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Mapping the stage-specific plasma p53 interactome reveals colorectal cancer progression signatures and therapeutic vulnerabilities

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ABSTRACT

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide, yet the molecular changes that occur in the bloodstream as the disease advances remain poorly understood. In particular, how p53—a key tumor suppressor—interacts with circulating proteins at different stages of CRC has not been well characterized. In this discovery-phase study, we analyzed plasma samples from patients with CRC stages I–IV using high-resolution LC–MS/MS. Stage-specific proteins were identified and cross-validated with transcriptomic data, revealing TP53 as a central hub in multiple functional networks. Enrichment analyses highlighted progressive changes in pathways linked to immune surveillance, complement activation, and cellular stress responses. Early-stage disease showed signals consistent with tumor suppression and immune regulation, while advanced stages were enriched in proteins associated with metastasis and therapy resistance. Notably, KLHL40 (Stage I) and FKBP1A (Stage III) emerged as potential stage-specific circulating biomarkers with functional links to p53 signaling. These findings outline a plasma-based molecular map of p53-associated protein networks across CRC progression and point to candidate biomarkers that warrant validation in larger, independent, and longitudinal studies.

1. Introduction

Colorectal cancer (CRC) is a major global cause of cancer-related morbidity and mortality. Disease progression—from localized lesions to metastatic dissemination—is driven by complex molecular changes, including disruption of the tumor suppressor p53. As a master regulator of genomic stability, p53 controls critical processes such as cell cycle regulation, apoptosis, DNA repair, senescence, and immune signaling [1]. TP53 is one of the most frequently mutated genes in CRC, with mutation rates reaching approximately 50–60 % in advanced-stage tumors.

While tissue-based genomic profiling remains the gold standard, indirect monitoring of p53 pathway activity in circulation may provide valuable complementary insights. This is particularly relevant in liquid biopsy applications, where the sensitivity of mutation detection can be limited. Although TP53 mutations are common in CRC, their clinical utility for prognostication or therapeutic decision-making remains restricted. Increasing evidence indicates that p53 function is highly context-dependent: wild-type and mutant forms engage distinct protein–protein interaction (PPI) networks, resulting in divergent cellular outcomes [2].

Most existing studies have focused on tumor tissue or cell lines, overlooking systemic alterations reflected in the plasma proteome. Given that CRC progression involves both tumor-intrinsic changes and tumor microenvironment remodeling [3], profiling circulating p53-interacting proteins offers a unique opportunity to capture systemic

Abbreviations: CRC, Colorectal Cancer; PPI, Protein–Protein Interaction.

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disease dynamics. Such analyses may enable minimally invasive biomarker discovery for stage-specific monitoring and reveal therapeutic vulnerabilities associated with p53 network rewiring.

In this study, we conducted stage-stratified plasma proteomics using high-resolution ESI-nanoLC-MS/MS in CRC patients across stages I–IV. We integrated these proteomic data with curated p53 interaction networks and functional pathway annotations using STRING, GeneMANIA, and Enrichr-based transcriptomics. Our findings reveal a dynamic plasma p53 interactome that mirrors CRC progression, identifies candidate diagnostic biomarkers, and highlights stage-specific p53 pathway vulnerabilities.

2. Materials and methods

Motivated by the functional specificity of p53 and its role in tumor progression, we sought to investigate its status and interactions across CRC stages. This approach aims to enhance understanding of disease progression, facilitate earlier detection, and uncover potential therapeutic vulnerabilities. To this end, we initiated a discovery-phase proteomics study to identify circulating, stage-specific proteins. An overview of the experimental setup and workflow is shown in Workflow 1, while detailed methodologies are provided in the Supplementary Section.

3. An overview

3.1. Methods – sample collection and processing

Plasma samples were obtained from 12 treatment-naïve CRC patients, stratified by TNM stage: Stage I (n = 1), Stage II (n = 3), Stage III (n = 4), and Stage IV (n = 3), along with 3 healthy controls (two males, one female). All participants were of Asian ethnicity and recruited prior to any therapeutic intervention. Venous blood was collected into EDTA tubes, processed within 2 h by centrifugation at 1500×*g* for 15 min at 4 °C, and aliquots stored at −80 °C until analysis.

3.2. Methods – proteomic analysis

High-resolution electrospray ionization nano–liquid chromatography tandem mass spectrometry (ESI-nanoLC–MS/MS) was performed on stage-stratified plasma samples. Peptides were separated on a C18 analytical column using a linear acetonitrile gradient, followed by MS acquisition in data-dependent mode. Protein identification and quantification were conducted using Progenesis QI for Proteomics v4.2 (Non-Linear Dynamics, Waters), applying a 1 % FDR at the peptide and protein levels, with at least one unique peptide required for identification.

