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Factor VIII as a Novel Biomarker for Diagnosis, Prognosis, and Therapy Prediction in Human Cancer and Other Disorders

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Abstract

Coagulation factor VIII (FVIII) is an essential cofactor in the coagulation cascade, encoded by the *F8* gene on the long arm of chromosome X (Xq28). FVIII is normally circulated in complex with Von Willebrand factor (VWF) and has relevant emerging extracoagulative functions. Dysregulation of FVIII is associated with tumor progression, and could be used as a novel biomarker for tumor screening and monitoring. In breast cancer, bladder cancer, colorectal carcinoma, esophageal carcinoma, hepatocellular carcinoma and lung cancer, *F8* is regarded as an oncogene. In coronary heart disease, hemophilia A and liver disease, *F8* dysregulation has been recognized as a potential biomarker for disease diagnosis and prognosis. However, the basis of these differential expression levels remains to be understood. In this review, which is a mixture of literature review and bioinformatics analysis we described the biological functions and characteristics of FVIII, and also its expression level in non-malignant disorders and various cancers.

Keywords: Biomarkers, Cancer, Factor VIII, Prognosis
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Introduction

Coagulation factor VIII (FVIII), a significant multidomain glycoprotein component of the intrinsic coagulation pathway, consists of six domains: heavy chain (A1-A2) and light chain (A3-C1-C2) [1]. The coagulation FVIII is encoded by the *F8* gene on the long arm of chromosome X (Xq28). *F8* gene consists of 26 exons and is approximately 186 *Kb* [2,3]. The 3D structure of FVIII is constructed by the SWISS PDB viewer (https://www.rcsb.org/3d-view/3CDZ/1), and is shown in figure 1A. There are several proteins that interact with FVIII. The protein-protein interaction of FVIII is analyzed by STRING (https://string-db.org), and is represented in figure 1B. Mounting evidence states that there are over 3000 mutations in *F8* gene, including small deletions, insertions, large deletions, nonsense, and mis-sense (substitutions). Additionally, intron 1 and 22 inversions make up approximately 50% of total mutations [4,5].

FVIII initially binds to von Willebrand factor (vWf), which is a serine protease in plasma that preserves FVIII from degradation and clearance by Dendritic Cells (DCs). In response to injury, FVIII is cleaved by thrombin and forms activated FVIII (FVIIIa) which is then separated from vWf and joins the activated platelets *via* C1-C2 domains. FVIIIa binds to activated FIX (FIXa), another coagulation factor to generate intrinsic tenase complex. This complex then leads to the formation of activated factor X (fXa) and also thrombin. Free FVIII is an unstable molecule with a labile structure with a half-life of about two hours. Generation of the FVIII-vWF improves the FVIII half-life time in the blood circulation [6]. FVIII and VWF production are not localized in the same tissues. While sites of VWF production have been discovered across the vascular endothelium, especially in the lung and brain, FVIII is largely generated in the liver, specifically in sinusoidal endothelial cells with almost different expressions [7-10]. In addition, the expression of FVIII is different in various tissues. Overall, the biological functions, and also expression changes of FVIII remain unknown during decades.

Several investigations have reported high levels of circulating FVIII in cancer patients [11-13]. Notably, FVIII is independently associated with increased risk

