Unraveling the Anemia Nexus: Bioinformatics-Driven Discovery of Key Protein Interactions and Therapeutic Targets

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Abstract- Anemia, characterized by a deficiency in red blood cells or their oxygen-carrying capacity, is a prevalent condition with significant health impacts. This study utilizes a bioinformatics approach to identify key proteins involved in anemia, leveraging multiple centrality metrics within the anemia protein interaction network to uncover potential therapeutic targets. By analyzing genomic and proteomic data, we identified critical proteins using centrality metrics, including Degree, Closeness, Betweenness, and Radiality. The study focused on five key proteins: GAPDH, EEF2, TPI1, ACO1, and RPS13. These proteins were assessed for their roles in cellular processes related to anemia. Our findings highlight GAPDH's multifunctional roles in glycolysis and iron homeostasis, EEF2's regulation of protein synthesis under stress, TPI1's crucial function in glycolysis and its link to hemolytic anemia, ACO1's dual role in the TCA cycle and iron regulation, and RPS13's importance in protein synthesis and erythropoiesis. Each protein was identified as a significant node within the network, indicating its potential as a biomarker and therapeutic target. The integration of genomic, proteomic, and clinical data revealed that these proteins play pivotal roles in the molecular mechanisms underlying anemia. GAPDH interacts with iron-regulatory proteins, EEF2 modulates protein synthesis, TPI1 mutations lead to hemolytic anemia, ACO1 regulates iron homeostasis and is linked to sideroblastic anemia, and RPS13 contributes to erythropoiesis. This study explores how specific proteins may contribute to the development and progression of anemia. Rather than reinforcing existing models, it introduces fresh biological clues that could reshape how clinicians interpret and treat this condition. These findings point toward personalized treatment options and offer a more refined lens for evaluating patient needs.

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Introduction

Anemia poses a widespread challenge to global health, impacting vast populations and contributing to serious illness and premature death. (1). Characterized by a reduction in red blood cells or hemoglobin levels, anemia impairs the blood's oxygen-carrying capacity. This condition arises from various etiologies, including nutritional deficiencies, chronic diseases, genetic disorders, and infections, reflecting its multifactorial nature (2).

Recent advancements in molecular biology have highlighted the role of moonlighting proteins in various biological processes, including those related to anemia (3). Moonlighting proteins are multifunctional proteins that perform two or more distinct biological functions within a single polypeptide chain (4). Certain proteins perform surprising and unrelated tasks beyond their conventional biological roles, a phenomenon known as “moonlighting.” Their ability to shift functions stems from their flexible structures, which enable them to take on diverse functional capacities, including enzymatic

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processes, cellular structural organization, and modulation of signaling pathways, all shaped by their surroundings. These adaptive changes are influenced by conditions like protein modifications after synthesis, interactions with other molecules, or shifts in the cellular surroundings (5,6).

The function a moonlighting protein performs is determined by its biological surroundings. Factors such as its spatial distribution within the cell and fluctuations in external conditions influence the range of roles it can adopt. Moonlighting proteins can exhibit different functions in various cellular tissues (5). The presence of these proteins across a wide array of organisms over evolutionary time points to their functional flexibility as a trait preserved by natural selection. The ability to perform multiple functions with a single gene product is advantageous for organisms, enhancing energy efficiency and facilitating rapid adaptation to changing environments (7). Moonlighting proteins can function as dynamic signaling mediators, integrating distinct biological routes and enabling communication between diverse cellular mechanisms. They play crucial roles in several cellular processes, including metabolic pathways, structural integrity of cells or organelles, regulation of various cellular processes, and cellular stress response (8).

Studies have shown that moonlighting proteins affect multiple aspects of hematopoiesis and iron metabolism, which are critical in the pathogenesis of anemia (9). For instance, specific moonlighting proteins regulate hemoglobin synthesis. Proteins primarily functioning in metabolic pathways may interact with transcription factors or signaling pathways that regulate genes involved in hemoglobin production, and disruption in these pathways can lead to anemia (10). Moreover, moonlighting proteins are involved in iron metabolism, essential for red blood cell production (8). While ferritin is best known for storing iron within cells, it also plays an active role in modulating cellular signaling pathways and influencing the transcriptional activity of genes involved in iron balance. Disruption in these regulatory mechanisms can contribute to iron-deficiency anemia (11).

Moonlighting proteins extend their influence into immune regulation and inflammatory signaling, assuming functions not traditionally associated with their primary roles. Chronic inflammation can lead to anemia of chronic disease, where the body sequesters iron from pathogens, reducing its availability for red blood cell production. Proteins that modulate inflammatory responses indirectly affect the development of anemia (12). Moonlighting proteins are also directly involved in erythropoiesis (13). Proteins involved in cellular metabolism may influence the proliferation and differentiation of erythroid progenitor cells, and deficiencies in these proteins can impair red blood cell production, leading to anemia (14).

Furthermore, genetic mutations affecting moonlighting proteins can result in hereditary forms of anemia. Mutations in proteins with dual roles in metabolism and erythropoiesis disrupt normal blood cell formation (15). Compared to previous research, our study delves deeper into the intricate roles of moonlighting proteins in anemia. Prior studies have primarily focused on individual functions of these proteins in specific pathways (16). However, our research will comprehensively analyze their multifunctional roles and the interplay among them in anemia. This study draws upon core concepts from distinct yet interconnected domains—such as hemoglobin production, iron balance mechanisms, cellular development within the erythroid lineage, and patterns of genetic variation—to construct a unified analytical perspective. This synthesis aims to elucidate the complex biological factors underlying anemia in a comprehensive, structured manner (15,17). This study aims to determine interactions among candidate proteins in anemia using bioinformatics methods and to predict crosstalk pathways in the constructed networks to generate hypotheses. We propose that the expression profiles of specific candidate proteins within the anemia-associated functional network diverge significantly from those observed in physiologically normal individuals. In addition, we hypothesize that the structural configurations and functional dynamics of the regulatory proteins HAMP and EPO within their associated molecular networks exhibit notable divergence in individuals with anemia compared with those with normal physiological profiles. By addressing the gap in understanding the complex roles of moonlighting proteins in anemia, this research employs advanced bioinformatics and systems biology approaches to elucidate the interactions and pathways underpinning these proteins' multifunctionality. The outcomes of this investigation are intended to shed light on prospective molecular intervention points and support the advancement of more efficient therapeutic strategies for anemia management.