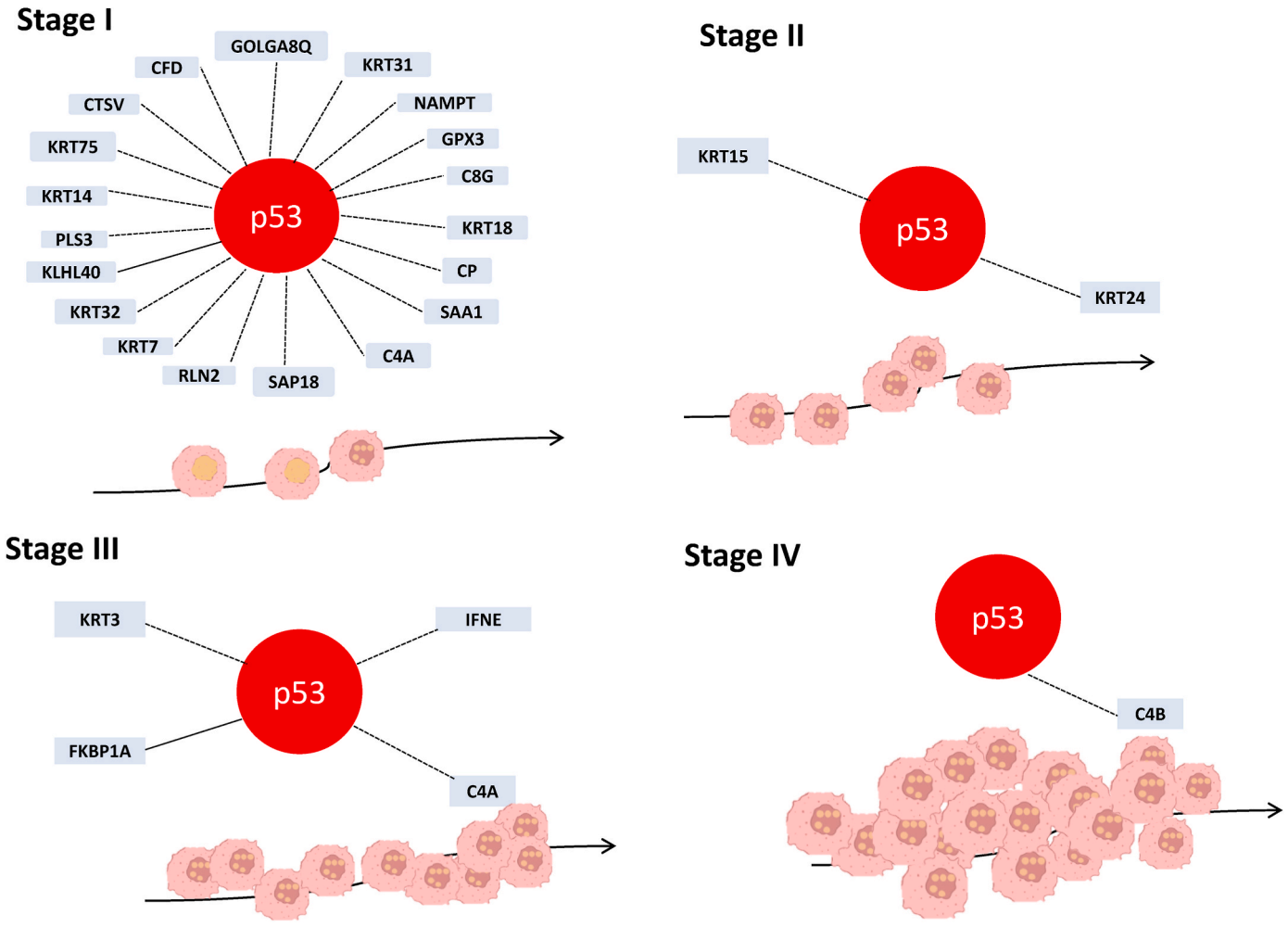


Fig. 1. Stage-Specific Plasma Proteomic Landscape of p53-Associated Networks in Colorectal Cancer
Stage-specific plasma proteomic landscape of p53-associated networks in colorectal cancer (CRC). High-resolution LC–MS/MS profiling of plasma samples from TNM stages I–IV identified distinct p53-associated ligand–target modules. Stage-specific proteins were validated by transcriptomic enrichment (Enrichr, MSigDB Hallmark 2020) and mapped onto interaction networks using STRING and GeneMANIA. Panels highlight progression-associated shifts in epithelial remodeling, immune regulation, apoptosis, and stress response, with direct TP53 associations annotated.

3.3. Methods – bioinformatics and network analysis

Stage-specific proteins were identified using Venn diagram analysis to extract unique protein sets for each stage. Transcriptomic enrichment was performed using Enrichr (MSigDB Hallmark 2020 gene sets) to identify p53-associated gene expression signatures. PPI networks were mapped using STRING and GeneMANIA, with annotations distinguishing direct and indirect TP53 associations. Functional modules were categorized into epithelial remodeling, immune regulation, apoptotic signaling, and stress response.

4. Results and discussion

4.1. Identification of circulating stage-specific proteins in CRC

Across all TNM stages, high-resolution LC–MS/MS identified 236 proteins in Stage I, 144 in Stage II, 154 in Stage III, and 149 in Stage IV plasma samples. To define stage-specific protein signatures, we performed Venn diagram analysis (Fig. 2), yielding 84 proteins unique to Stage I, 2 to Stage II, 9 to Stage III, and 5 to Stage IV.

Stage I: Of the 84 stage-specific proteins, transcriptomic enrichment analysis identified six ligands (SAA1, KRT18, KRT75, KRT14, KRT7, KLHL40) with significant enrichment (adjusted $p < 0.05$). Dominant pathways included epithelial structural integrity and remodeling (KRT18, KRT75, KRT14, KRT7), acute-phase inflammation and extracellular matrix (ECM) remodeling (SAA1), and apoptotic/stress-response regulation (KRT18, KRT7).

Stage II: Among the two stage-specific proteins, KRT15 showed significant enrichment (adjusted $p = 0.0113$). Enriched pathways involved epithelial cytoskeleton maintenance and p53-related transcriptional regulation, through overlap with KRT17, ITGB4, CLCA2, SFN, and SERPINB5.