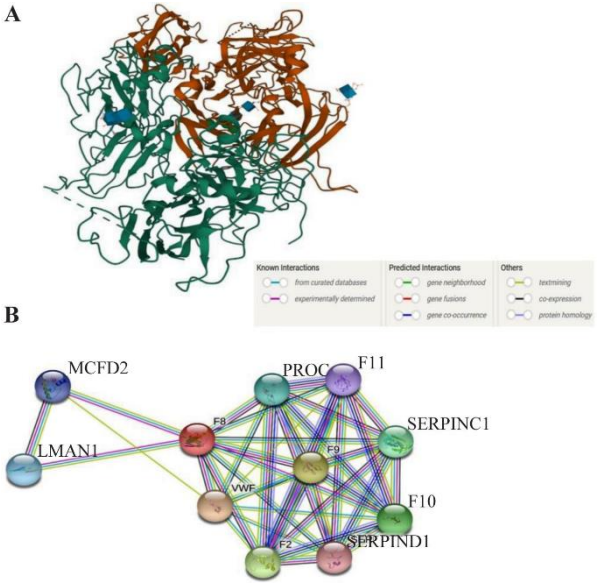

Figure 1. A) The 3D structure of FVIII protein. The red color represents the coagulation FVIII light chain, and the green represents the coagulation FVIII heavy chain. The 3D structure was constructed by the SWISS PDB viewer (https://www.rcsb.org/3d-view/3CDZ/1). B) The protein-protein interaction with FVIII. The data were analyzed by STRING (https://string-db.org). VWF: Von Willebrand factor; F9: Coagulation factor IX; F2: Prothrombin; F10: Coagulation factor X; SERPINC1: Antithrombin-III; PROC: Vitamin K-dependent protein C; LMAN1: Lectin, mannose binding 1; MCFD2: Multiple coagulation factor deficiency protein 2; F11: Coagulation factor XI; SERPIND1: Heparin cofactor 2.

of venous thromboembolism in these patients. In evaluating the potential causes of increased circulating FVIII levels in cancer patients, the following parameters should be considered: the physiological and cancer-related sites of FVIII production, and the body's response to cancer. The latter has several shared components with wound healing and inflammatory proteins [11-13].

In this review, we have mainly focused on the differential expression of FVIII in human disorders, especially cancer, to improve our understanding of its biological functions, and importance in disorders. Also, the FVIII potential to be identified as a novel diagnostic, prognostic, and therapeutic biomarker has been clarified.

The Role of FVIII in Human Diseases (Table 1)

Non-Malignant Diseases

Coronary Heart Disease: In multiple prospective researches, the function of FVIII as a risk factor for incident Coronary Heart Disease (CHD) and stroke has been hypothesized. However, it is still unknown if the FVIII connection is independent of the other Cardiovascular Disease (CVD) risk factors [14-18]. FVIII is significantly linked to risk factors associated with atherosclerosis, including age, high Body Mass Index (BMI), diabetes, and inflammatory mediators [19].

In an experiment performed by Raffield *et al* [20], 3493 African-American patients were studied in the Jackson Heart Study. According to this study, compared to other subclinical outcomes, elevated FVIII was substantially linked to Left Ventricular Hypertrophy (LVH) ($p=0.01$). In Cox models that were moderately modified for gender and age, sustained FVIII level was substantially linked with general hard CHD, Heart Failure (HF), and death, in contrast to stroke. After adjusting for conventional CVD risk factors, such as CRP, these relationships were thus diminished. Raffield *et al* observed a model adjusted for CVD risk factors, such as CRP. The results showed that the p values for the correlation with HF and death were significant ($p<0.05$); while the correlation with hard CHD was marginally significant ($p=0.05$). Numerous other multiracial investigations have produced results indicating that FVIII is more strongly correlated with mortality than incident CVD [14,21].

Hemophilia A: Hemophilia A (HA) is an inherited X chromosome-linked hemorrhagic condition that either results in the down expression of the *FVIII* gene or the synthesis of an abnormal FVIII protein [22,23].

Jankowska *et al* [24] studied the HEK-293T cell line using an MS2-TRAP assay. In order to conduct this experiment, they transfected HEK-293T cells with either MS2 alone (negative control) or MS2 fused to the FVIII. The RNA samples were isolated from cells transfected with MS2 alone and MS2 fused with FVIII. The second batch of cells had a 5-fold increase in miR readings overall. Of the 64 miRNAs that Jankowska *et al* [25] found to be linked to the FVIII mRNA, 22 likewise expressed at higher levels (higher than 2-fold) in HA patients compared to healthy controls, and 8 were observed at lower levels (less than 0.5-fold) in HA patients, particularly miRNA-19b-3p and miR-186-5p. These two miRNAs were also detected in the experimental pull-down assay with the highest read count. One explanation for high FVIII levels is the downregulation of the miRNAs that typically suppress the *FVIII* gene. Moreover, upregulation of the so-called miRNAs can reduce the expression of FVIII, leading to severe HA [26-29].