Materials and Methods

This analytical study examined expression data for moonlighting proteins in patients with anemia compared

with a control group of healthy individuals. The initial data for network construction were extracted from various bioinformatics databases, including MoonProt, NCBI, SWISSprot, and Diseasome, using samples from both anemic patients and healthy individuals. The data recorded in these bioinformatics databases serve as the gold standard, encompassing all verified protein expression data from healthy individuals and anemia patients.

To identify candidate proteins involved in anemia, a comprehensive review and database search were conducted across MoonProt, NCBI, GeneCards, Swiss-Prot, and Diseasome.

Proteins implicated in the disease were selected based on evidence from at least one of the following methods: in vivo, in vitro, or in silico studies. These proteins were then considered as candidate proteins. Expression data were collected from these bioinformatics databases, and the expression data from both groups were standardized against the control group to compare the results and test the study hypotheses.

Subsequently, an interaction network was constructed from expression data for candidate proteins and human moonlighting proteins in both the patient and control groups. The networks were plotted separately for each group in MATLAB. Structural parameters of the expression data networks were calculated and compared between the two groups. Statistically significant parameters can be proposed as potential biomarkers.

All statistical calculations in this study were performed using R and MATLAB software. Data analysis employed advanced descriptive and inferential statistical methods, as well as machine learning techniques based on advanced bioinformatics algorithms. These methods were used to compute network features for network data analysis and to identify biomarkers associated with the network's structural characteristics.

By employing these methodologies, this study aims to elucidate the complex roles of moonlighting proteins in anemia, providing insights into potential therapeutic targets and contributing to the development of effective treatments.

This analytical study aims to investigate the network relationships of proteins involved in anemia and moonlighting proteins, and to calculate essential parameters. Initially, text mining methods were employed to identify proteins implicated in anemia. The association of these proteins with anemia was confirmed using at least one of the following methods: in vivo, in vitro, and in silico.

First, the most probable genes encoding these proteins were identified using bioinformatics databases such as MoonProt, NCBI, SWISSProt, and Diseasome. The set of target proteins involved in anemia was then ranked using the Gene-Disease Association (GDA) score. The GDA score reflects the strength of the association between each gene and anemia, with higher scores indicating a stronger relationship. These scores were calculated based on multiple sources of evidence, including in vivo, in vitro, and in silico studies, as well as literature references. Formally, the GDA score is defined as:

GDA=C+M+I+LGDA = C + M + I + L

Where:

- C=0.6C= 0.6 if Nsourcesi>2N_{\text{sources}}
- (18)_i} > 2
- C=0.5C=0.5 if Nsourcesi=2N_{\text{sources}_i} = 2
- C=0.3C=0.3 if Nsourcesi=1N_{\text{sources}_i} = 1
- C=0C=0 otherwise

Here, NsourcesiN_{\text{sources}_i} represents the number of specialized sources confirming the association of the gene with the target disease (CTD, UNIPROT, PSYGENET, CGL, GENOMICS, CLINGEN, ORPHANET).

- M=0.2M=0.2 if Nsourcesi>0N_{\text{sources}_i}>0 in databases like CTD, MGD, RGD
- M=0M = 0 otherwise
- I=0.1I=0.1 if Nsourcesi>0N_{\text{sources}_i} > 0 in databases like HPO, CLINVAR, GWASCAT, GWASDB
- I=0I=0 otherwise
- L=0.1L=0.1 if Npubs>9N_{\text{pubs}} > 9
- L=Npubs×0.01L=N_{\text{pubs}} \times 0.01 if Npubs<9N_{\text{pubs}} < 9

NpubsN_{\text{pubs}} refers to the number of publications confirming the gene-disease association in databases like BEFREE, LHGDN. After calculating the GDA score, the candidate genes for anemia were ranked as shown in Table 1 (19,20).

Table 1. GDA Scores for anemia protein interaction network

Gene Name	GDA score
HBA2	1.0
HBA1	1.0
HBB	0.95
HAMP	0.95
EPO	0.90
ITPA	0.90
CSF2	0.85
SLC11A2	0.85
CSF3	0.85
TF	0.85

In the next step, some of the moonlighting proteins in human samples were extracted from the MoonProt database. These proteins are multifunctional and play diverse roles in cellular processes, making them significant in the study of diseases such as anemia. Table 2 lists representative examples of these moonlighting proteins identified in human samples, highlighting their diverse functional roles and relevance to anemia research.

Table 2. Some of the moonlighting proteins in human samples

Protein Name	Index
1-Cys Peroxiredoxin	1
Hsp60	2
Enolase	3
Lactose Synthase	4
Delta-Aminolevulinic Acid Dehydratase	5
Leukotriene A-4 Hydrolase	6
Lysyl Hydroxylase	7

Then, using the Gephi platform, we constructed the interaction network between the candidate proteins and moonlighting proteins. We determined the interaction network structure among candidate proteins and calculated network centrality measures to identify essential proteins. In this network, edge weights were determined based on the expression levels of the corresponding proteins (21).

In the constructed interaction network (Figure 1).

Each node represents a protein, and the edges denote physical or functional interactions, identified through at least one of the methods: in vivo, in vitro, or in silico studies, as well as genes computed based on the GDA criterion (Table 1).