Stage III: Of the nine stage-specific proteins, two ligands (KRT3, IFNE) were significantly enriched (adjusted $p < 0.05$), with pathways dominated by epithelial adaptation during invasion (KRT3) and immune signaling/microenvironmental crosstalk (IFNE). In addition, FKBP1A emerged as a direct p53 interactor associated with apoptosis regulation and immune modulation. Although its adjusted p value (0.2086) did not meet statistical significance, its established links to p53-regulated apoptotic pathways (via BAX, BAK1, and TAX1BP3) highlight it as a biologically relevant candidate warranting further validation.

Stage IV: None of the five stage-specific proteins reached the adjusted $p < 0.05$ threshold. The top non-significant enrichment signals involved complement activation and systemic immune remodeling (C4B).

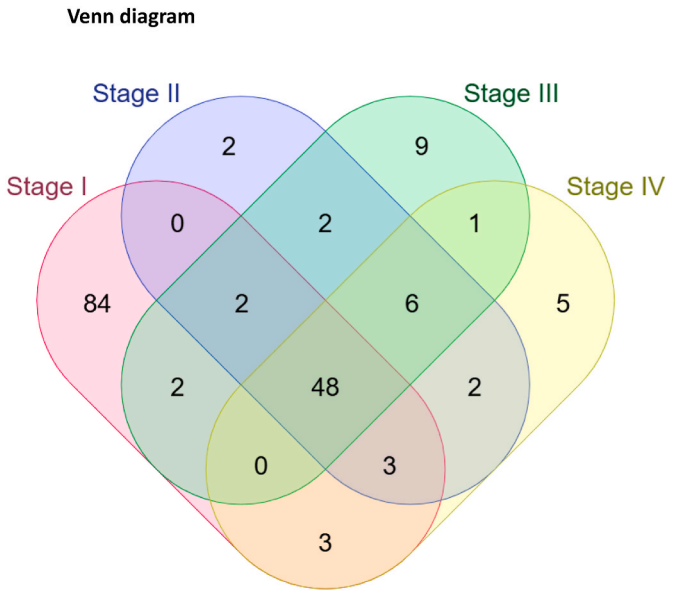


Fig. 2. Venn Diagram of Stage-Specific and Shared Circulating Proteins in Colorectal Cancer Plasma
This Venn diagram depicts the distribution and overlap of circulating proteins identified via LC-MS/MS-based plasma proteomics across colorectal cancer (CRC) stages I–IV. Each segment quantifies proteins uniquely associated with a specific stage (e.g., 84 for Stage I, 2 for Stage II, 9 for Stage III, and 5 for Stage IV) or shared across multiple stages, including 48 proteins consistently detected in all stages. The stage-specific subsets form the foundation for subsequent transcriptomic enrichment and p53-centered network analyses. This visualization supports the identification of liquid biopsy–accessible protein signatures that reflect CRC progression and may serve as noninvasive biomarkers for disease staging and therapeutic stratification.

Collectively, these findings (Table 1) reveal a progressive shift in the circulating p53 interactome: early stages are characterized by epithelial structural and inflammatory pathways, mid-stages show increased immune modulation and apoptosis-related signals, and late-stage profiles feature systemic immune remodeling.

4.2. Stage I: transcriptomic p53 network enrichment and KLHL40 as a candidate plasma interactor

Plasma proteomic profiling of Stage I CRC using high-sensitivity ESI-nanoLC-MS/MS revealed a distinct set of differentially abundant proteins. Enrichr-based transcriptomic mapping demonstrated significant overlap between several stage-specific proteins and canonical p53-regulated gene sets (Table 2). Notably, KRT7, KRT14, KRT18, KRT31, KRT32, and KRT75 showed strong enrichment for p53-associated pathways (adjusted $p < 0.02$; combined score >100), aligning with key p53 targets such as SFN, PERP, TP63, and EPHA2.

PPI mapping using GeneMANIA identified KLHL40 as the only Stage I plasma protein with a predicted direct interaction with p53, positioned apart from the core keratin/inflammatory protein cluster (Fig. 3A). Despite its modest enrichment score (adjusted $p = 0.63$), KLHL40 showed transcriptomic co-expression with TNNI1, a known p53-responsive gene, suggesting potential functional relevance in early tumorigenesis. Interestingly, KLHL40's interaction with p53 was absent in the STRING network, where it appeared disconnected from the p53-centered cluster (Fig. 3B).

This discrepancy likely reflects methodological differences: GeneMANIA integrates co-expression, pathway prediction, and genetic interaction data, whereas STRING prioritizes experimentally validated biochemical interactions. KLHL40 may therefore represent a context-specific, transcriptionally co-regulated node rather than a direct biochemical interactor. Given its reported role in E3 ubiquitin ligase regulation and muscle homeostasis [4], its detection in early-stage CRC plasma—alongside its unique network behavior—suggests that KLHL40 could act as a Stage I-specific molecular sentinel within the broader p53 regulatory landscape.

In contrast, the STRING-based network displayed a densely interconnected set of indirect p53-associated proteins, including CP, NAMPT, GPX3, CTSV, RLN2, and a broad keratin cluster (KRT7/14/18/31/32/75). Although these proteins do not directly bind p53, their co-enrichment and network proximity indicate integration within p53-regulated transcriptional programs. These modules likely reflect early epithelial stress responses, inflammatory priming, and compensatory remodeling during initial tumor development.

While several Stage I proteins demonstrated strong statistical enrichment (e.g., SAA1, KRT18, KRT75, KRT14, KRT7; adjusted $p < 0.05$), others, such as KLHL40, were identified primarily based on network positioning and potential functional relevance rather than statistical stringency (adjusted $p = 0.63$). Their inclusion reflects the

Table 1
Summary of stage-specific protein counts, enrichment significance, and dominant pathways in the p53 interactome.