In another study by Sarachana *et al* [30], 15 HA patients were examined. They extracted the total RNA from the whole blood sample collected from HA patients and the healthy control group. Then they carried out an analysis of non-coding RNA (ncRNA) expression profiling, observing that numerous ncRNAs are dysregulated in HA patients. The hsa-miR-1246 was found to be the most significantly dysregulated miR. Sarachana *et al* also found a hsa-miR-1246 binding site on the *FVIII* gene, suggesting that it can regulate the *FVIII* gene expression. Their experiment showed that overexpression of has-miR-1246 leads to suppression of *FVIII*, although the effect of this ncRNA on the clinical manifestations of HA varies in different patients. Moreover, some of the other overexpressed miRNAs

Table 1. The expression changes and roles of FVIII in human diseases

Category	Disease type	Expression	Samples	Cell lines	Animals	Function	Ref
Non-malignant diseases							
	Coronary Heart Disease (CHD)	Up	3,493 African/ Americans involved in Jackson Heart Study (JHS)	-	-	FVIII is associated with incidence of heart failure	(20)
		Down	-	HEK-293T cells	-	miR-19b-3p and miR-186-5p downregulates F8 gene and leads to FVIII deficiency and severe bleeding in HA	(24)
	Hemophilia A (HA)	Down	15 HA patients	-	-	miR-1246 suppresses F8 gene that leads to HA pathobiology	(30)
		Up (in HKB11 compared with other cell lines)	-	HKB11	-	Because HKB11 demonstrates elevated FVIII levels compared with other cell lines, it considers as an efficient cell line for FVIII expression in HA	(32)
	Liver disease	Up	19 patients with liver disease	-	-	FVIII plasma levels in liver cirrhosis are increased, while its mRNA levels are decreased due to over growing larger vessels and over producing FVIII protein	(33)
Cancers							
	Breast Cancer (BC)	Up	235 stage I–IIA BC patients	-	-	FVIII is associated with poor survival rate and is a biomarker for coagulation activation in BC	(38)
	Bladder Cancer (BLC)	Up	-	ECV-304	-	FVIII is a risk factor for independent vascular thromboembolism in BLC	(41)
	Triple-negative Breast cancer	Up	360 invasive ductal BC patients	-	-	FVIII is significantly associated with overall survival, tumor recurrence, and metastatic nodes	(42)
	Colorectal Carcinoma (CRC)	Up	79 CRC patients	-	-	FVIII is a potential biomarker for CRC and its different stage identification, but not tumor metastasis	(43)
	Esophageal Carcinoma (EC)	Up	50 EC patients	-	-	FVIII increased level is a risk factor for venous thromboembolism in EC following chemotherapy	(44)
	Hepatocellular Carcinoma (HCC)	Up	-	Hepal-6, HepG2, HUVEC	Male C57BL/6J mice	Dihydrodiosgenin regulate HCC cells proliferation via suppressing FVIII level	(45)
	Lung Cancer (LC)	Up	115 LC patients and 98 controls	-	-	Elevated FVIII levels facilitate the thrombotic events, metastasis and invasion of LC cells	(51)

seen in HA patients include hsa-miR-374b-3p, hsa-miR-5581-3p, hsa-miR-6803-3p, hsa-miR-30c-3p, and hsa-miR-542-3p. Mutations in the 3′UTR of *FVIII* alter the process of splicing and expression of mRNAs, resulting in downregulation of *FVIII*, which can cause HA [31].

Additionally, Mei *et al* [32] experimented on several cell lines to identify the most efficient for the *FVIII* gene expression. The results showed that the FVIII levels in the HKB11 cells were significantly higher than the other cell lines, both intracellular and on the cell surface. Mei *et al* also investigated the HKB11 cells to discover the mechanism of which the *FVIII* expression is regulated. HKB11 cells were a hybrid of human embryonic renal cells and human B lymphocytes, suggesting that the overexpression of *FVIII* gene in these cells is due to HKB11 cell inheriting the embryonic characteristics of kidney in transcription and translation of FVIII mRNA, and also the characteristics of B cells in the process of protein secretion.

