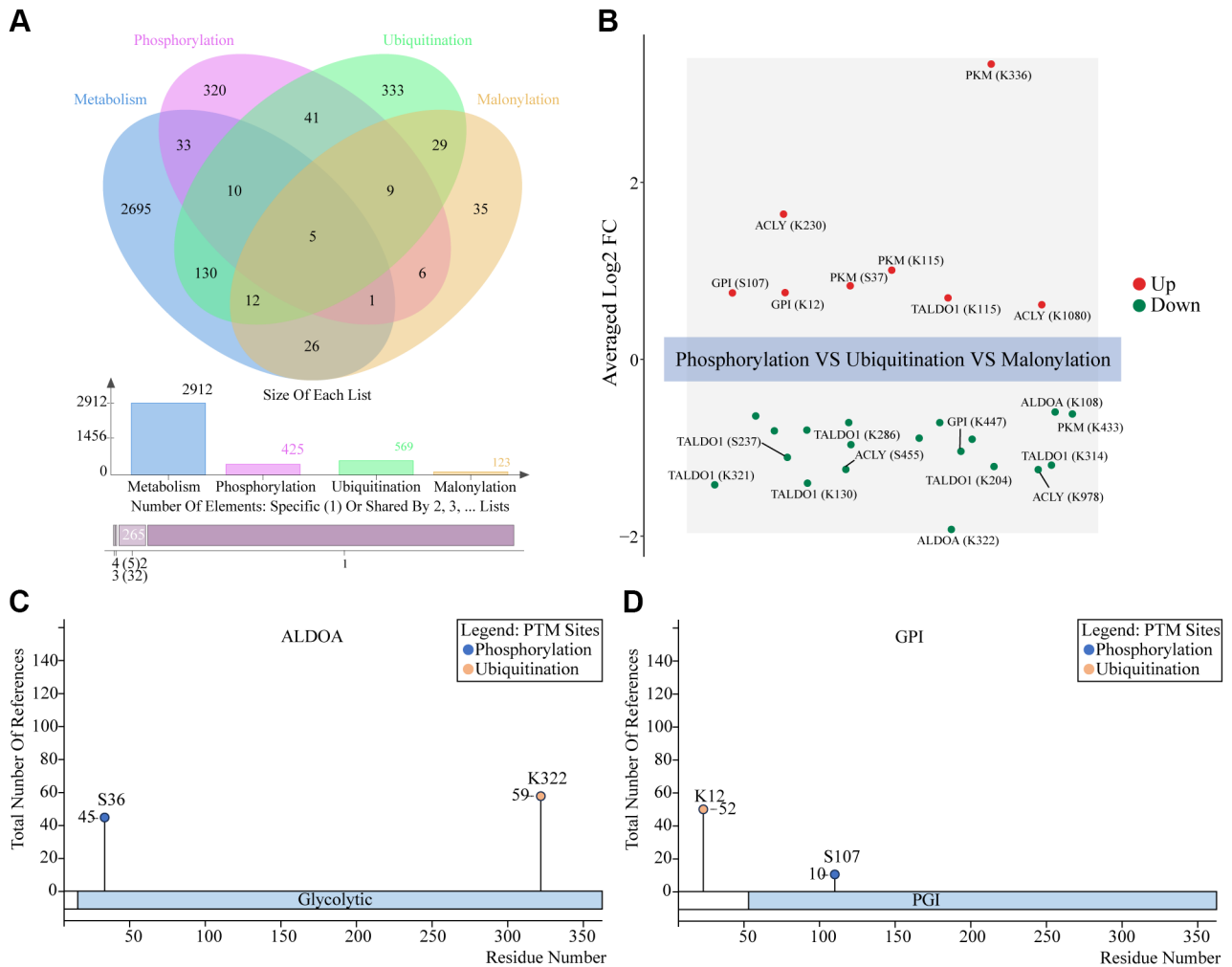


Figure 2. Continued

linked to multiple PTM layers. The proteins that are changed by these PTMs likely play a key role in the changes to CRC metabolism, coordinating how enzymes work, where proteins are located, and maintaining protein balance. To further investigate how different PTMs regulate key metabolic enzymes, we analyzed the site-specific log₂ FC for five enzymes: ACYL, ALDOA, GPI, PKM, and TALDO1 (Figure 2B). The results of this study demonstrate that each of these proteins is modified by phosphorylation, ubiquitination, and malonylation in CRC. ACYL, an important enzyme in fat production, is mostly switched off by phosphorylation and malonylation, while several

ubiquitination sites are switched on, suggesting a possible way to deal with the problem through protein breakdown. In contrast, ALDOA shows consistent downregulation across all PTM types, which may suggest a potential inhibitory effect on glycolytic flux. Both GPI and PKM exhibit elevated phosphorylation and ubiquitination, but reduced malonylation. This pattern could be associated with enhanced glycolytic activity and a reduction in mitochondrial oxidation. Notably, the K336 site of PKM, a rate-limiting enzyme in glycolysis, shows a substantial alteration with a log₂ FC of 3.33, which appears to be linked to ubiquitination. The present findings may be indicative of

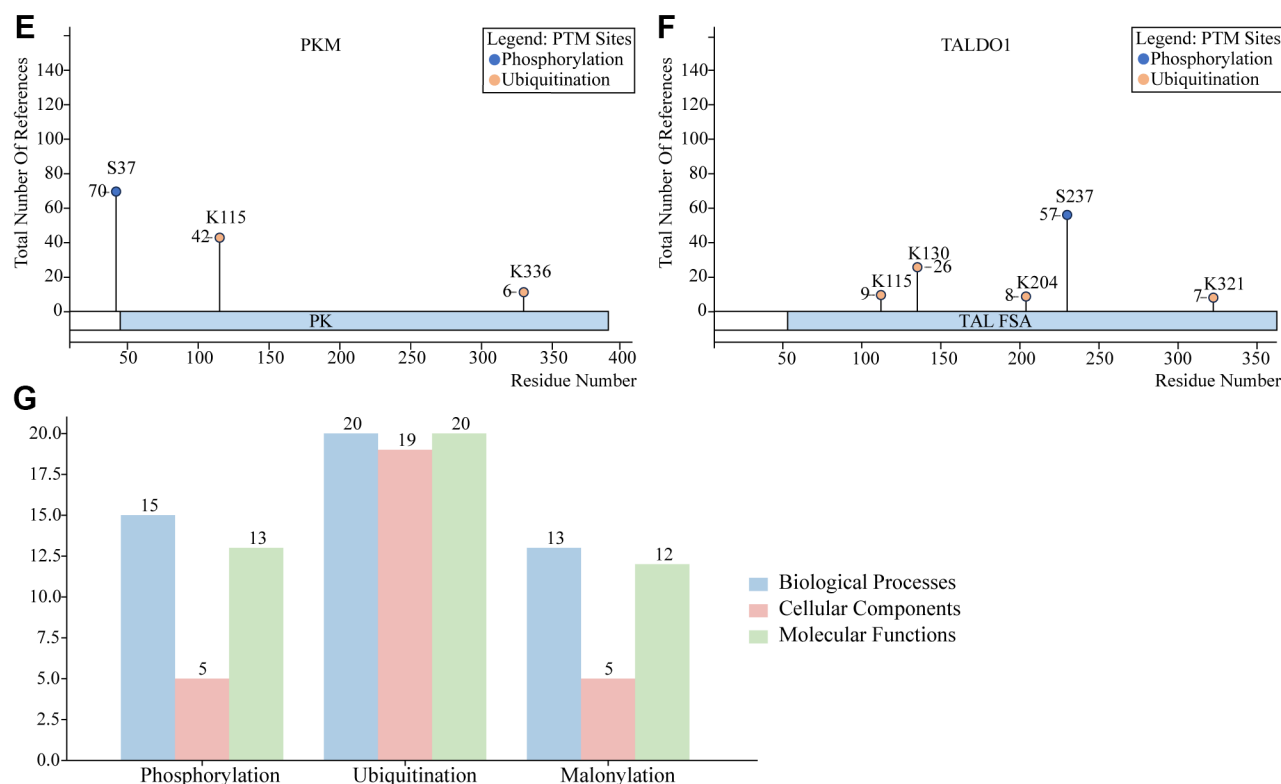


Figure 2. Intersection features of phosphorylation, ubiquitination, and malonylation in metabolic proteins and their distribution in core metabolic pathways. (A) Venn diagram showing the overlap of phosphorylation (purple), ubiquitination (green), malonylation (orange), and metabolism (blue) related proteins. (B) $\log_2$ FC of phosphorylation, ubiquitination, and malonylation modifications in proteins modified by all three PTM types. Red dots indicate upregulation ($\log_2$ FC > 0.58), and green dots indicate downregulation ($\log_2$ FC < 0.58). (C-F) Distribution of phosphorylation (blue) and ubiquitination (orange) modification sites across key metabolic enzymes: (C) ALDOA, (D) GPI, (E) PKM, and (F) TALDO1. Each plot shows the total number of references for each modification site along the protein sequence, with residue numbers on the x-axis and the total number of references on the y-axis. (G) Bar chart showing the enrichment of Biological Processes (blue), Cellular Components (red), and Molecular Functions (green) for proteins modified by phosphorylation, ubiquitination, and malonylation. The y-axis represents the number of enriched terms, and each bar corresponds to the number of terms associated with the respective PTM type. PTM, Post-translational modification.

either increased protein stability or regulation through non-degradative signaling pathways (26). TALDO1, involved in the pentose phosphate pathway, shows consistent downregulation in both ubiquitination and malonylation, with modest changes in phosphorylation, indicating that NADPH production is suppressed.