Stage	Raw proteomics count	Venn diagram unique proteins	Significant (adj. $p < 0.05$)	Direct p53 interactors	Dominant pathways
I	236	84	6 (SAA1, KRT18, KRT75, KRT14, KRT7, KLHL40)	KLHL40	Epithelial structural integrity/remodeling; acute-phase inflammation and ECM remodeling; apoptotic/stress-response regulation
II	144	2	1 (KRT15)	–	Epithelial cytoskeleton maintenance; p53-related transcriptional regulation
III	154	9	3 (KRT3, IFNE, FKBP1A)	FKBP1A	Epithelial adaptation during invasion; apoptosis regulation; immune signaling and epithelial–microenvironment crosstalk
IV	149	5	0	–	Complement activation; systemic immune remodeling (non-significant enrichment)

Table 2
Stage-Specific Plasma Ligands –p53 Validated via Transcriptomic Enrichment (Enrichr) and MSigDB Hallmark 2020, combining STRING and GeneMANIA.

Stage	Ligand	P-value	Adjusted p-value	Odds Ratio	Combined score	Overlap gene/s	Direct p53 Interaction	Functional Implication
I	SAP18	0.6349	0.6349	1.00	0.45	HINT1	No	Promotes proliferation and invasion
I	C4A	0.6348	0.6348	1.0	0.4543	EPHX1	No	Complement activation, opsonization, and immune surveillance
I	SAA1	0.0005	0.001	6.48	49.19	KRT17; PERP; CLCA2; SDC1; EPS8L2; TM4SF1	No	Inflammation, ECM remodeling
I	CP	0.6348	0.6348	1.0	0.4543	GPX2	No	Iron metabolism and oxidative stress
I	CFD	0.0789	0.1929	3.09	7.86	CEBPA; S100A4; IFI30	No	Complement activation, immune regulation
I	KRT18	0.0033	0.0192	5.32	30.35	PERP; SDC1; SFN; EPS8L2; EPHA2	No	Apoptotic regulation, epithelial remodeling
I	C8G	0.6348	0.6348	1.0	0.4543	DCXR	No	Modulates immune-mediated cell lysis
I	GPX3	0.6348	0.6348	1.0	0.4543	EPHX1	No	Oxidative stress response
I	NAMPT	0.6348	0.6348	1.0	0.4543	MXD1	No	Metabolic rewiring, p53 suppression
I	KRT31	0.0789	0.3156	3.09	7.86	CLCA2; KLK8; TP63	No	Epithelial integrity; linked to EMT and cancer invasiveness
I	GOLGA8Q	0.6348	0.6348	1.0	0.4543	ERCC5	No	role in vesicle transport and chromosomal instability
I	CTSV	0.2642	0.4152	2.0307	2.7027	SFN; KLK8	No	Degrades ECM components; facilitates tumor invasion and metastasis.
I	KRT75	0.0033	0.01662	5.32	30.35	KRT17; CLCA2; SFN; KLK8; SERPINB5	No	Epithelial barrier, structural adaptation
I	KRT14	0.0005	0.0025	6.48	49.19	KRT17; CLCA2; SFN; KLK8; TP63; SERPINB5	No	EMT regulation, epithelial differentiation
I	RLN2	0.6348	0.6348	1.0	0.4543	GLS2	No	ECM-remodeling hormone; promotes cancer cell migration, angiogenesis, and metastasis.
I	KRT7	0.000007	0.00005	8.93	105.49	KRT17; ITGB4; PERP; SDC1; SFN; EPS8L2; TM4SF1; EPHA2	No	Epithelial integrity, stress response
I	PLS3	0.2642	0.3963	2.0307	2.7027	PTPN14; EPHA2	No	Involved in cytoskeletal dynamics and metastatic potential.
I	KLHL40	0.6349	0.6349	1.00	0.45	TNNI1	Yes	Potential noncanonical coexpression with TP53
I	KRT32	0.0789	0.2683	3.09	7.86	CLCA2; SFN; KLK8	No	Ectopic expression may indicate abnormal differentiation in colorectal cancer.
II	KRT15	0.0033	0.01130	5.32	30.35	KRT17; ITGB4; CLCA2; SFN; SERPINB5	No	Maintained p53 transcriptional activity
II	KRT24	0.6348	0.6348	1.0	0.4543	CLCA2	No	Limited relevance; weak transcriptomic overlap
III	KRT3	0.0033	0.01662	5.32	30.35	KRT17; CLCA2; SFN; KLK8; SERPINB5	No	Epithelial adaptation during invasion
III	C4A	0.6348	0.6348	1.0	0.4543	EPHX1	No	Complement suppression, immune evasion
III	FKBP1A	0.0789	0.2086	3.09	7.86	BAX; TAX1BP3; BAK1	Yes	Apoptosis regulation, immune modulation
III	IFNE	0.0180	0.0466	4.1887	16.8108	PROCR; PTPN14; TM4SF1; EPHA2	No	Immune signaling, epithelial-microenvironment crosstalk
IV	C4B	0.6348	0.6348	1.0	0.4543	EPHX1	No	Immune remodeling, systemic stress adaptation

Table 2: presents a stage-specific analysis of plasma-derived ligands validated through transcriptomic enrichment using Enrichr, in the context of colorectal cancer progression. The ligands were identified based on LC-MS/MS proteomics and further integrated with network analyses (STRING and GeneMANIA) to map their association with p53 signaling. For each ligand, the corresponding interacting protein(s), statistical enrichment values (adjusted p-value, odds ratio, and combined score), and overlap genes from the enriched gene sets are listed. The column "Direct p53 Interaction" denotes whether the ligand or its interacting partner is known to directly interact with p53. Functional implications are summarized based on known roles in epithelial remodeling, immune modulation, apoptosis, or metastasis, highlighting the evolving p53 network landscape across CRC stages.

exploratory nature of this discovery-phase study, where biological plausibility within the p53 interactome was weighed alongside statistical metrics. KLHL40, despite its modest statistical support, emerged as a direct TP53 interactor in network analyses, warranting further investigation. These findings should therefore be interpreted as hypothesis-generating, with independent validation in larger, stage-stratified CRC cohorts required to confirm their biomarker or mechanistic value.