Identification of essential nodes

In the context of interaction networks, the term "hub" describes nodes that are essential according to one of the network centrality measures. In biological networks, selecting hub nodes is crucial for identifying influential components that significantly affect the network. These hub nodes can be pivotal for understanding the network's structure and dynamics. By pinpointing these critical nodes, researchers can identify key genes or proteins (Essentials) within the network that could serve as biomarkers. These biomarkers are invaluable for diagnosing or treating diseases. By analyzing structural centrality measures, we aim to identify essential nodes in the protein interaction network, thereby providing insights that can aid in the development of effective therapeutic strategies (18,22).

Results

Structural parameters of the network

Maximum neighborhood component (MNC)

Each node, such as node aa, has several neighbors directly connected to it, denoted as N(a)N(a). The MNC score for node aa is defined as the size of the largest connected component to which node aa belongs. Based on this parameter, the highest MNC scores are attributed to the following biomarkers (Table 3).

The interaction network is visualized as follows:

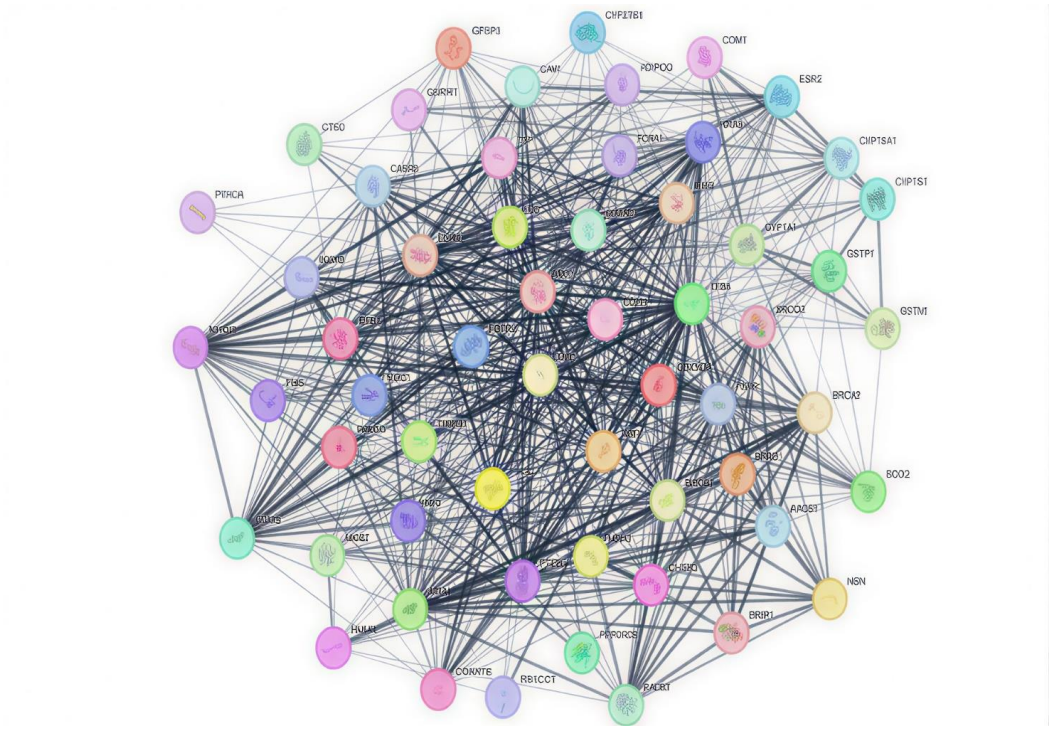

Figure 1. Interaction network of candidate proteins and moonlighting proteins in anemia. This figure illustrates the interaction network constructed between candidate proteins and moonlighting proteins, identified in patients with anemia and compared to a control group of healthy individuals. Each node represents a protein, and the edges depict physical or functional interactions, determined through in vivo, in vitro, or in silico studies. The network visualization was created using Gephi, with edge weights based on the expression levels of the corresponding proteins. Structural centrality measures were computed to identify essential nodes, which could serve as potential biomarkers for anemia diagnosis and treatment

Table 3. MNC scores for anemia protein interaction network including moonlighting proteins

Biomarker	Rank
GAPDH	1
PGK1	2
TPI1	3
EEF2	4
ACO1	5
RPS3	6
RPS13	7
RPS14	8
HSPD1	9
KARS1	10

Degree centrality

Degree centrality is defined as the number of edges connected to a node. Based on this criterion, the most influential biomarkers in the anemia protein interaction network, including moonlighting proteins, are as follows (Table 4):

The interaction network for these 10 components with the highest degree scores is visualized as "(Figure 2)

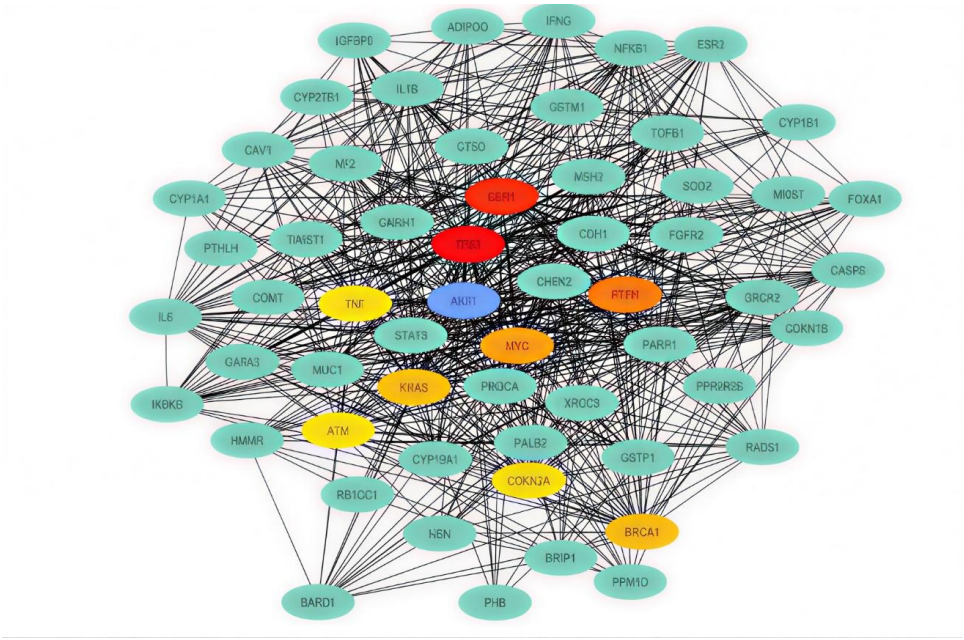

Figure 2. Interaction network of candidate proteins and moonlighting proteins in anemia