To further investigate how different PTMs are organized within the protein structure, we analyzed the residue-specific localization of phosphorylation and ubiquitination sites on four representative metabolic enzymes (ALDOA, GPI, PKM, and TALDO1) (Figure 2C-F). ALDOA displays phosphorylation at Ser36 and

ubiquitination at Lys322, located at the N- and C-termini, respectively. These modifications may regulate different aspects of ALDOA's function, such as enzyme activity and degradation. GPI shows phosphorylation at residue 107 and ubiquitination at residue 12, indicating a potential separation of catalytic and regulatory domains. Ubiquitination at the N-terminus may influence subcellular localization or protein turnover, while phosphorylation likely modulates catalytic function. PKM, as a rate-limiting enzyme in glycolysis, exhibits three key PTM sites: phosphorylation at Ser37 and ubiquitination at Lys316 and Lys336. The accumulation of ubiquitination

sites at the C-terminus may enhance protein stability or facilitate interactions with metabolic complexes. TALDO1, modified at five sites, including phosphorylation at Ser237 and four ubiquitination sites spanning Lys115 to Lys321, demonstrates a complex regulation pattern, likely coordinating enzyme stability, localization, and NADPH-related metabolic output. These PTM modifications indicate specific functional domains within metabolic enzymes that are subject to differential regulation in CRC. The observed distribution patterns of these modifications serve to emphasize the precision of PTM-mediated metabolic regulation, thus identifying potential “hotspots” for the control of key metabolic proteins.

Finally, we performed GO enrichment analysis of the biological process (BP), cellular component (CC), and molecular function (MF) associated with PTM-modified metabolic proteins (Figure 2G). The results demonstrated that ubiquitinated proteins were significantly enriched across all three GO categories, suggesting their critical role in maintaining protein homeostasis and regulating organelle-specific metabolic processes. Phosphorylation and malonylation, while also showing notable enrichment in BP and MF, were less represented in CC. This finding suggests that these modifications may be more focused on regulating enzyme activity and metabolic function.

Spatial clustering of multi-modified sites on enzyme structural domains reveals potential coordinated regulatory regions. PTMs play a crucial role in cellular metabolic adaptation by regulating enzyme activity, stability, and protein interactions (27). However, the systematic study of the impact of PTM regulation of CRC metabolic enzymes on glycolytic processes remains to be undertaken. Therefore, in this study, we integrated protein structures predicted by AlphaFold2 with experimentally identified PTM sites to analyze the PTMs of four key metabolic enzymes: Aldolase A (ALDOA), glucose-6-phosphate isomerase (GPI), pyruvate kinase M (PKM), and transaldolase 1 (TALDO1), and their regulatory effects on enzyme function.

The key PTM sites on ALDOA include phosphorylation at Ser36, ubiquitination at Lys322, and malonylation at

Lys108 (Figure 3A). Phosphorylation at Ser36 may regulate ALDOA's enzymatic activity by altering its conformation, which impacts substrate binding and catalytic efficiency, thereby influencing glycolytic flux. Ubiquitination at Lys322 may target ALDOA for proteasomal degradation, thereby modulating its turnover and stability in response to metabolic changes. Malonylation at Lys108 could regulate ALDOA's interaction with other glycolytic enzymes and its subcellular localization, playing a pivotal role in the metabolic network. In GPI, the key PTM site phosphorylation at Ser107 may regulate GPI's catalytic activity by inducing conformational changes, thus affecting substrate interactions, particularly in glucose metabolism and rapid energy production in cancer cells (Figure 3B). Ubiquitination at Lys12 may target GPI for proteasomal degradation, modulating its stability. The malonylation of Lys252 and Lys447 in GPI may affect its stability and subcellular localization, thereby influencing its interactions with other glycolytic enzymes and contributing to energy production. These observations suggest that GPI is subject to fine regulation through phosphorylation, ubiquitination and malonylation, which ensures its ability to adapt to fluctuating metabolic demands, particularly within glycolysis. Several critical PTM sites have been identified for PKM, including phosphorylation at Ser37, ubiquitination at Lys115 and Lys336, and malonylation at Lys135 and Lys433 (Figure 3C). The activity of PKM during glycolysis is subject to regulation by its conformation, which can be altered by phosphorylation at Ser37 in response to metabolic cues. It is postulated that ubiquitination at Lys115 and Lys336 govern PKM stability and turnover by promoting proteasomal degradation. Meanwhile, malonylation at Lys135 and Lys433 may influence PKM stability, localization, and PPIs, potentially contributing to the regulation of glycolysis. Potential alterations to substrate interactions within the pentose phosphate pathway may be influenced by phosphorylation at Ser237 in TALDO1 (Figure 3D). Ubiquitination of lysine residues, such as Lys115, Lys204 and Lys130, may promote proteasomal degradation, thereby regulating TALDO1 stability and turnover in order to maintain the correct level

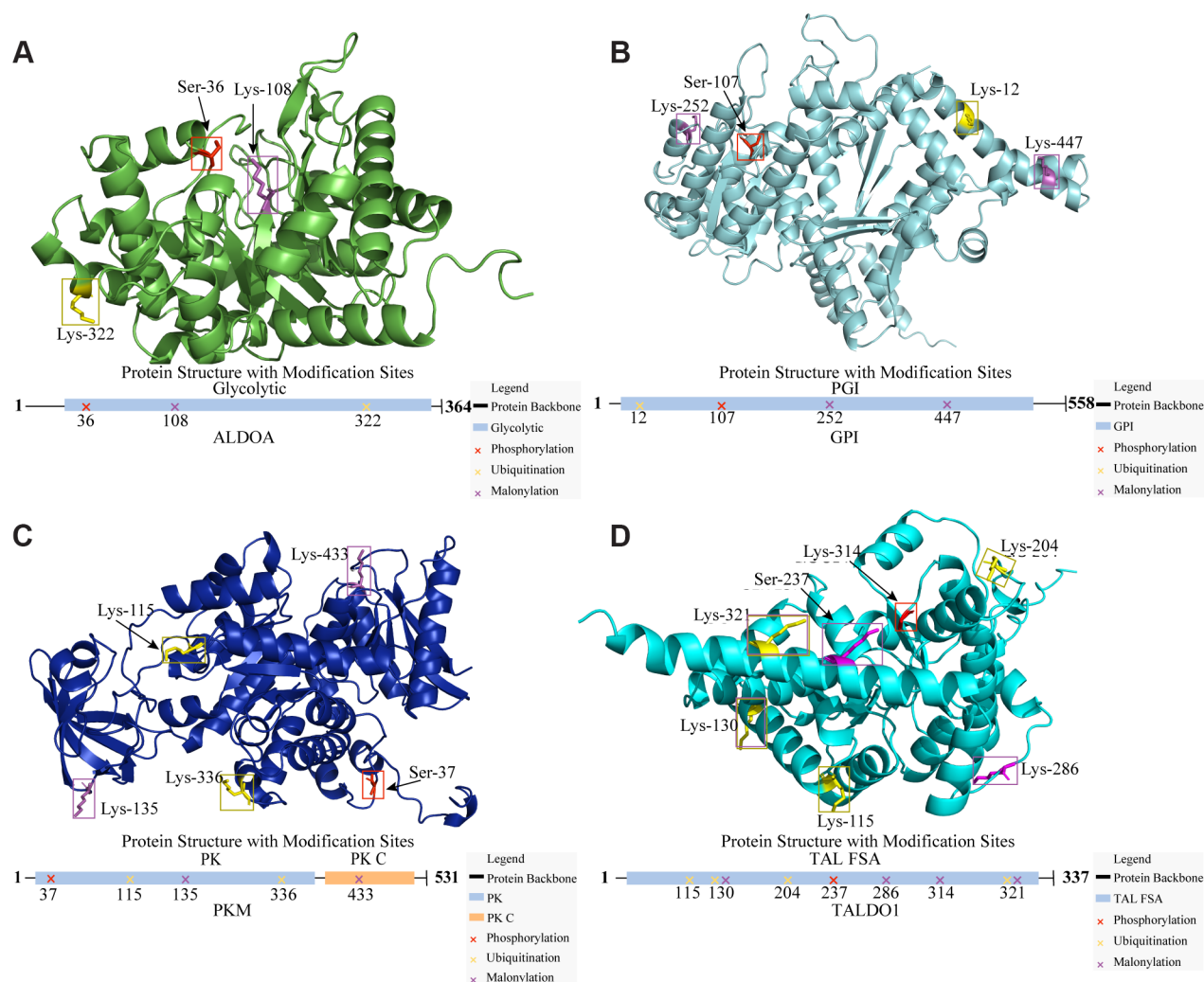


Figure 3. PTM site localization on 3D structures of key glycolytic enzymes. (A-D) 3D structures of four key glycolytic enzymes, showing the locations of phosphorylation (red), ubiquitination (yellow), and malonylation (purple) modification sites. Each modification site is marked on the protein backbone, with residue numbers indicated. The color coding represents the type of PTMs at each site. (A) ALDOA, (B) GPI, (C) PKM, and (D) TALDO1. PTM, Post-translational modification.