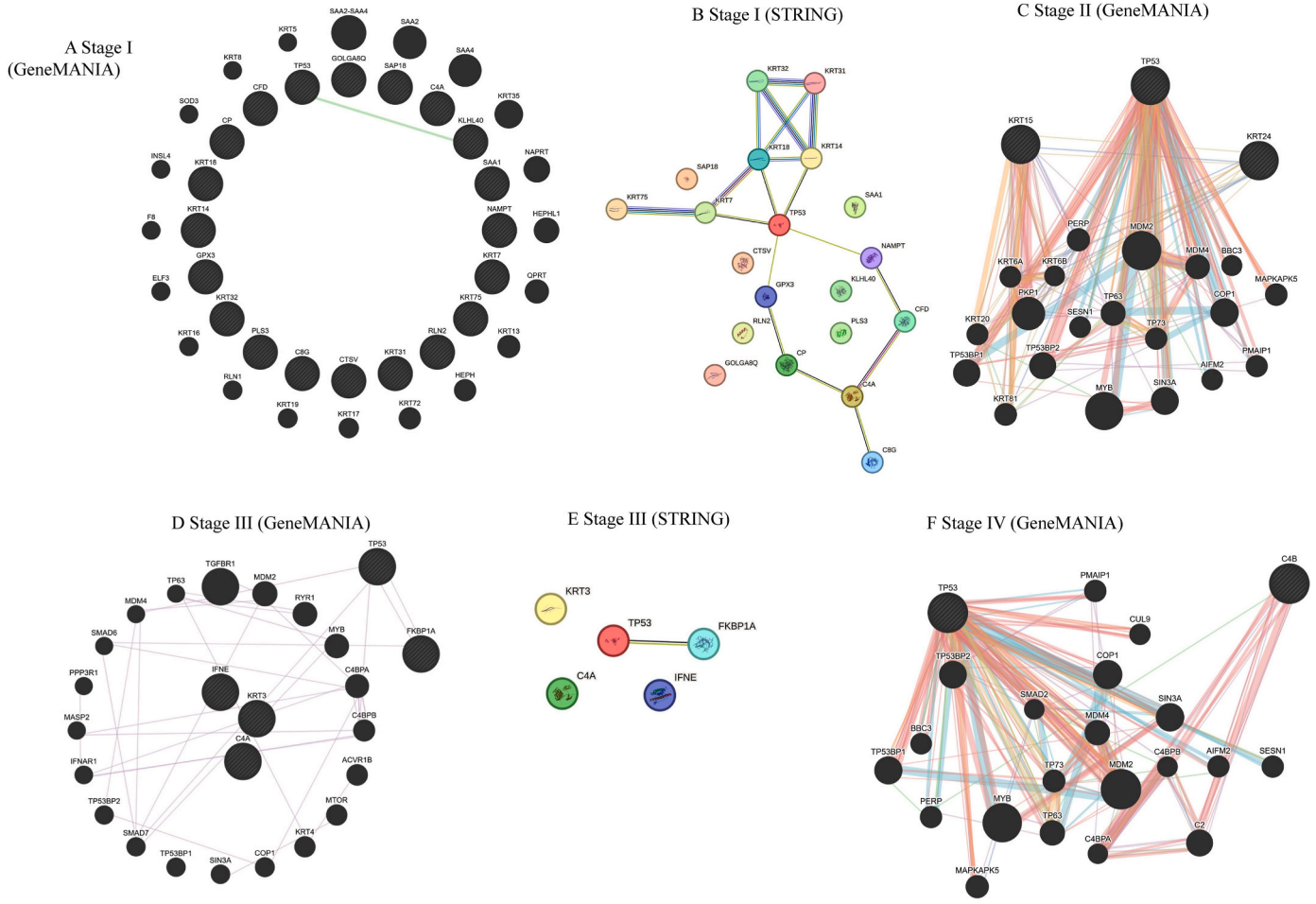


Fig. 3. Stage-Specific Plasma Protein Networks Reflect the Dynamic Remodeling of Circulating p53-Associated Signatures in Colorectal Cancer
(A–B) *Stage I CRC:* Comparative network analysis of KLHL40 in relation to TP53. The GeneMANIA network (A) predicts a direct interaction between KLHL40 and TP53, distinct from the keratin/inflammatory cluster. In contrast, the STRING network (B) shows no KLHL40–TP53 connection, instead highlighting indirect associations between p53 and keratins, NAMPT, GPX3, and stress-response proteins—reflecting early epithelial remodeling and immune priming in the plasma proteome.
(C) *Stage II CRC:* Plasma p53 interaction map reveals KRT15 and KRT24 as stage-specific proteins lacking direct connectivity to TP53 in either network platform. However, Enrichr-based transcriptomic enrichment links KRT15 to p53-regulated gene sets, indicating a transcriptional, albeit indirect, relationship with p53 signaling that persists in intermediate disease stages.
(D–E) *Stage III CRC:* Network integration identifies FKBP1A as a direct interactor of TP53 in the GeneMANIA network (D), with connections to pro-apoptotic and immunoregulatory pathways. STRING analysis (E) embeds FKBP1A and IFNE within a broader p53-centered network, indicating sustained apoptotic signaling and emerging immune–tumor crosstalk in the circulating proteome.
(F) *Stage IV CRC:* C4B is the sole stage-specific plasma protein identified. While no direct interaction with TP53 is observed in either network, Enrichr transcriptomic data suggests a weak association via shared enrichment with the oxidative stress response gene EPHX1, pointing to late-stage divergence or exhaustion of p53 signaling pathways in circulation.