This figure illustrates the interaction network constructed between candidate proteins and moonlighting proteins, identified in patients with anemia and compared to a control group of healthy individuals. Each node represents a protein, and the edges depict physical or functional interactions, determined through in vivo, in vitro, or in silico studies. The network visualization was created using Gephi, with edge weights based on the expression levels of the corresponding proteins. Structural centrality measures were computed to identify essential nodes, which could serve as potential biomarkers for anemia diagnosis and treatment

Closeness centrality

Closeness centrality is defined as the sum of the shortest paths from a node to all other nodes in a connected network. This measure is crucial for identifying biomarkers in biological networks, as it indicates how close a protein is to all other proteins. Based on this criterion, the most significant biomarkers in the anemia protein interaction network, including moonlighting proteins, are as follows (Table 5):

The interaction network for anemia based on closeness centrality is visualized as follows (Figure 4).

Table 4. Degree scores for anemia protein interaction network including moonlighting proteins

Biomarker	Rank
GAPDH	1
EEF2	2
PGK1	3
TPI1	4
ACO1	5
RPS3	6
RPS13	7
HSPD1	8
RPS14	9
KARS1	10

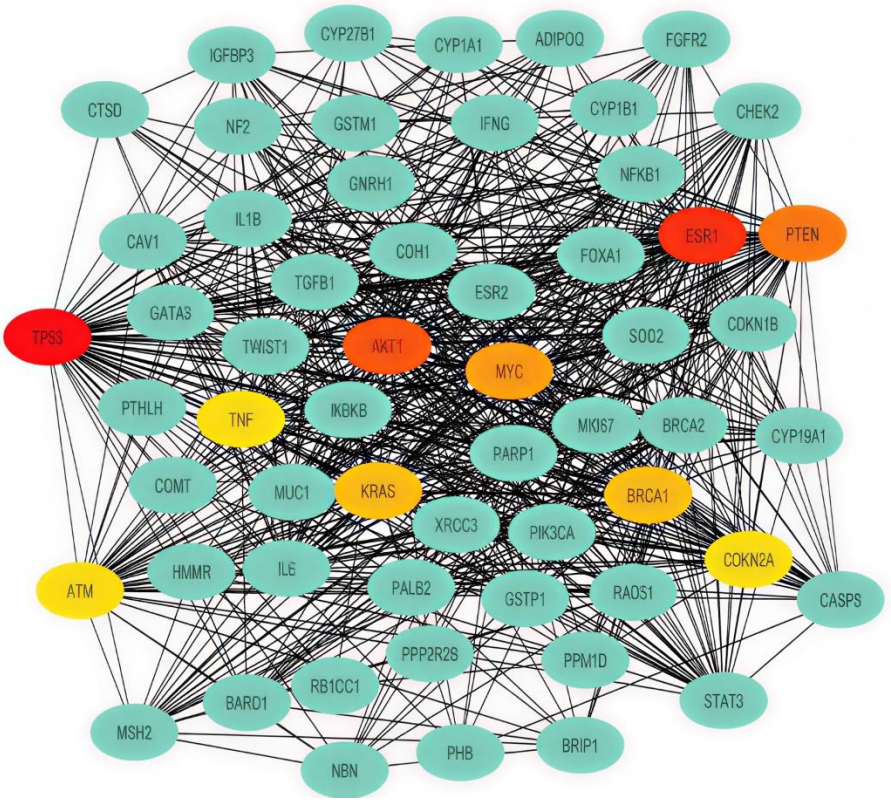

Figure 3. Interaction network of Top 10 biomarkers by Degree Centrality. This figure illustrates the network structure of the top 10 biomarkers with the highest degree scores, highlighting their central roles and interconnections within the anemia protein interaction network

Radiality

Radiality measures a node's proximity to all other nodes in its neighborhood, identifying the nodes with the shortest paths to others. Based on this criterion, the highest radiality scores for proteins in the anemia network, including moonlighting proteins, are as follows (Table 6):

The interaction network for anemia based on radiality is visualized as follows (Figure 5)

Betweenness centrality

Betweenness centrality measures the extent to which a node lies on the shortest path between other nodes in the network. Nodes with high betweenness centrality are crucial for information transfer within biological networks. The removal of these nodes could disrupt the overall network communication. Based on this criterion, the highest betweenness scores for proteins in the anemia network, including moonlighting proteins, are as follows (Table 7):

The interaction network for anemia based on betweenness centrality is visualized as follows (Figure 6):

Table 5. Closeness scores for anemia protein interaction network including moonlighting proteins

Biomarker	Rank
GAPDH	1
EEF2	2
PGK1	3
TPI1	4
RPS13	5
ACO1	6
RPS3	7
RPS14	8
HSPD1	9
YBX1	10