Liver disease: Liver disorders are linked to significantly high levels of FVIII, while several other proteins and coagulative factors are decreased. Although, the pathological process of FVIII elevation is still unclear [33,34]. In a study by Hollestelle *et al* [33], 19 patients with liver disease were studied. The FVIII level was increased in 13 out of 19 patients. Also, individuals with hepatic cirrhosis had significantly lower levels of FVIII mRNA than control patients (p=0.01). Therefore, it would seem that rising levels of FVIII did not correspond to rising *FVIII* gene transcription; instead, it

appears that there was an inverse correlation observed, but it was not significant. Additionally, there were no variations in the cellular distribution pattern of FVIII between patients. Nonetheless, they observed that bigger vessels in cirrhotic tissue appeared to overgrow sinusoidal endothelial cells that generate FVIII. This could clarify why FVIII mRNA expression was lower in the cirrhotic tissues than in non-cirrhotic. Raffield *et al* also evaluated at the expression of VWF at the mRNA and protein levels to gain understanding into the seemingly different cellular expression of *FVIII* gene and plasma FVIII levels. It is widely known that VWF plays a key role in controlling plasma FVIII concentrations. FVIII is protected by VWF from premature clearance and proteolytic degradation. Therefore, according to the increase in VWF in liver disease, it is obvious that overexpression of VWF leads to an elevation in FVIII levels [35-37].

Cancers

Breast cancer: Several previous studies have highlighted the prognostic effects of coagulative factors in Breast Cancer (BC) treatment outcomes. Mandoj *et al* [38] conducted a study on 235 patients with BC staged I to IIA. The serum level of coagulation activation factors including FVIII were measured before therapeutic procedures. The levels of FVIII in patients with early stages of BC were considerably elevated. Mandoj *et al* found that high levels of FVIII are correlated with intermediate mortality risk [38].

Bladder cancer: It has been demonstrated that cancer-related thrombosis with high risks of Venous Thromboembolism (VTE) happens in Bladder Cancer (BLC) [39,40]. Although the mechanism is still somewhat unclear, FVIII is believed to be an independent risk factor for VTE in several cancers [20-22]. Walker *et al* [41] performed an experiment on the cell line ECV-340. They found that in 7 out of 9 urothelial carcinomas the FVIII levels were elevated in the cancer cells. The highest expression of the *FVIII* gene was detected in invasive BLC cells. Also, the *FVIII* gene was found to be overexpressed in the cancer cells compared to the healthy adjacent tissue. Walker *et al* chose the BLC cell lines for the experiment because of the high VTE incidence rate in the BLC cells [39,40].

Triple-negative breast cancer: Gujam *et al* [42] studied 360 patients with invasive ductal BC to determine the prognostic value of Lymphovascular Invasion (LVI) and Blood Vessel Invasion (BVI) in node-negative and triple-negative BC. The experiment showed that both lymphatic and blood vessels were persistently positive for FVIII. The results suggested FVIII as a predictive factor for tumor recurrence, cancer-specific survival rate, and metastatic node formation.

Colorectal carcinoma: Schellerer *et al* [43] studied 79 patients with Colorectal Cancer (CRC). Following measuring the plasma levels of FVIII, they found an elevation in the FVIII level in CRC cells compared to healthy cells. Schellerer *et al* detected a significant elevation in the FVIII levels in the cells with stage II-IV cancer, while cells with stage I cancer showed FVIII levels in the normal range. Their results showed that staging and differentiation of the tumor have significant impacts on the FVIII levels. But there was no connection between FVIII levels and the occurrence of metastases.

Esophageal carcinoma: Byrne *et al* [44] performed a study on 50 patients with Esophageal Cancer (EC) after the chemoradiotherapy cycles. Measurement of FVIII levels showed a significant elevation in FVIII levels following the chemoradiotherapy sessions. Additionally, increased FVIII levels might cause primary or recurrent VTE. Although the exact pathogenetic process of this elevation is still unclear.