4.3. Stage II: limited but selective p53 pathway enrichment in plasma proteome

Proteomic profiling of Stage II CRC plasma identified two stage-specific proteins: KRT15 and KRT24. Neither exhibited direct interactions with TP53 in GeneMANIA or STRING PPI databases (Fig. 3C). However, Enrichr-based transcriptomic enrichment revealed notable alignment between KRT15 and p53-regulated gene sets (adjusted $p = 0.0113$; combined score = 30.35), suggesting an indirect but functionally relevant association with p53 signaling. The KRT15-linked gene set included SFN, CLCA2, SERPINB5, ITGB4, and KRT17—canonical epithelial and tumor suppressor genes involved in cell cycle arrest, apoptosis, and epithelial integrity, all hallmark processes regulated by p53 [5]. In contrast, KRT24 demonstrated minimal enrichment (adjusted $p = 0.63$) and shared only CLCA2 as a common target, suggesting peripheral involvement in p53-associated transcriptional networks. Although direct protein-level interactions were absent, the selective enrichment of KRT15 and KRT24 within the plasma proteome and its transcriptomic overlap with p53-responsive genes indicate persistent activation of indirect p53 regulatory circuits at this intermediate disease stage. These findings suggest that p53-mediated control of epithelial plasticity and early stress responses may still influence tumor biology in Stage II CRC, even in the absence of overt network connectivity.

4.4. Stage III: FKBP1A as a direct p53 interactor and modulator of Tumor–Immune crosstalk

Stage III CRC plasma proteomics identified four unique stage-specific proteins, including KRT3 and C4A. While C4A showed no significant interaction with TP53 in either STRING or GeneMANIA, KRT3 exhibited strong transcriptomic enrichment for p53-associated gene sets (adjusted $p = 0.0166$; combined score = 30.35). Its overlap with canonical p53-regulated genes—such as KRT17, SFN, KLK8, and SERPINB5—suggests

a role in epithelial remodeling and stress adaptation.

A particularly notable finding was FKBP1A (FK506-binding protein 1A), which emerged as a direct p53 interactor in GeneMANIA (Fig. 3D) and was densely integrated within the p53-centered STRING network (Fig. 3E). Although its transcriptomic enrichment did not reach statistical significance (adjusted $p = 0.2086$; combined score = 7.86), FKBP1A was co-expressed with key p53-regulated apoptotic genes including BAX, BAK1, and TAX1BP3. FKBP1A is a peptidyl-prolyl cis–trans isomerase with established roles in calcium signaling, TGF-β receptor trafficking, and immune checkpoint regulation [6,7]. Its direct interaction with p53 suggests dual functions in maintaining apoptotic fidelity and modulating immune tolerance within the tumor microenvironment. Previous studies have implicated FKBP1A in stabilizing pro-apoptotic machinery, suppressing TGF-β signaling, and inhibiting NF-κB—all pathways intersecting with p53's tumor-suppressive activity [8].

Another transcriptomically enriched protein, interferon epsilon (IFNE), showed weaker protein-level linkage to p53 but significant enrichment (adjusted $p = 0.0467$; combined score = 16.81) through co-expression with EPHA2, PTPN14, PROCR, and TM4SF1—genes associated with epithelial integrity, immune signaling, and cellular adhesion [9] (Fig. 4A–D). Collectively, these results suggest that Stage III CRC represents a transition point in the circulating p53 interactome, shifting from predominantly epithelial stress responses (Stage I/II) toward apoptosis regulation and immune modulation.

Previous CRC liquid biopsy studies have primarily centered on TP53 mutations and related DNA damage response (DDR) genes detected in