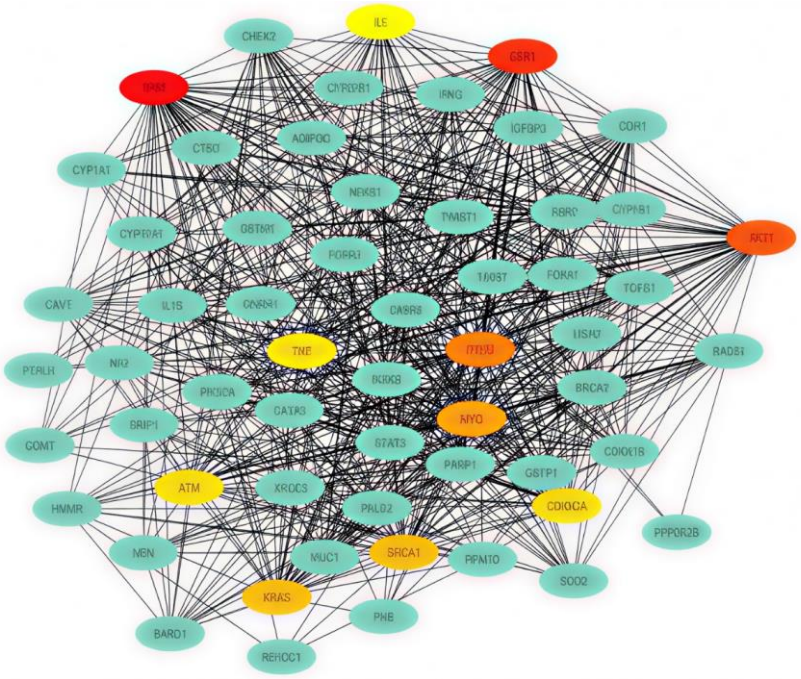

Figure 4. Anemia interaction network based on closeness centrality. This figure illustrates the network structure of the top biomarkers ranked by closeness centrality, highlighting their central roles and interconnections within the anemia protein interaction network. This analysis helps identify key proteins crucial to maintaining the network's integrity and function, making them potential targets for therapeutic intervention

Table 6. Radiality scores for anemia protein interaction network including moonlighting proteins

Biomarker	Rank
GAPDH	1
EEF2	2
YBX1	3
PGK1	4
TPI1	5
RPS13	6
MRPS7	7
RPS14	8
RPS3	9
ACO1	10

Table 7. Betweenness scores for anemia protein interaction network including moonlighting proteins

Biomarker	Rank
GAPDH	1
EEF2	2
YBX1	3
MAPK1	4
ACO1	5
ATF2	6
HSPD1	7
PARP1	8
MIRPL13	9
CSF2	10