of the enzyme under changing metabolic conditions. Furthermore, malonylation of residues such as Lys130, Lys286, Lys314 and Lys321 may regulate TALDO1 stability, subcellular localization and protein interactions.

The PTM modification sites on ALDOA, GPI, PKM, and TALDO1 suggest a potential role of phosphorylation, ubiquitination, and malonylation in regulating these enzymes' activity, stability, and protein interactions. These modifications ensure that the enzymes can adapt

to changes in cellular metabolic demands, regulating glycolytic flux. In cancer cells, metabolic reprogramming is a hallmark of tumor growth and survival, with these enzymes playing key roles in this process (28).

Enrichment analysis of PTMs reveals their roles in metabolic regulation and subcellular localization. It is evident that glycolytic enzymes play a pivotal role in cellular metabolism, particularly in the metabolic reprogramming of cancer cells

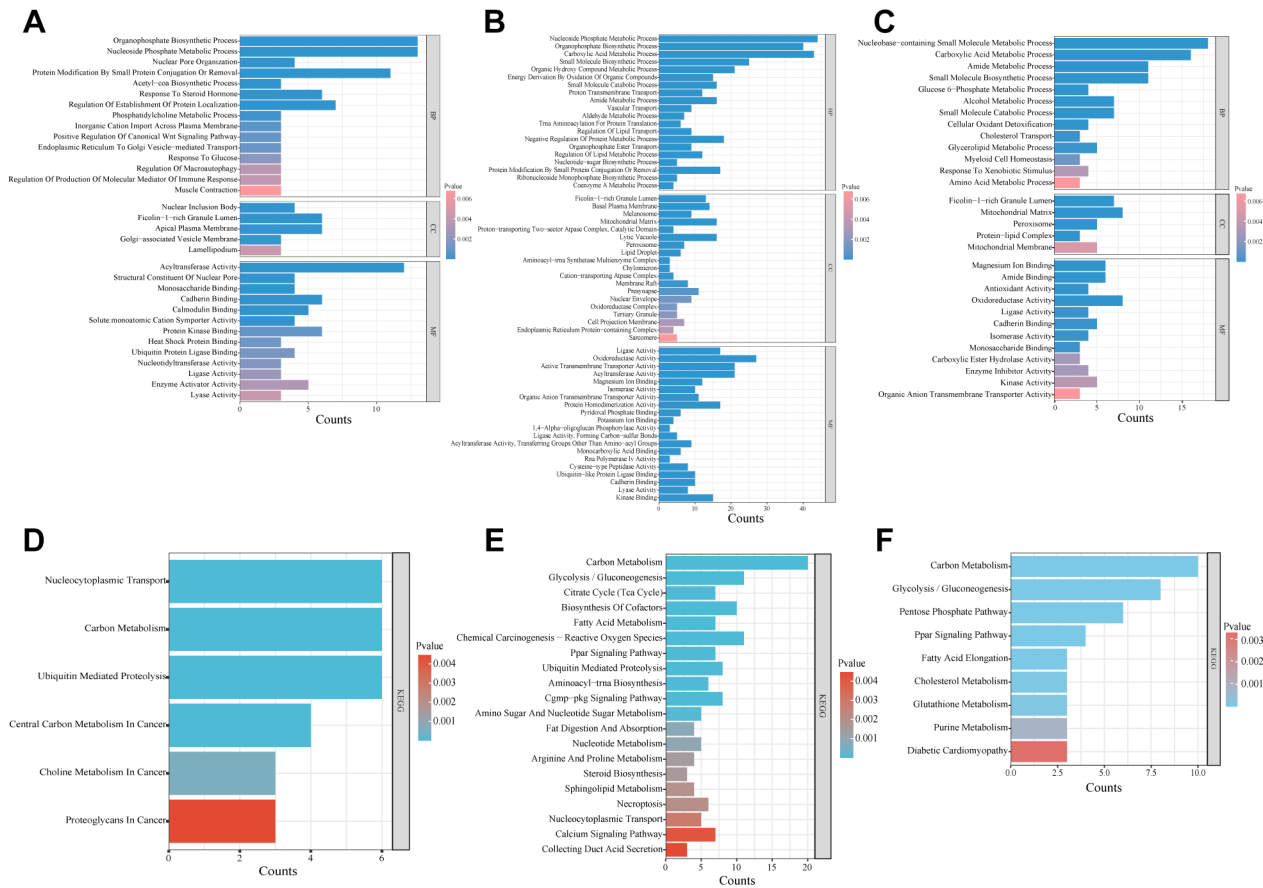


Figure 4. Continued

(29). We investigated how PTMs regulate their activity by conducting enrichment analyses with a focus on phosphorylation, ubiquitination and malonylation.

GO enrichment analysis revealed that all three types of modification were associated with a range of biological processes, cellular components, and molecular functions, highlighting the varied functions of PTMs in the regulation of glycolytic enzymes (Figure 4A). Phosphorylation was notably increased in the endoplasmic reticulum and the Golgi apparatus, suggesting a potential role in regulating protein function across subcellular compartments. Ubiquitination was enriched in pathways related to protein degradation and the stress response, which may indicate its involvement in maintaining enzyme stability and turnover in response to environmental changes (Figure

4B). Malonylation has been found to be potentially linked to energy metabolism and cellular responses to metabolic stress. Its higher concentration in mitochondria and peroxisomes may reflect its role in regulating these organelles and adapting to metabolic changes (Figure 4C). When these findings are considered collectively, it appears that phosphorylation, ubiquitination, and malonylation may collectively influence glycolytic enzymes across different parts of the cell. This coordinated regulation could assist glycolytic enzymes in responding to fluctuations in metabolic rate, thereby supporting energy metabolism and potentially contributing to the metabolic switch that is characteristic of cancer cells.

Taken together, these findings suggest that phosphorylation, ubiquitination, and malonylation may