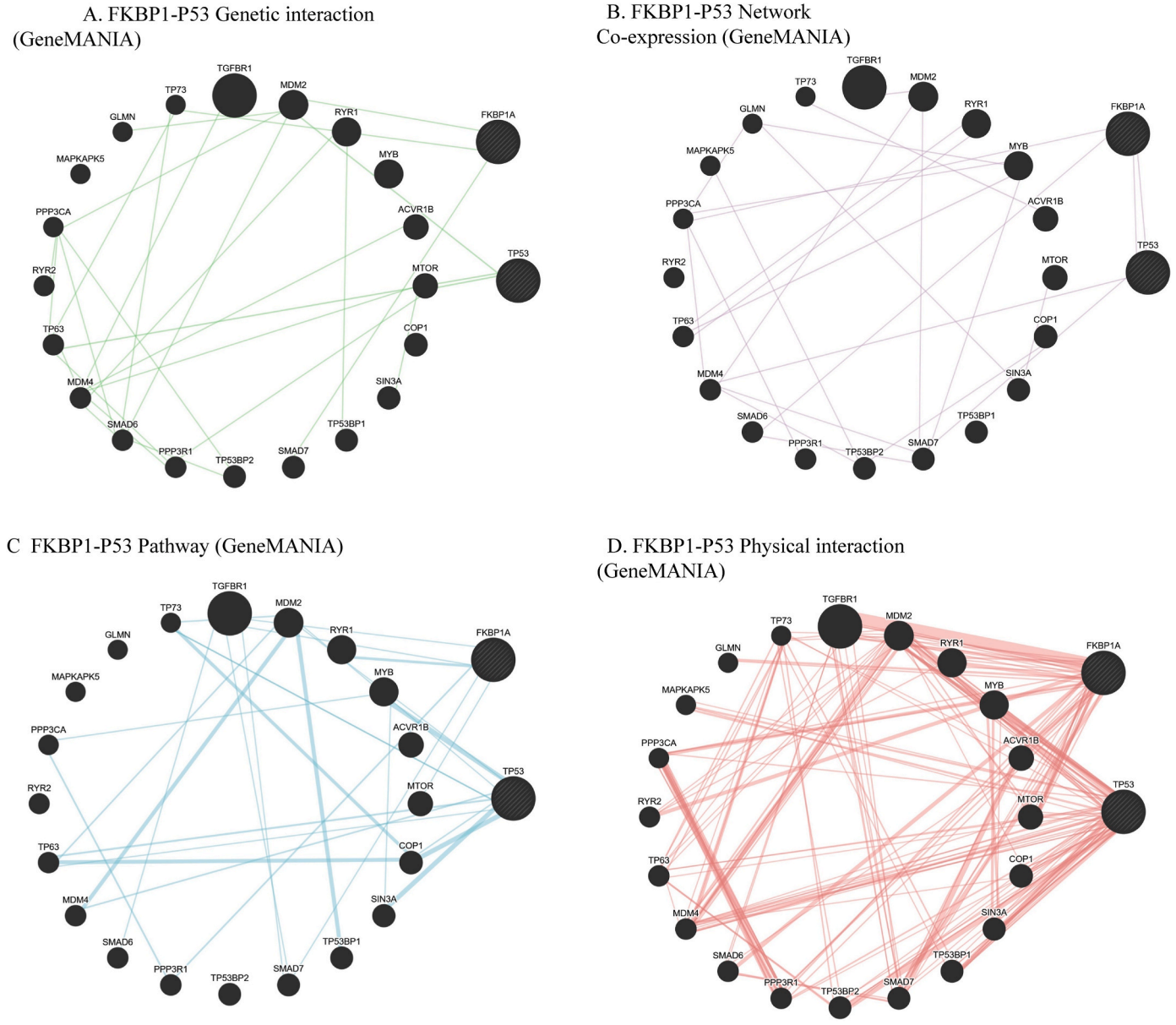
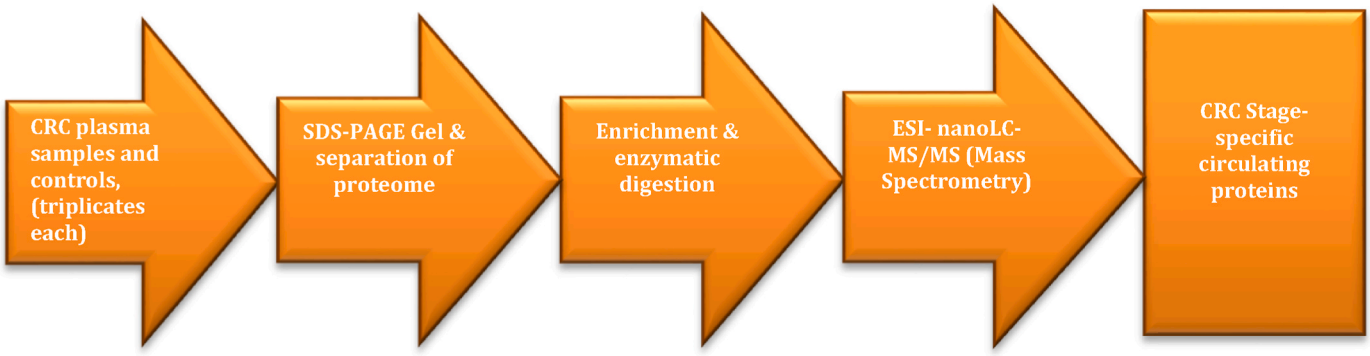


Fig. 4. Network-Based Co-Expression Mapping of TP53 and FKBP1A in Stage III Colorectal Cancer Plasma Proteomics
(A–D) A co-expression-based interaction network centered on TP53 was constructed using GeneMANIA to explore functional associations in the Stage III colorectal cancer (CRC) plasma proteome. Node size represents interaction connectivity, and edge thickness indicates confidence based on integrated genomic and transcriptomic datasets. FKBP1A emerged as a key node co-expressed with TP53, yet lacking evidence of direct interaction through curated physical, genetic, or pathway associations. Core regulators of the TP53 axis—including *MDM2*, *MDM4*, *TP63*, *TP73*, and members of the *SMAD* family—were also mapped, illustrating the broader signaling context. The observed TP53–FKBP1A co-expression, in the absence of canonical interactions, suggests a condition-dependent or context-specific regulatory link detectable through liquid biopsy–based proteomics. This network supports the potential of FKBP1A as a circulating modulator of TP53-linked apoptotic and immune pathways, relevant to tumor–immune dynamics in advanced CRC.