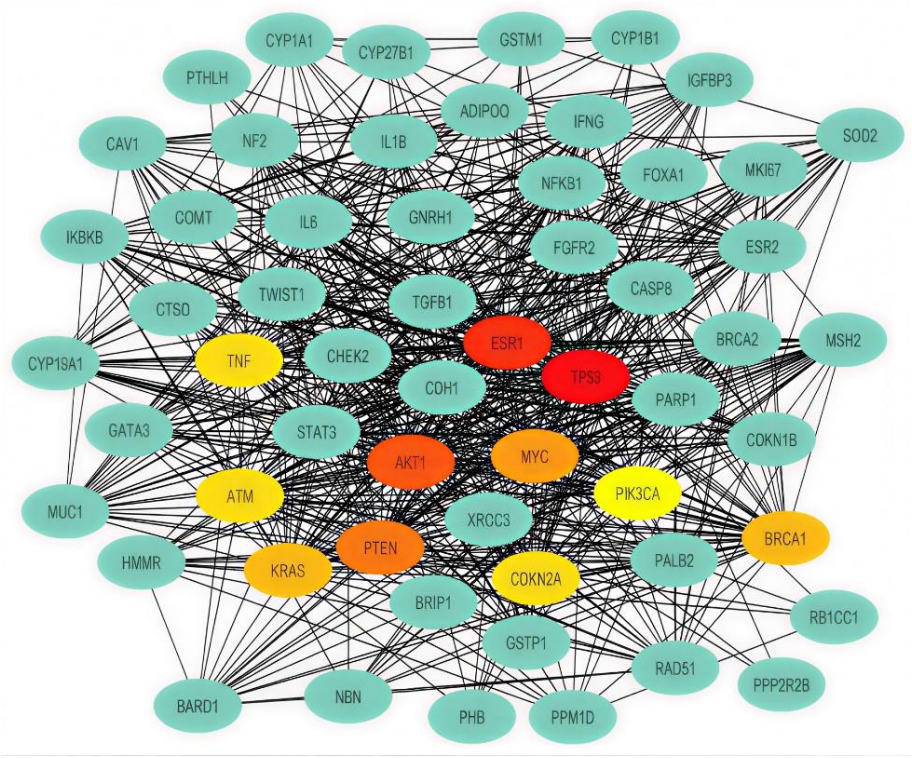

Figure 5. The interaction network for anemia based on radiality is visualized

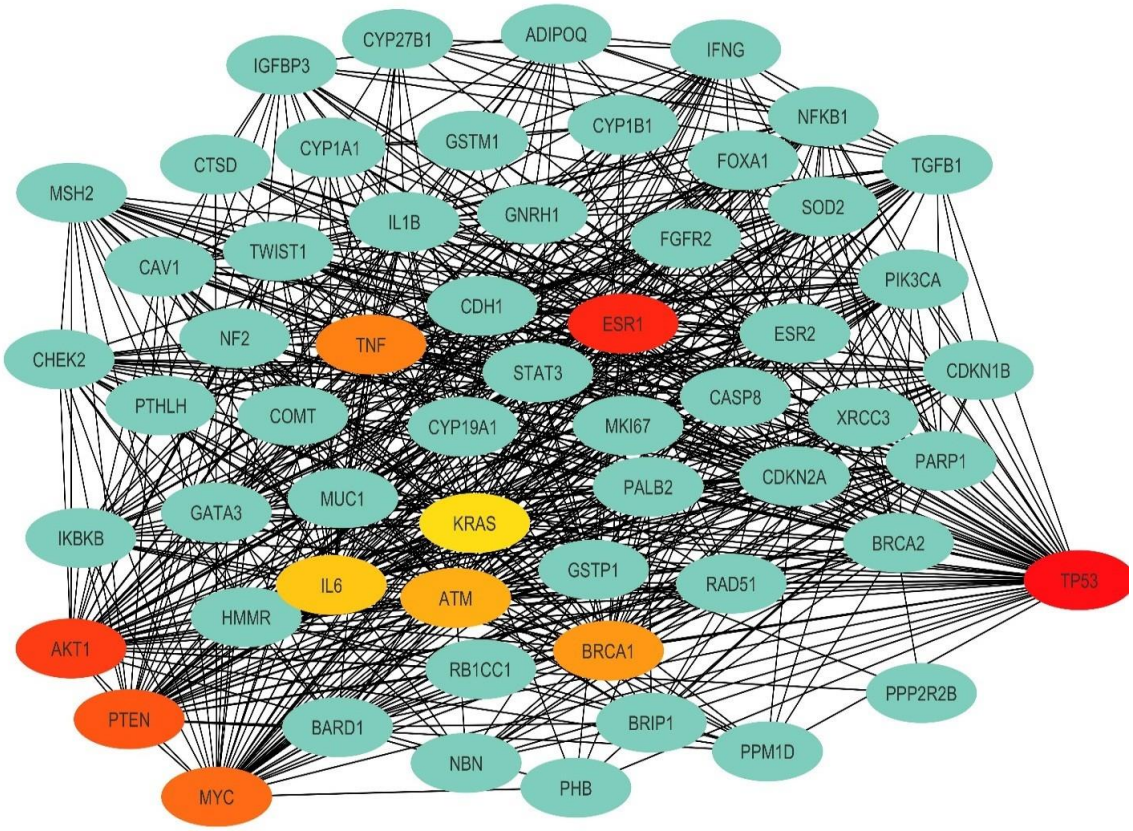

Figure 6. Anemia interaction network based on betweenness centrality. This figure highlights the network structure of the top biomarkers ranked by betweenness centrality, emphasizing their pivotal roles and interconnections within the anemia protein interaction network. This analysis is crucial for identifying key proteins that maintain the network's integrity and function, making them potential targets for therapeutic intervention

Based on network analysis of candidate proteins in anemia, using five centrality metrics (Maximum Neighborhood Component, Degree, Betweenness, Closeness, and Radiality), GAPDH, EEF2, TPI1, ACO1, and RPS13 were identified as having the highest consistency across these metrics.

- **GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)**: Located on chromosome 12p13.31, it is predominantly expressed in skeletal muscle. Diseases related to this gene include hemolytic anemia due to triosephosphate isomerase deficiency. GAPDH is involved in DNA synthesis inhibition, chemotherapy, and acts as a strong pro-apoptotic anticancer agent by activating caspase 3.
- **EEF2 (Eukaryotic translation elongation factor 2)**: Found on chromosome 19p13.3, this protein is highly expressed in lymph nodes. Related diseases include anemia, sideroblastic anemia, and spinocerebellar ataxia. EEF2 functions as a high-affinity adenosine receptor agonist.
- **TPI1 (Triosephosphate isomerase 1)**: Located on chromosome 12p13.31, TPI1 is highly expressed in blood. It is associated with hemolytic anemias and is a key component in the synthesis and maintenance of cell membranes.
- **ACO1 (Aconitase 1)**: Found on chromosome 9p21.1, this protein is predominantly expressed in blood. It is related to hemochromatosis and functions as an antioxidant and mucolytic agent.
- **RPS13 (Ribosomal protein S13):** Located on chromosome 11p15.1, it is highly expressed in blood and associated with hemolytic anemia due to triosephosphate isomerase deficiency. It functions as an antioxidant and mucolytic agent.

These proteins were identified as essential due to their high repetition and confirmation across the five centrality metrics, making them significant in the study of anemia. Their roles and mechanisms provide valuable insights into potential therapeutic targets for anemia.

Table 8. Common proposed essential proteins based on 5 bioinformatics metrics. This table presents the essential proteins identified using five centrality metrics in the bioinformatics analysis of the anemia protein interaction network. The table includes the protein names, their full names, chromosomal locations, tissues with the highest expression, associated diseases, and the mechanisms involved

Protein Name	Full Name	Chromosome	Highest Expression	Associated Diseases	Mechanism	Ref
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	12p13.31	Skeletal Muscle	Hemolytic anemia due to triosephosphate isomerase deficiency	Inhibition of DNA synthesis, chemotherapy, strong pro-apoptotic anticancer agent; activates caspase 3	(23)
EEF2	Eukaryotic Translation Elongation Factor 2	19p13.3	Lymph Nodes	Anemia, sideroblastic anemia, spinocerebellar ataxia	High-affinity adenosine receptor agonist	(24,25)
TPI1	Triosephosphate Isomerase 1	12p13.31	Blood	Hemolytic anemias	Key component in the synthesis and maintenance of cell membranes	(26,27)
ACO1	Aconitase 1	9p21.1	Blood	Hemochromatosis	Antioxidant; mucolytic agent	(28,29)
RPS13	Ribosomal Protein S13	11p15.1	Blood	Hemolytic anemia due to triosephosphate isomerase deficiency	Antioxidant; mucolytic agent	(30,31)

Discussion

Anemia is characterized by a reduction in the number or quality of red blood cells, leading to decreased oxygen transport in the body. Leveraging computational methodologies, this study advances the molecular dissection of anemia by uncovering novel regulatory patterns and diagnostic indicators. Integrating diverse biological datasets enables a refined exploration of anemia's molecular determinants, fostering the development of tailored therapeutic frameworks grounded in precision medicine. Bioinformatics uses computational tools to analyze biological data, aiding the study of complex diseases such as anemia (32,33). The convergence of multi-omic datasets enables a more refined dissection of anemia's molecular etiology, laying the groundwork for bespoke therapeutic strategies aligned with the principles of precision medicine. This precision-driven approach informs individualized treatment strategies by aligning molecular diagnostics

with targeted therapeutic interventions.