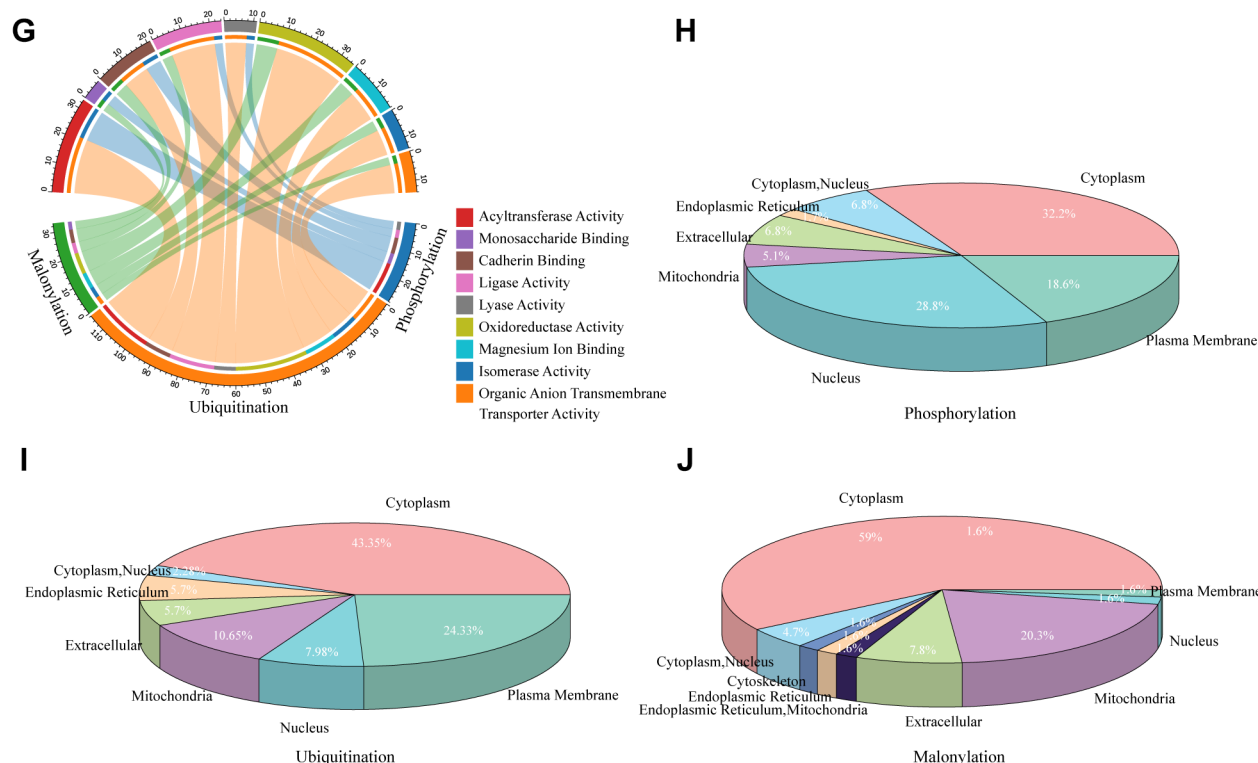


Figure 4. GO and KEGG pathway enrichment analysis of PTMs in glycolytic enzymes. (A-C) GO enrichment analysis showing the enrichment of biological process (BP), cellular component (CC), and molecular function (MF) for phosphorylation (A), ubiquitination (B), and malonylation (C). The color gradient from blue to red indicates the p-value significance, with blue representing lower p-values (higher significance) and red representing higher p-values (lower significance). (D-F) KEGG pathway analysis of phosphorylation (D), ubiquitination (E), and malonylation (F). Color intensity indicates the degree of enrichment in each pathway, with darker colors representing higher enrichment. (G) Chord diagram illustrating the relationships between functional pathways and the types of modifications (phosphorylation, ubiquitination, and malonylation). (H-J) Subcellular localization of PTMs: (H) Phosphorylation, (I) Ubiquitination, and (J) Malonylation, with colored regions indicating the distribution of each PTM type in different cellular compartments: Cytoplasm, Nucleus, Mitochondria, and Plasma membrane. PTM, Post-translational modification; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

act in concert to regulate glycolytic enzymes across distinct cellular environments. This coordinated regulation could facilitate the dynamic response of glycolytic enzymes to metabolic fluctuations, thereby potentially supporting energy metabolism and contributing to the metabolic reprogramming that is characteristic of cancer cells. In addition, KEGG pathway enrichment analysis further supports the central role of these PTMs in regulating glycolytic enzymes and broader metabolic pathways (Figure 4D-F). Phosphorylation, in particular, has been demonstrated to regulate enzyme activity, stability, and metabolic adaptation, with the potential to influence cancer cell function. It is hypothesized that ubiquitination

plays a significant role in the maintenance of protein turnover and enzyme homeostasis, thereby facilitating the adaptation of enzymes to fluctuating metabolic demands. Malonylation has been identified as a key component in a number of essential metabolic processes, including lipid metabolism, redox regulation, and nutrient sensing. These processes have the potential to impact cellular energy homeostasis. The associations of PTMs with molecular functions were mapped in order to further explore their potential relevance. The resulting diagram illustrates the connections between PTMs, specific enzymatic activities, and binding events within different metabolic pathways (Figure 4G).

We investigated subcellular localization patterns by analyzing the distribution of phosphorylation, ubiquitination, and malonylation across different cellular compartments (Figure 4H-J). Phosphorylation was predominantly enriched in the cytoplasm (32.2%) and the nucleus (28.8%), highlighting its important role in signal transduction and enzymatic regulation. Ubiquitination was primarily localized to the cytoplasm (43.35%) and the plasma membrane (24.33%), with smaller amounts present in the nucleus (7.98%) and the extracellular space (5.7%), which highlights its important role in protein turnover and cellular homeostasis. Malonylation showed the highest enrichment in the cytoplasm (59%), followed by the mitochondria (20.3%), and lower levels were observed in the extracellular space (7.8%) and the nucleus (1.6%). This distribution suggests its potential importance in lipid metabolism and mitochondrial regulation.

Collectively, these data on localization suggest the potential roles of phosphorylation, ubiquitination, and malonylation in regulating cell function. Phosphorylation has been shown to facilitate signal transduction and enzymatic control in the cytoplasm and nucleus. The process of ubiquitination has been implicated in the degradation of proteins and the maintenance of homeostatic balance. Malonylation has been demonstrated to contribute to the regulation of lipid metabolism and may play a role in mitochondrial energy production. These PTMs appear to function within discrete subcellular environments, a property that may facilitate dynamic enzymatic responses to fluctuating metabolic demands, a phenomenon particularly evident in tumour cells undergoing metabolic reprogramming. This analysis lends support to the context-dependent regulatory roles of PTMs, thereby highlighting their potential importance in the metabolic adaptation of cancer cells.

Interaction network and subnetwork analysis of multi-modified proteins. The interaction networks of these proteins were analyzed to further explore their potential role in cellular metabolism, as well as their possible applications in the treatment of metabolic diseases and

cancer. This analysis underscores the potential significance of multi-PTM proteins in regulating cellular metabolism and posits possible therapeutic targets for metabolic diseases and cancer. To identify these proteins, we used the Metascape database to annotate the differentially modified proteins and then generated their interaction networks (Figure 5A). The resulting network illustrates the complex interactions between proteins modified by phosphorylation, ubiquitination, and malonylation. This network suggests the interconnectedness and potential roles of these modified proteins in regulating cellular metabolism, maintaining homeostasis, and responding to metabolic fluctuations.

To further investigate the interactions between glycolytic enzymes and TCA (Tricarboxylic Acid) cycle enzymes, we constructed a PPI network. This network reveals tight interactions between PKM, GAPDH, ALDOA, PGK1, LDHA, and TCA cycle enzymes such as IDH1 and SDHA (Figure 5B). It is important to note that IDH1 functions as a core node in this network, thus emphasizing its critical role in metabolic reprogramming, particularly in the integration of glycolysis and oxidative phosphorylation to meet the elevated energy demands of tumour cells (30). In addition, we analyzed a PPI network focusing on nuclear transport and transcriptional regulation (Figure 5C). This network prominently features interactions between nucleoporins and nuclear transport factors, such as NUP93, NUP98, NUP153, NUP205, RANBP2, and TPR, emphasizing their collective importance in regulating nuclear import/export processes. The network also includes isolated nodes such as AK2, POLR2B, and NME2, which may be involved in transcriptional and metabolic regulation. The central roles of nuclear transport factors in cell function, gene expression, and metabolic regulation are further underscored.

Figure 5D displays the PPI network of key E3 ubiquitin ligases involved in protein degradation, highlighting several E3 ligases, including BIRC6, UBR5, and HUWE1. UBA1, as the central node, connects to various ligases, underscoring its pivotal role in the ubiquitination process. The dense interactions between these proteins suggest their essential involvement in regulating protein stability