Workflow 1. Proteomic LC-MS/MS analysis of colorectal cancer plasma samples.

cfDNA. For example, Vasseur et al. (2024) [10] reported that over half of advanced cancer patients harbored liquid biopsy–only mutations not seen in matched tissue, with TP53 and DDR genes comprising more than 90 % of these alterations. While clinically relevant, these findings also highlighted interpretive challenges such as low variant allele frequencies and tissue–plasma discordance. Similarly, Garrido-Navas et al. (2020) [11] reviewed TP53 mutation detection in cfDNA and circulating tumor cells (CTCs) from breast, colon, and lung cancers, noting variable concordance with tissue-based profiling and emphasizing the influence of sample type, tumor biology, and analytic platform.

Our proteomics-driven approach complements these mutation-centric studies by focusing on functional protein networks associated with p53 signaling, independent of mutational status. By integrating high-resolution LC–MS/MS data with transcriptomic enrichment (Enrichr, MSigDB Hallmark 2020) and network mapping (STRING, GeneMANIA), we identified stage-specific plasma proteins converging on p53-regulated pathways. This network-level perspective captures progressive remodeling of immune surveillance, complement activation, epithelial remodeling, and apoptotic regulation across CRC stages. Notably, biomarkers such as KLHL40 (Stage I) and FKBP1A (Stage III) emerged that would be missed by cfDNA mutation screening alone, yet may reflect functionally important p53 pathway perturbations. These results support the integration of multi-omic approaches—combining mutation profiling, proteomic mapping, and transcriptomic context—to more comprehensively assess p53 pathway integrity in CRC.

4.5. Stage IV: C4B as an indirect transcriptomic correlate of p53 signaling

Plasma proteomic profiling in Stage IV CRC identified complement component C4B as the sole stage-specific protein (Fig. 3F). Despite the absence of direct interactions between C4B and p53 in GeneMANIA or STRING network analyses, transcriptomic enrichment via Enrichr indicated a weak but notable association with the p53 signaling pathway (adjusted $p = 0.63$; combined score = 0.45), mediated through the shared gene EPHX1. EPHX1, an oxidative stress–response gene and secondary effector within p53-associated pathways [12], may act as an indirect functional bridge. Although statistical support was modest, this connection suggests a subtle shift in p53 pathway activity, potentially reflecting the evolving immunoregulatory landscape of advanced CRC. In this context, p53's classical tumor-suppressive role may be increasingly subverted by chronic inflammation and immune evasion mechanisms. The absence of direct interactors in plasma further implies that, by Stage IV, canonical p53-related biomarkers are diminished or obscured. This supports the hypothesis that functional inactivation or diversion of p53 signaling contributes to metastatic progression and systemic immune modulation.

4.6. Cross-stage synthesis and translational implications

The identification of KLHL40 in Stage I and FKBP1A in Stage III highlights candidate stage-specific effectors within the circulating p53 interactome, each representing distinct opportunities for translational follow-up. KLHL40, despite modest enrichment in this discovery-phase analysis, demonstrated direct TP53 network connectivity and may represent a previously underexplored early-stage marker. In contrast, FKBP1A was statistically enriched and directly interacted with p53, aligning with apoptotic regulation and immune modulation pathways characteristic of more advanced disease.

Future validation will require larger, stage-balanced patient cohorts to ensure reproducibility and generalizability. Longitudinal sampling could clarify whether KLHL40 levels decline or persist through progression, and whether FKBP1A emerges progressively as disease advances. Multi-omic integration—particularly combining plasma proteomics with circulating tumor DNA (ctDNA) profiling and transcriptomic datasets—could enhance biological plausibility by linking protein abundance changes to mutational status, gene expression profiles, and pathway activation states. Such integrative approaches could determine whether these proteins complement existing biomarkers, refine liquid biopsy panels, or serve as early indicators of p53 pathway disruption in CRC.

5. Conclusion

This study provides a stage-specific systems view of circulating p53-associated proteins in CRC, integrating LC–MS/MS plasma proteomics with transcriptomic enrichment and interaction network analyses. Our results reveal a progressive remodeling of p53 signaling from early to late disease stages, reflecting transitions in epithelial regulation, apoptosis, and immune modulation.

In early-stage CRC (Stage I), p53-associated transcriptional activity is prominent, with strong epithelial and inflammatory signatures, and KLHL40 emerges as a potential transcriptional interactor indicating early regulatory involvement. By Stage II, p53 network connectivity diminishes, suggesting a transitional state of partial pathway suppression. In Stage III, FKBP1A appears as a direct p53 interactor linked to apoptotic regulation and immune modulation, suggesting residual pathway functionality in tumor–immune adaptation. In contrast, Stage IV shows a near-complete loss of p53-associated interactions, consistent with metastatic progression and systemic immune reprogramming.

Although direct detection of p53 in plasma remains technically challenging due to its low abundance, complex post-translational modifications, and aggregation-prone nature, our findings underscore the value of surrogate interactors and network-based mapping in capturing tumor suppressor pathway dynamics [13]. The identification of KLHL40 and FKBP1A as stage-specific effectors highlights their potential as biomarker candidates and therapeutic targets, pending validation in larger, independent, and longitudinally sampled cohorts.

Our findings suggest that network-guided plasma proteomics, when integrated with transcriptomic and interaction analyses, may complement mutation-based approaches by providing a framework to explore

stage-specific p53 pathway alterations and guide future precision monitoring strategies in CRC.

Author contribution

R.A.N. conceived the study, conducted the research and wrote the manuscript.

R.A.N., V.B., S.K., J.R., and R.S. analyzed the data and provided the lab infrastructures.

Statements and declarations

This submission is original, has not been published or submitted elsewhere, and has been approved by all authors. We have no conflicts of interest to declareThe authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlb.2025.100329.

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