Additionally, analyzing protein expression profiles in anemic patients can reveal biomarkers indicating disease severity or response to treatment (34). Bioinformatics integrates proteomic data with clinical outcomes, enhancing diagnostic accuracy. Furthermore, it enables researchers to explore metabolic and signaling pathways involved in erythropoiesis (red blood cell production), thereby identifying new therapeutic targets for treating anemia (35).

The investigation focused on the transcriptional landscapes of anemia-associated proteins, underscoring the critical function of bioinformatic methodologies in their systematic identification and molecular characterization. Using resources like the GeneCards database, this research integrates genomic, proteomic, and clinical data to provide a comprehensive understanding of anemia's molecular mechanisms. This study employs computational analysis to investigate molecular determinants of anemia, demonstrating how bioinformatics enables the systematic identification and functional annotation of relevant proteins. By harmonizing multidimensional biological datasets, this framework deepens mechanistic comprehension of anemia and fosters the emergence of individualized therapeutic models rooted in precision medicine.

GAPDH

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), classically recognized for its catalytic function in the glycolytic conversion of glyceraldehyde-3-phosphate through oxidative phosphorylation, was identified in this study as a key integrative component within the anemia-associated protein interaction network. Its centrality implies a potentially expansive role in the molecular orchestration of iron-related pathophysiological processes.

GAPDH interacts with the iron regulatory protein IRP2, impacting iron metabolism, which is crucial because iron deficiency is a common cause of anemia (36,37). This molecular association may exert regulatory control over the transcription of genes involved in iron assimilation, intracellular depot formation, and metabolic deployment, potentially shaping the pathophysiology of iron-deficiency anemia. GAPDH has also been implicated in the modulation of fetal hemoglobin (HbF) synthesis, a process of clinical relevance given HbF's capacity to attenuate the pathological effects associated with sickle cell disease. Modulating GAPDH activity could thus have therapeutic potential in managing sickle cell disease. The existing literature reinforces the present observations by demonstrating GAPDH's involvement in diverse regulatory pathways, thereby supporting its multifaceted role in cellular control mechanisms.

In our analysis, GAPDH emerged as a significant biomarker based on its high centrality metrics, suggesting an essential role in the network. Its high expression in skeletal muscle and its association with hemolytic anemia underscore its importance. While GAPDH's primary role lies in glycolysis, emerging evidence suggests broader regulatory roles in anemia (38). This research expands the functional profile of GAPDH, highlighting its relevance beyond traditional roles and proposing it as a viable molecular target for therapeutic intervention. The insights gained offer a refined understanding of GAPDH's involvement in anemic pathology and inform the development of more effective treatment modalities.

EEF2

Eukaryotic Translation Elongation Factor 2 (EEF2) plays a critical role in protein synthesis by facilitating the translocation of tRNA and mRNA during translation. Through integrative network analysis, EEF2 emerged as a pivotal node within the anemia-associated protein interaction landscape, as determined by multiple centrality metrics. This study harnesses computational genomics to pinpoint genetic disruptions linked to distinct anemic conditions, such as sickle cell disease and thalassemia. By decoding these molecular aberrations, the approach enables phenotype-specific stratification and informs the design of tailored therapeutic regimens within a precision medicine framework.

Recent studies have begun exploring the potential link between EEF2 and various forms of anemia, driven by EEF2K regulation. In response to metabolic stress, eukaryotic elongation factor 2 kinase (EEF2K) becomes activated and phosphorylates EEF2, disrupting its role in ribosomal translocation during the elongation phase of translation. This regulatory mechanism imposes a translational checkpoint that limits protein synthesis, conserving cellular energy and potentially impairing red blood cell production under stress-induced hematopoietic conditions. This translational attenuation may impair erythropoietic output by limiting the biosynthetic capacity required for red blood cell production, particularly during stress-induced hematopoietic responses. Given that erythropoiesis is a highly energy-dependent process, disruption of translation by EEF2K activation can contribute to anemia

Additionally, EEF2K has a role in regulating immune responses. Inflammatory conditions can lead to anemia of chronic disease (ACD), where red blood cell production

is inhibited. Modulating EEF2K activity may affect the inflammatory response, influencing the development of ACD.

Our findings align with previous research, emphasizing EEF2's importance in protein synthesis and its regulation under stress. The identification of EEF2 as a key node in the anemia protein network highlights its potential as a therapeutic target. Understanding the effects of EEF2K inhibitors, such as A484954, on EEF2 activity could provide insights into their potential impact on anemia, especially in patients with comorbid cardiovascular issues. These findings enhance our understanding of EEF2's role in anemia and suggest new avenues for therapeutic intervention.

TPI1

Triosephosphate Isomerase 1 (TPI1) is an essential enzyme in the glycolytic pathway, responsible for the interconversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Our study identified TPI1 as a significant protein in the anemia protein interaction network, highlighting its role based on multiple centrality metrics. This finding underscores TPI1's critical function in red blood cell metabolism and its potential impact on anemia (39).

TPI1 deficiency is primarily associated with congenital hemolytic anemia. This condition arises from reduced enzyme activity, leading to the accumulation of dihydroxyacetone phosphate (DHAP) and subsequent metabolic disturbances in red blood cells (39). Impaired glycolytic function reduces energy production, which is crucial for maintaining red blood cell integrity and lifespan. Consequently, patients with TPI1 deficiency experience hemolysis and reduced red blood cell counts, contributing to anemia (40).