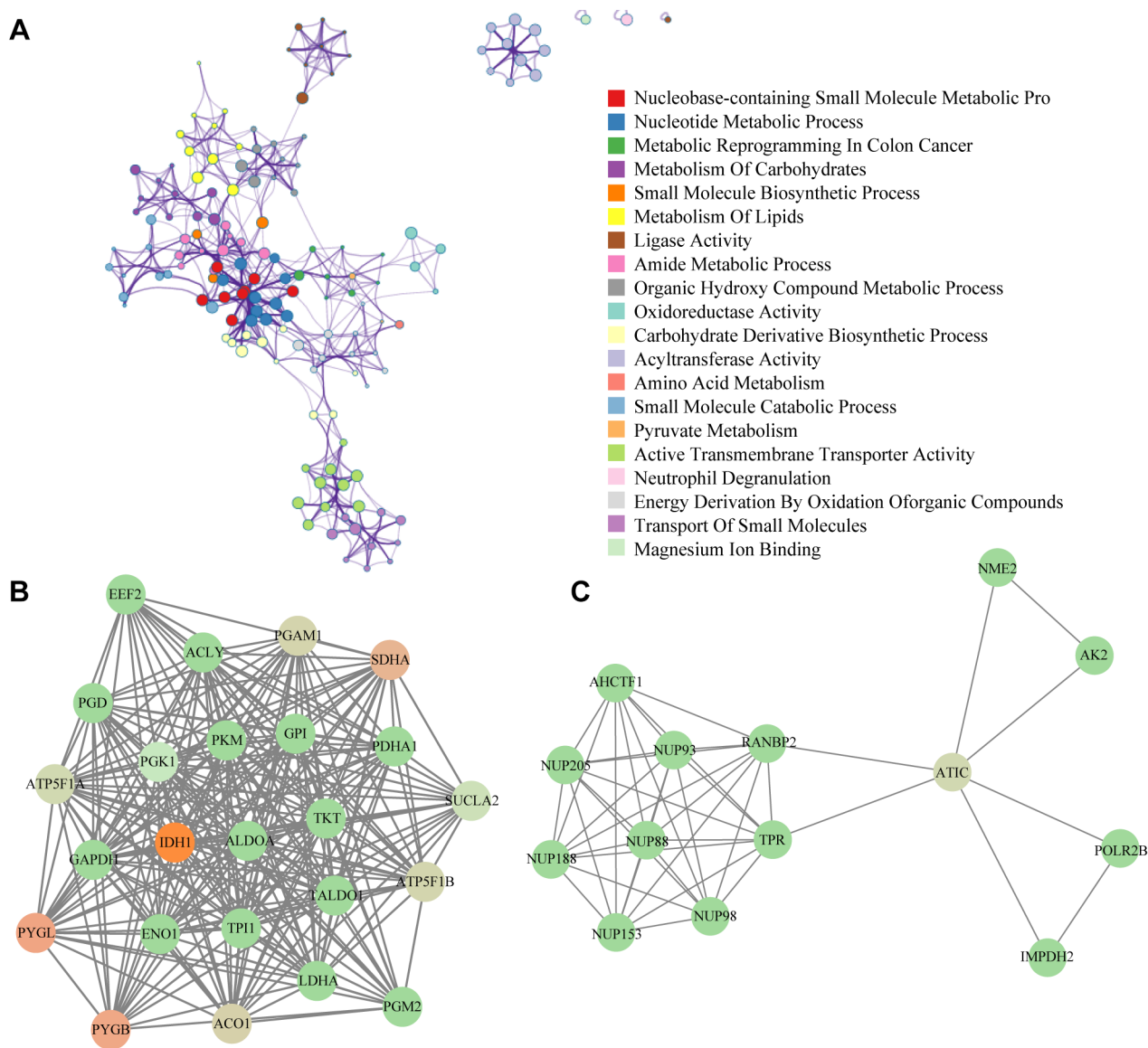


Figure 5. Continued

and cellular homeostasis *via* the proteasomal degradation pathway. Figure 5E illustrates the interaction network of key enzymes involved in cholesterol metabolism, with HMGCS2 as the central node. It connects with enzymes such as FDPS, LSS, and CYP51A1, emphasizing its critical role in the mevalonate pathway. Furthermore, the observation that ACSL5 and ACSL4 interact with other enzymes suggests a potential interconnection between the

processes of fatty acid activation and cholesterol biosynthesis. These interactions may highlight the interdependence of cholesterol biosynthesis and lipid metabolism, with HMGCS2 potentially playing a central role in regulating these processes.

PTM-mediated modulation of metabolic signaling pathways in CRC. To better understand the metabolic

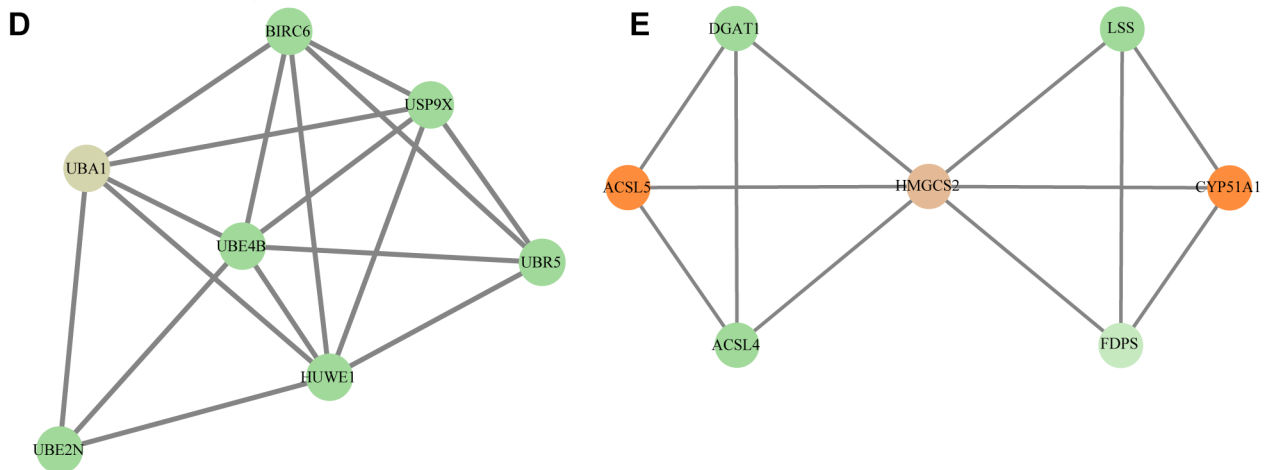


Figure 5. PPI network of multi-modified proteins in metabolic regulation. (A) The functional cluster network showing the interactions between key metabolic enzymes, including glycolytic enzymes, mitochondrial enzymes, and other metabolic regulators. (B) PPI network of key metabolic enzymes, with green nodes representing proteins involved in glycolysis and metabolic regulation, and orange nodes highlighting enzymes with critical modifications such as phosphorylation, ubiquitination, or malonylation. (C) PPI network for enzymes associated with metabolic and cellular functions, with green nodes representing proteins involved in general metabolic regulation and yellow nodes marking proteins like ATIC that are involved in more specific processes. (D) PPI network of proteins associated with ubiquitination, with green nodes indicating stable proteins involved in cellular processes, and orange nodes identifying those with significant modifications that impact stability and interactions. (E) PPI network focusing on enzymes involved in lipid metabolism, with green nodes representing general metabolic regulators and orange nodes highlighting proteins like HMGCS2 that are modified by key PTMs and play central roles in metabolic regulation. PPI, Protein-protein interaction.

reprogramming mechanisms in CRC driven by key PTMs, we systematically analyzed differentially modified proteins and performed functional annotation using the GO and KEGG databases, alongside an extensive literature review. By integrating protein-protein interactions and exploring biological pathway crosstalk, we constructed a potential metabolic regulatory network that illustrates the role of PTMs in shaping CRC metabolism (Figure 6). This network emphasizes how PTMs influence critical metabolic shifts linked to tumorigenesis, cancer cell proliferation, and immune evasion.

The altered activity of key metabolic enzymes in CRC is largely driven by PTMs. For instance, mutations in IDH1 lead to the production of 2-hydroxyglutarate (2-HG), an oncometabolite that inhibits α -ketoglutarate-dependent enzymes, resulting in DNA and histone methylation (31). This epigenetic alteration not only promotes tumor formation but also rewires the metabolic landscape of cancer cells. Furthermore, the ubiquitination of IDH1 facilitates its degradation, which further impacts cellular

metabolism by maintaining a balance between metabolic intermediates and ensuring the proper functioning of the TCA cycle and other key processes (32). In a similar manner, LDHA, which is also modified by ubiquitination, plays a crucial role in maintaining the glycolytic flux by converting pyruvate to lactate. This step is of particular importance in the context of CRC, as it facilitates the rapid energy demands of proliferating cancer cells. The ubiquitin-mediated regulation of LDHA has been demonstrated to enhance lactate production, in addition to playing a role in immune evasion, a hallmark of cancer metabolism (33). These processes underscore the significant interplay between protein modifications and metabolic shifts, enabling CRC cells to adapt to their rapidly changing environment. Moreover, it has been established that the activity of PDHA1, a further pivotal enzyme within the metabolic network, is subject to suppression by means of phosphorylation at the hands of PDK1. This process gives rise to a diminution in acetyl-CoA production (34). This alteration shifts the cellular metabolism away from

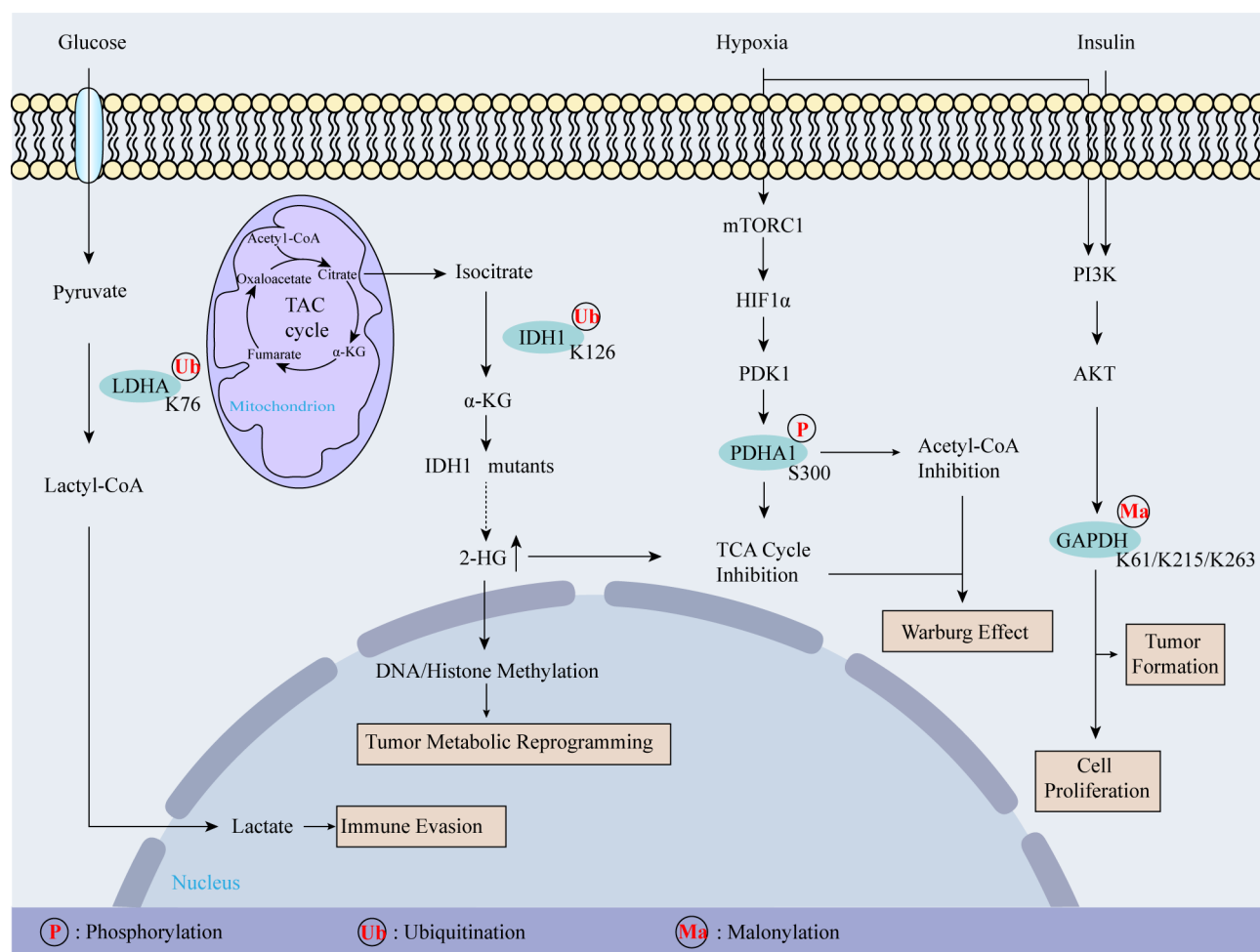


Figure 6. Mechanistic models of metabolic pathway regulation in CRC via PTMs. This diagram illustrates key metabolic enzymes and their PTMs (ubiquitination, phosphorylation, and malonylation) in CRC metabolism. IDH1 mutation produces 2-HG, leading to DNA/histone methylation and epigenetic changes. Ubiquitination of IDH1 enhances its degradation. LDHA is ubiquitinated, promoting lactate production and supporting glycolysis. PDHA1, phosphorylated by PDK1, inhibits the TCA cycle and promotes glycolysis via the Warburg effect. GAPDH undergoes malonylation, modulating glycolytic activity. CRC, Colorectal cancer; PTM, post-translational modification.

oxidative phosphorylation towards glycolysis, a phenomenon known as the Warburg effect (35). This metabolic shift further fuels the rapid proliferation of CRC cells, making it a crucial driver of tumor growth. Moreover, GAPDH, a key enzyme in glycolysis, undergoes malonylation, which fine-tunes its enzymatic activity and may contribute to the metabolic adaptability of cancer cells (36). This modification highlights the flexibility of metabolic pathways in CRC, where enzymes are constantly remodeled to meet the demands of cell growth and survival.

The integration of these key metabolic pathways is further coordinated by complex signaling networks involving PI3K/AKT, mTORC1, and HIF-1α. It is hypothesized that these pathways may regulate both the enzymes and their PTMs, potentially driving metabolic adaptations that could play a role in tumour formation and progression. The crosstalk between these pathways may enable CRC cells to fine-tune their metabolism, particularly under hypoxic conditions, thereby supporting their survival and proliferation in adverse environments.

Discussion

The complex interplay between PTMs and metabolic reprogramming in CRC is an emerging area of significant interest for research in the field. This study provides a comprehensive analysis of how phosphorylation, ubiquitination, and malonylation, as key PTMs, contribute to CRC metabolism and tumour progression. The findings of this study demonstrate a complex interplay between these PTMs in regulating key metabolic pathways, with significant implications for understanding CRC pathophysiology and identifying potential therapeutic targets.

One of the most striking observations of this study is the distinctive PTM landscape observed in CRC tissues compared to adjacent normal tissues. Ubiquitination, in particular, exhibited the highest number of differentially modified proteins, which may reflect the dynamic regulation of protein turnover in cancer cells. This finding suggests that the maintenance of protein homeostasis through the process of ubiquitin-mediated degradation may play a significant role in the adaptability of CRC cells to metabolic stress. The pronounced variability observed in ubiquitination modifications further highlights the complexity of its regulatory role, which may reflect the diverse cellular contexts and the heterogeneous nature of CRC. Phosphorylation and malonylation are also very important, but they affect specific metabolic enzymes in a more targeted way. For example, the important control of enzymes involved in glycolysis, such as PKM and ALDOA, through the addition of phosphates and malonates, suggests that these changes are key drivers of the Warburg effect seen in CRC. The process of malonylation being reduced, along with ubiquitination being increased, suggests a way in which CRC cells can make the most of energy production when there is not much nutrition available. This metabolic adaptation is essential for tumor survival and proliferation, particularly in the context of rapidly growing tumor masses that often experience hypoxic and nutrient-deprived environments (37).

Moreover, the present study underscores the significance of multi-layered PTM regulation in

orchestrating metabolic flux. The identification of enzymes such as ACLY, ALDOA, GPI, PKM, and TALDO1 as convergence points for multiple PTMs underscores their central roles in CRC metabolic reprogramming. These enzymes are of critical importance within the metabolic network, where alterations in their PTM profiles can lead to substantial changes in cellular metabolism, including glycolysis, lipid metabolism, and energy production. For example, the phosphorylation of PKM and its subsequent regulation *via* ubiquitination are indicative of the fine-tuning required to balance glycolytic activity with mitochondrial oxidative processes, supporting the cancer cell's ability to rapidly adapt to metabolic fluctuations (38). In addition to the regulation of enzyme activity, PTMs also impact protein stability and subcellular localization. The results of this study demonstrate that PTMs such as phosphorylation and ubiquitination regulate enzyme activity by altering their conformation and interactions with other cellular proteins. The spatial distribution of these PTMs further reinforces their role in subcellular compartmentalization, where phosphorylation is predominantly enriched in the cytoplasm and nucleus, reflecting its role in signal transduction and enzymatic regulation. On the other hand, the enrichment of malonylation in mitochondria and peroxisomes may point to its potential function in lipid metabolism and mitochondrial energy production, further suggesting the role of PTMs in maintaining cellular metabolic homeostasis.

The integration of these findings into a broader metabolic regulatory network provides novel insights into how PTMs influence CRC metabolism and tumor progression. For instance, the altered activity of key metabolic enzymes, such as IDH1, LDHA, and PDHA1, *via* PTMs, contributes to the metabolic shift that drives CRC cell proliferation and survival. This shift, characterized by a preference for glycolysis over oxidative phosphorylation (the Warburg effect), is a hallmark of cancer metabolism and is essential for tumor growth (39). Furthermore, the involvement of PTMs in regulating immune evasion mechanisms underscores their broader significance in the metastatic potential of CRC cells.