Metabolic stress activates EEF2K, leading to EEF2 phosphorylation and disruption of its role in ribosomal translocation, thereby dampening protein synthesis. This post-translational modification impairs its role in coordinating ribosomal movement during peptide chain elongation, thereby imposing a translational restraint that conserves cellular energy and may disrupt erythroid biosynthesis under stress-adaptive conditions. This stress-responsive modulation of translational machinery imposes a biosynthetic constraint that may compromise erythroid lineage maturation and red blood cell output. The severity of the disease can lead to significant complications, and most affected individuals do not survive beyond early childhood without intervention (41). Our network-based analysis identified TPI1 as a key regulatory node within the anemia-associated protein interaction framework, reinforcing its established role in sustaining erythrocyte integrity and its pathogenic relevance to hemolytic anemia. The Glu104Asp variant, the most prevalent mutation linked to TPI1 deficiency, accounts for the majority of documented cases and is strongly associated with severe clinical outcomes. Other mutations like E105D have also been identified, contributing to the phenotypic diversity observed in patients. Diagnostic evaluation of TPI1 deficiency relies on a combination of molecular and biochemical approaches, particularly in individuals exhibiting unexplained hemolytic anemia and neurological impairments. Comprehensive diagnosis of TPI1 deficiency integrates molecular screening for pathogenic variants with functional assays quantifying enzymatic activity in red blood cells. This integrative diagnostic strategy enhances clinical accuracy by linking genotypic alterations with measurable enzymatic deficits in erythrocytes. Elevated concentrations of dihydroxyacetone phosphate (DHAP) serve as an additional biochemical hallmark. As no curative therapy currently exists, clinical management centers on supportive interventions, including transfusion-based correction of anemia and treatment of secondary complications, such as recurrent infections. Our findings contribute to this understanding, highlighting the need to recognize TPI1's role in anemia for better diagnosis and management (42).

ACO1

ACO1 encodes a bifunctional cytosolic protein that operates as both a metabolic enzyme within the tricarboxylic acid (TCA) cycle and a post-transcriptional regulator of iron homeostasis. Under iron-replete conditions, ACO1 incorporates a [4Fe-4S] cluster and functions enzymatically as aconitase, catalyzing the isomerization of citrate to isocitrate. Conversely, in iron-deficient states, the protein adopts an RNA-binding conformation, interacting with iron-responsive elements (IREs) on transcripts encoding ferritin and the transferrin receptor to modulate iron uptake and storage. Network analysis from our study positioned ACO1 as a central node within the anemia-associated protein interaction landscape, underscoring its pivotal role in coordinating iron metabolism with erythropoietic demand (43,44). ACO1 is pivotal in maintaining iron homeostasis, and mutations or dysregulation can lead to iron deficiency anemia. It also plays a role in sideroblastic anemia, characterized by ringed sideroblasts in bone marrow due to disruptions in iron metabolism, leading to ineffective erythropoiesis.

Furthermore, ACO1 is involved in anemia of chronic disease (ACD), often associated with inflammatory conditions. Inflammatory cytokines can modulate ACO1 activity, altering iron distribution and contributing to ACD development (45). Our findings support previous research, which has established ACO1's dual role as both a metabolic enzyme and an iron regulator. Understanding ACO1's function highlights its potential as a therapeutic target. Modulating ACO1 activity could improve iron availability for red blood cell production, offering new avenues for treating various forms of anemia (46).

RPS13

RPS13, or Ribosomal Protein S13, is a component of the ribosome essential for protein synthesis in cells. Although the direct relationship between RPS13 and anemia is not well documented, understanding the broader context of ribosomal proteins and their roles in cellular function can provide insights into potential connections. Ribosomal proteins, such as RPS13, play indispensable roles in erythropoiesis by supporting the translational demands of proliferating erythroid precursors. As integral components of the ribosomal machinery, they ensure efficient protein synthesis, which is required for hemoglobin production and cellular maturation during red blood cell development. Disruptions in ribosomal protein function can lead to various forms of anemia, particularly those associated with ineffective erythropoiesis (47).

Conditions such as Diamond-Blackfan anemia (DBA) are linked to mutations in ribosomal protein genes, leading to reduced red blood cell production (48). Although RPS13 is not directly involved in DBA, its role in ribosome assembly and function suggests that abnormalities in ribosomal proteins can contribute to anemia. Ribosomal proteins also play a role in the cellular response to erythropoietin, a hormone that stimulates red blood cell production. Compromise in ribosomal architecture can attenuate cellular responsiveness to erythropoietin, thereby disrupting the proliferation and differentiation of erythroid progenitors. This translational inefficiency may culminate in suboptimal red blood cell formation and contribute to an anemic phenotype (49).

Our study identified RPS13 as a significant protein in the anemia protein interaction network, based on multiple centrality metrics, emphasizing its role in cellular processes. While specific studies directly linking RPS13 to anemia are limited, several indirect connections can be considered. Anemia, especially in conditions like sickle cell disease, is often exacerbated by oxidative stress and inflammation (50). RPS13 may influence the stress-adaptive behavior of erythroid progenitors by modulating ribosomal performance under adverse physiological conditions. In parallel, folate plays a central role in nucleotide biosynthesis and cell cycle fidelity; its insufficiency disrupts DNA replication, impairs erythroblast proliferation, and culminates in megaloblastic anemia. Although RPS13 does not directly interact with folate metabolism, any ribosomal dysfunction can impact the synthesis of proteins involved in folate utilization, thereby affecting erythropoiesis (51).

This study pinpointed GAPDH, EEF2, TPI1, ACO1, and RPS13 as pivotal molecular nodes within the protein interaction landscape of anemia, each exerting specialized roles in regulating red blood cell formation and maintaining iron balance. By integrating multi-source genomic and proteomic data, we revealed how these proteins influence red blood cell formation, stress-adaptive translation, and iron-responsive signaling. Their network centrality and biological relevance suggest strong potential as clinical biomarkers for anemia subtyping and for monitoring anemia progression. Moreover, these findings provide a foundation for developing targeted therapies, such as small-molecule modulators or gene-based interventions, that could restore erythroid function or correct metabolic imbalances. The study’s systems-level approach bridges molecular insight with translational utility, paving the way for precision diagnostics and personalized treatment strategies in anemia management.

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