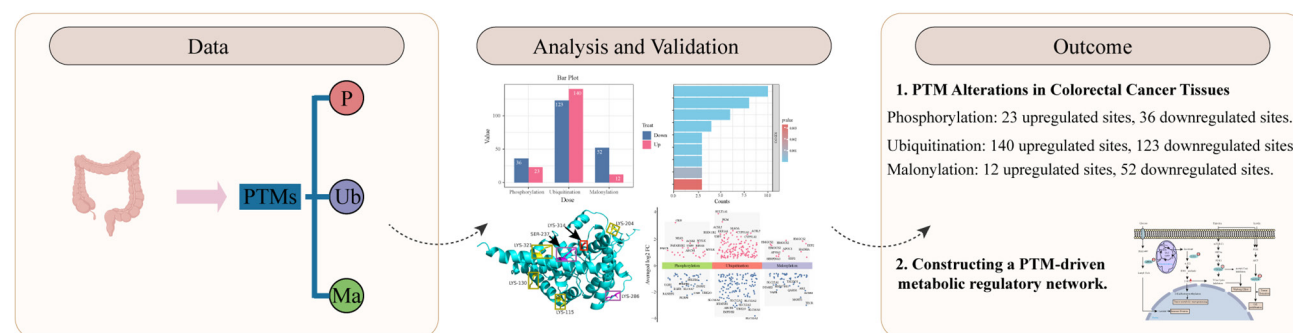


Figure 7. Schematic diagram of the research workflow and main findings. This figure outlines the comprehensive workflow used in this study, illustrating the integration of multi-omics data acquisition, PTM profiling, and bioinformatic analysis to construct a PTM-driven metabolic regulatory network in CRC. Key steps include the identification of differential PTMs, mapping of modified enzymes, and the construction of a metabolic regulatory network. These findings highlight how PTMs in enzymes like IDH1, LDHA, PDHA1, and GAPDH regulate metabolic pathways, supporting CRC tumor progression and the Warburg effect. CRC, Colorectal cancer; PTM, post-translational modification.

Despite the valuable insights provided by this study, several limitations should be acknowledged. The relatively small sample size ($n=8$) limits the statistical power and generalizability of the findings, and larger cohorts are needed to confirm these results. Additionally, technical variability in mass spectrometry, including potential biases in PTM enrichment and detection, may affect the identification of low-abundance modifications. Addressing these issues in future studies will help refine the sensitivity and accuracy of PTM analysis. Finally, while our study highlights the potential of PTMs as therapeutic targets in CRC, translating these findings into clinical applications presents several challenges. The development of specific inhibitors or modulators of PTM-regulating enzymes requires overcoming hurdles such as off-target effects, delivery issues, and the complexity of targeting enzymes that may have broader biological functions. Furthermore, the role of metabolic reprogramming in CRC is undeniably central, but it is also important to consider how other factors, such as nutrition and inflammation, may influence the effectiveness of therapeutic interventions. Recent studies have shown that targeting pathways like FSP1 and GPX4, which induce ferroptosis, could complement metabolic reprogramming therapies (40). However, these pathways and their interactions with PTMs and metabolic processes were not addressed in this study. Additionally,

assessing patients' nutritional and inflammatory status has been demonstrated to provide valuable prognostic information, potentially guiding more personalized treatment approaches (41). These considerations underscore the need to investigate how PTMs, metabolic pathways, and physiological factors like nutrition and inflammation interact to optimize CRC treatment strategies.

To facilitate comprehension of the research workflow and key findings, Figure 7 provides a schematic overview. The text delineates the multi-omics approach that has been utilized to profile PTMs (phosphorylation, ubiquitination, and malonylation) in CRC, with a view to identifying key metabolic enzymes and constructing a metabolic regulatory network. The figure summarizes the role of PTMs in the regulation of metabolic enzymes, the coordination of metabolic processes, and the promotion of CRC progression. In summary, the present study highlights the pivotal roles of phosphorylation, ubiquitination, and malonylation in shaping the metabolic landscape of CRC. These PTMs regulate key enzymes and signaling pathways, enabling CRC cells to adapt to metabolic challenges and sustain rapid growth. It is imperative to comprehend the precise mechanisms by which these PTMs regulate metabolic reprogramming in order to develop novel therapeutic strategies that target the metabolic vulnerabilities of CRC cells. Given the pivotal role of these modifications in

regulating cancer cell metabolism, future research should concentrate on elucidating the temporal and spatial dynamics of PTM regulation and exploring their therapeutic potential in clinical settings.

Conclusion

This study emphasizes the pivotal functions of phosphorylation, ubiquitination, and malonylation in regulating pivotal metabolic signaling pathways in CRC, particularly those associated with glycolysis, the tricarboxylic acid (TCA) cycle, and immune evasion. By regulating enzymes such as IDH1, LDHA, and PDHA1, these PTMs drive the metabolic shift towards glycolysis, a hallmark of cancer cell survival and proliferation. The findings of this study suggest the potential of targeting PTM-mediated metabolic reprogramming in the development of novel therapeutic strategies for CRC.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

WZ, JJD, TYL, and YD contributed to the conceptualization and methodology of the study. WZ, JD, EJH, and DY carried out the investigation and were responsible for data curation. TYL, WZ, YJZ, and FZ performed formal analysis and interpreted the data, with additional statistical analysis conducted by JQ, MF, and JJD. TYL, WZ, and JJD drafted the original manuscript. TYL, YD, and WZ reviewed and edited the manuscript. YD acquired funding, and YD and WZ supervised the study. All Authors contributed to the article and approved the submitted version.

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Artificial Intelligence (AI) Disclosure

During the preparation of this manuscript, a large language model (ChatGPT, OpenAI) was used for language polishing and enhancing the style of certain sections. No sections involving the generation, analysis, or interpretation of research data were produced by generative AI. All scientific content, including research data, was created and verified by the authors. Additionally, no figures or visual data were generated or modified using generative AI or machine learning-based image enhancement tools. Generative AI was not involved in any part of the data generation, analysis, or interpretation.

References

- 1 Xi Y, Xu P: Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 14(10): 101174, 2021. DOI: 10.1016/j.tranon.2021.101174
- 2 Kishore C, Bhadra P: Current advancements and future perspectives of immunotherapy in colorectal cancer research. *Eur J Pharmacol* 893: 173819, 2021. DOI: 10.1016/j.ejphar.2020.173819
- 3 Zhang J, Zou S, Fang L: Metabolic reprogramming in colorectal cancer: regulatory networks and therapy. *Cell Biosci* 13(1): 25, 2023. DOI: 10.1186/s13578-023-00977-w
- 4 Denisenko TV, Gorbunova AS, Zhivotovsky B: Mitochondrial Involvement in Migration, Invasion and Metastasis. *Front Cell Dev Biol* 7: 355, 2019. DOI: 10.3389/fcell.2019.00355
- 5 Dutta H, Jain N: Post-translational modifications and their implications in cancer. *Front Oncol* 13: 1240115, 2023. DOI: 10.3389/fonc.2023.1240115
- 6 Qi H, Ning X, Yu C, Ji X, Jin Y, McNutt MA, Yin Y: Succinylation-dependent mitochondrial translocation of PKM2 promotes cell survival in response to nutritional stress. *Cell Death Dis* 10(3): 170, 2019. DOI: 10.1038/s41419-018-1271-9
- 7 Zhao L, Yu N, Zhai Y, Yang Y, Wang Y, Yang Y, Gong Z, Zhang Y, Zhang X, Guo W: The ubiquitin-like protein UBTD1 promotes colorectal cancer progression by stabilizing c-Myc to upregulate glycolysis. *Cell Death Dis* 15(7): 502, 2024. DOI: 10.1038/s41419-024-06890-5
- 8 Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, Lythgoe K, Dong S, Lonial S, Wang X, Chen GZ, Xie J, Gu TL,

- Polakiewicz RD, Roesel JL, Boggon TJ, Khuri FR, Gilliland DG, Cantley LC, Kaufman J, Chen J: Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. *Sci Signal* 2(97): ra73, 2009. DOI: 10.1126/scisignal.2000431
- 9 Heyliger S, Turley TN, Roach T, Saulsbury MD, Taka E, Reynolds JP, Copland JA 3rd, Kase AM, Reams RR: Analysis of peroxisomal ABCD3 transporter as a prognostic factor in clear cell renal cell carcinoma. *Cancer Genomics Proteomics* 22(5): 698-715, 2025. DOI: 10.21873/cgp.20530
- 10 Im CM, Oh HH, Park SY, Park YL, Kim JS, Joo YE: Oxysterol-binding protein-like 3 promotes tumor progression by regulating apoptosis and angiogenesis in colorectal cancer. *Cancer Genomics Proteomics* 22(6): 1025-1043, 2025. DOI: 10.21873/cgp.20553
- 11 Zhang W, Yang Y, Lin L, He J, Dong J, Yan B, Cai W, Chen Y, Yin L, Tang D, Liu F, Dai Y: Comprehensive characterization of ubiquitinome of human colorectal cancer and identification of potential survival-related ubiquitination. *J Transl Med* 20(1): 445, 2022. DOI: 10.1186/s12967-022-03645-8
- 12 Zhang W, Tang D, Lin L, Fan T, Xia L, Cai W, Dai W, Zou C, Yin L, Xu Y, Dai Y: Integrative multiplatform-based molecular profiling of human colorectal cancer reveals proteogenomic alterations underlying mitochondrial inactivation. *Am J Cancer Res* 11(6): 2893-2910, 2021.
- 13 Zhang Y, Zhang W, Li T, Hao E, Du J, Feng M, Zhu F, Dai Y: A Multi-PTM omics atlas uncovers novel aging regulators in colorectal cancer. *BMC Cancer* 26(1): 140, 2025. DOI: 10.1186/s12885-025-15274-7
- 14 Meier F, Brunner AD, Koch S, Koch H, Lubeck M, Krause M, Goedecke N, Decker J, Kosinski T, Park MA, Bache N, Hoerning O, Cox J, Räther O, Mann M: Online parallel accumulation-serial fragmentation (PASEF) with a novel trapped ion mobility mass spectrometer. *Mol Cell Proteomics* 17(12): 2534-2545, 2018. DOI: 10.1074/mcp.TIR118.000900
- 15 Liao Z, Li D, Liao S, Zeng Z, Liu J, Xie T, Hu B, Wang W, Hong X, Liu D, Yin L, Tang D, Dai Y: Proteomics profiling and lysine malonylation analysis in primary Sjogren's syndrome. *J Proteomics* 287: 104977, 2023. DOI: 10.1016/j.jprot.2023.104977
- 16 Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G: clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Camb)* 2(3): 100141, 2021. DOI: 10.1016/j.xinn.2021.100141
- 17 Bader GD, Hogue CW: An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4: 2, 2003. DOI: 10.1186/1471-2105-4-2
- 18 Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY: cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol* 8(Suppl 4): S11, 2014. DOI: 10.1186/1752-0509-8-s4-s11
- 19 Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D: Highly accurate protein structure prediction with AlphaFold. *Nature* 596(7873): 583-589, 2021. DOI: 10.1038/s41586-021-03819-2
- 20 Rosignoli S, Paiardini A: Boosting the Full Potential of PyMOL with Structural Biology Plugins. *Biomolecules* 12(12): 1764, 2022. DOI: 10.3390/biom12121764
- 21 Hornbeck PV, Kornhauser JM, Tkachev S, Zhang B, Skrzypek E, Murray B, Latham V, Sullivan M: PhosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. *Nucleic Acids Res* 40(Database issue): D261-D270, 2012. DOI: 10.1093/nar/gkr1122
- 22 Wang D, Liu D, Yuchi J, He F, Jiang Y, Cai S, Li J, Xu D: MusiteDeep: a deep-learning based webserver for protein post-translational modification site prediction and visualization. *Nucleic Acids Res* 48(W1): W140-W146, 2020. DOI: 10.1093/nar/gkaa275
- 23 Orsburn BC: Proteome discoverer-a community enhanced data processing suite for protein informatics. *Proteomes* 9(1): 15, 2021. DOI: 10.3390/proteomes9010015
- 24 Bentzen SM, Vogelius IR: Using and understanding survival statistics - or how we learned to stop worrying and love the Kaplan-Meier estimate. *Int J Radiat Oncol Biol Phys* 115(4): 839-846, 2023. DOI: 10.1016/j.ijrobp.2022.11.035
- 25 Gu D, Ye M, Zhu G, Bai J, Chen J, Yan L, Yu P, Lu F, Hu C, Zhong Y, Liu P, He Q, Tang Q: Hypoxia upregulating ACS2 enhances lipid metabolism reprogramming through HMGCS1 mediated PI3K/AKT/mTOR pathway to promote the progression of pancreatic neuroendocrine neoplasms. *J Transl Med* 22(1): 93, 2024. DOI: 10.1186/s12967-024-04870-z
- 26 Koo SY, Park EJ, Noh HJ, Jo SM, Ko BK, Shin HJ, Lee CW: Ubiquitination Links DNA Damage and Repair Signaling to Cancer Metabolism. *Int J Mol Sci* 24(9): 8441, 2023. DOI: 10.3390/ijms24098441
- 27 Hu Y, Xu W, Chen L: Post-translational modifications and the reprogramming of tumor metabolism. *Discov Oncol* 16(1): 929, 2025. DOI: 10.1007/s12672-025-02674-1
- 28 Faubert B, Solmonson A, DeBerardinis RJ: Metabolic reprogramming and cancer progression. *Science* 368(6487): eaaw5473, 2020. DOI: 10.1126/science.aaw5473
- 29 Ghasemi F, Farkhondeh T, Samarghandian S, Ghasempour A, Shakibaie M: Oncogenic alterations of metabolism associated with resistance to chemotherapy. *Curr Mol Med* 24(7): 856-866, 2024. DOI: 10.2174/1566524023666230622104625
- 30 van Noorden CJF, Hira VVV, van Dijk AJ, Novak M, Breznik B, Molenaar RJ: Energy metabolism in IDH1 wild-type and IDH1-mutated glioblastoma stem cells: a novel target for therapy? *Cells* 10(3): 705, 2021. DOI: 10.3390/cells10030705

- 31 Liu X, Yamaguchi K, Takane K, Zhu C, Hirata M, Hikiba Y, Maeda S, Furukawa Y, Ikenoue T: Cancer-associated IDH mutations induce Glut1 expression and glucose metabolic disorders through a PI3K/Akt/mTORC1-Hif1 α axis. *PLoS One* 16(9): e0257090, 2021. DOI: 10.1371/journal.pone.0257090
- 32 Yu P, Li J, Feng W, Lei T, Jia W, He Y, Bi Y: SKP2 ubiquitylation modifies IDH1 to regulate hepatoblastoma cell cycle and glucose metabolism. *BMC Cancer* 25(1): 1304, 2025. DOI: 10.1186/s12885-025-14644-5
- 33 Zhang Q, Han Z, Liu J, Li M, Li W, Zhou X, Liu X: Hypoxia facilitates stemness of colon cancer cells *via* histone lactylation. *Biochim Biophys Acta Mol Basis Dis* 1871(8): 167993, 2025. DOI: 10.1016/j.bbadis.2025.167993
- 34 Jin L, Kim EY, Chung TW, Han CW, Park SY, Han JH, Bae SJ, Lee JR, Kim YW, Jang SB, Ha KT: Hemistepsin A suppresses colorectal cancer growth through inhibiting pyruvate dehydrogenase kinase activity. *Sci Rep* 10(1): 21940, 2020. DOI: 10.1038/s41598-020-79019-1
- 35 Liao M, Yao D, Wu L, Luo C, Wang Z, Zhang J, Liu B: Targeting the Warburg effect: A revisited perspective from molecular mechanisms to traditional and innovative therapeutic strategies in cancer. *Acta Pharm Sin B* 14(3): 953-1008, 2024. DOI: 10.1016/j.apsb.2023.12.003
- 36 Zhang JY, Zhang F, Hong CQ, Giuliano AE, Cui XJ, Zhou GJ, Zhang GJ, Cui YK: Critical protein GAPDH and its regulatory mechanisms in cancer cells. *Cancer Biol Med* 12(1): 10-22, 2015. DOI: 10.7497/j.issn.2095-3941.2014.0019
- 37 Koizume S, Miyagi Y: Adaptation mechanisms in cancer: Lipid metabolism under hypoxia and nutrient deprivation as a target for novel therapeutic strategies (Review). *Mol Med Rep* 31(4): 83, 2025. DOI: 10.3892/mmr.2025.13448
- 38 Ross KE, Zhang G, Akcora C, Lin Y, Fang B, Koomen J, Haura EB, Grimes M: Network models of protein phosphorylation, acetylation, and ubiquitination connect metabolic and cell signaling pathways in lung cancer. *PLoS Comput Biol* 19(3): e1010690, 2023. DOI: 10.1371/journal.pcbi.1010690
- 39 Zhong X, He X, Wang Y, Hu Z, Huang H, Zhao S, Wei P, Li D: Warburg effect in colorectal cancer: the emerging roles in tumor microenvironment and therapeutic implications. *J Hematol Oncol* 15(1): 160, 2022. DOI: 10.1186/s13045-022-01358-5
- 40 Yasui C, Kono Y, Ishiguro R, Yagyu T, Kyoichi K, Yamamoto M, Matsunaga T, Takano S, Tokuyasu N, Sakamoto T, Hasegawa T, Umekita Y, Fujiwara Y: New treatment modalities for colorectal cancer through simultaneous suppression of FSP1 and GPX4. *Anticancer Res* 44(11): 4905-4914, 2024. DOI: 10.21873/anticancer.17316
- 41 Aoyama T, Yukawa N, Saito A: Clinical impact of nutrition and inflammation assessment tools in colorectal cancer treatment. *Anticancer Res* 44(4): 1335-1351, 2024. DOI: 10.21873/anticancer.16930