Hepatocellular carcinoma: Zhuang *et al* [45] conducted an experiment on the human cell lines Hepal-6, HepG2, and HUVEC, and the mice cell line C57BL/6J to determine whether the dydio controlling coagulative biomarkers plays a role in the metastasis of Hepatocellular Carcinoma Cells (HCC). Dihydrodiosgenin, a parent aglycone of diosgenyl saponin, commonly known as dydio, has anticancer, anti-inflammatory, and antithrombotic mechanisms [46-48]. Diosgenin derivatives have been shown in prior investigations to considerably lower FVIII levels in animal models [49,50]. Moreover, liver diseases such as HCC are correlated with high levels of FVIII [33]. Accordingly, it appears that dydio could control the progress of HCC by regulating the level of FVIII. Zhuang *et al* demonstrated that dydio decreased HCC metastasis by preventing platelet activation and lowering FVIII levels [45].

Lung cancer: In a survey by Liu *et al* [51], 115 patients with Lung Carcinoma (LC) were studied. Blood samples were collected for measuring the plasma levels of coagulative factors. The results showed a significant elevation in FVIII levels in LC patients compared to healthy individuals regardless of existent metastasis. These findings are in accordance with prior research that claimed patients with metastatic diseases had a higher propensity to develop coagulative and fibrinolytic disorders [52-54]. Even though the biological relevance of hemostatic disorders in cancer is yet unknown, findings demonstrate that activation of the coagulative-fibrinolytic pathway by cancer cells may enhance invasiveness and metastases [55]. Additionally, higher coagulative biomarker levels have been linked to a poor prognosis in LC and failure to treatment [56]. Table 1 represents the expression changes and roles of FVIII in human non-malignant and malignant disorders.

Database analyses

GEPIA2: GEPIA2 (Gene Expression Profiling Interactive Analysis 2) database, analyzes the data of RNA

sequencing expression for approximately nine thousand normal and tumor samples from the projects GTEx and TCGA with the help of standard processing pipelines [57]. We examined the connection between the overall prognosis and the F8 expression levels in a variety of cancer patients. We also used this database to investigate the link between the expression of *FVIII* and immunological responses and tumor markers in particular. Using GEPIA2 databases, the expression of *F8* was significantly increased in normal controls than in tumor tissues in Bladder Urothelial Carcinoma (BLCA), Breast invasive Carcinoma (BRCA), Colon Adenocarcinoma (COAD), Kidney Chromophobe (KICH), Lung Squamous Cell Carcinoma (LUSC), Ovarian serous cystadenocarcinoma (OV), Rectum Adenocarcinoma (READ), Testicular Germ Cell Tumors (TGCT), Uterine Corpus Endometrial Carcinoma (UCEC), and Uterine Carcinosarcoma (UCS). In contrast, the expression of *F8* was significantly decreased in normal control than in tumor tissues in Thymoma (THYM). Figure 2A represents the expression of *F8* in different cancers based on GEPIA2 database.

In addition, GEPIA2 is a web tool for investigating the impact of genes on survival in different cancer types. A log-rank p-value <0.05 is considered statistically significant. As shown in figure 3, the survival analysis by the GEPIA2 database indicated that over-expression or down- regulation of *FVIII* was not associated with overall survival in lung, breast or BLCs.

TIMER: The TIMER database [58] was also used to compare the F8 expression in normal and tumor tissues, and the results are represented in figure 2B. Besides, we investigated the infiltration of immune cells in several tumors with the help of TIMER database. We included the data on immune infiltration in cancers, which were derived by statistical techniques and verified by pathological tests. We investigated the link between the expression of *FVIII* and the intensity of infiltration using particular immune cell subsets and various immune invasion assessment algorithms. Lastly, correlations between the expression of *FVIII* and the immune cells, including CD8+ T cells, macrophages, neutrophils, and Natural Killer (NK) cells were examined, and figure 4 represents the results. For instance, in BRCA, *F8* had no significant correlation with macrophages and CD8+ T cells and a positive correlation with neutrophils, and NK cells, based on the MCP-counter algorithm.

cBioPortal: The cBioPortal database provides nu-

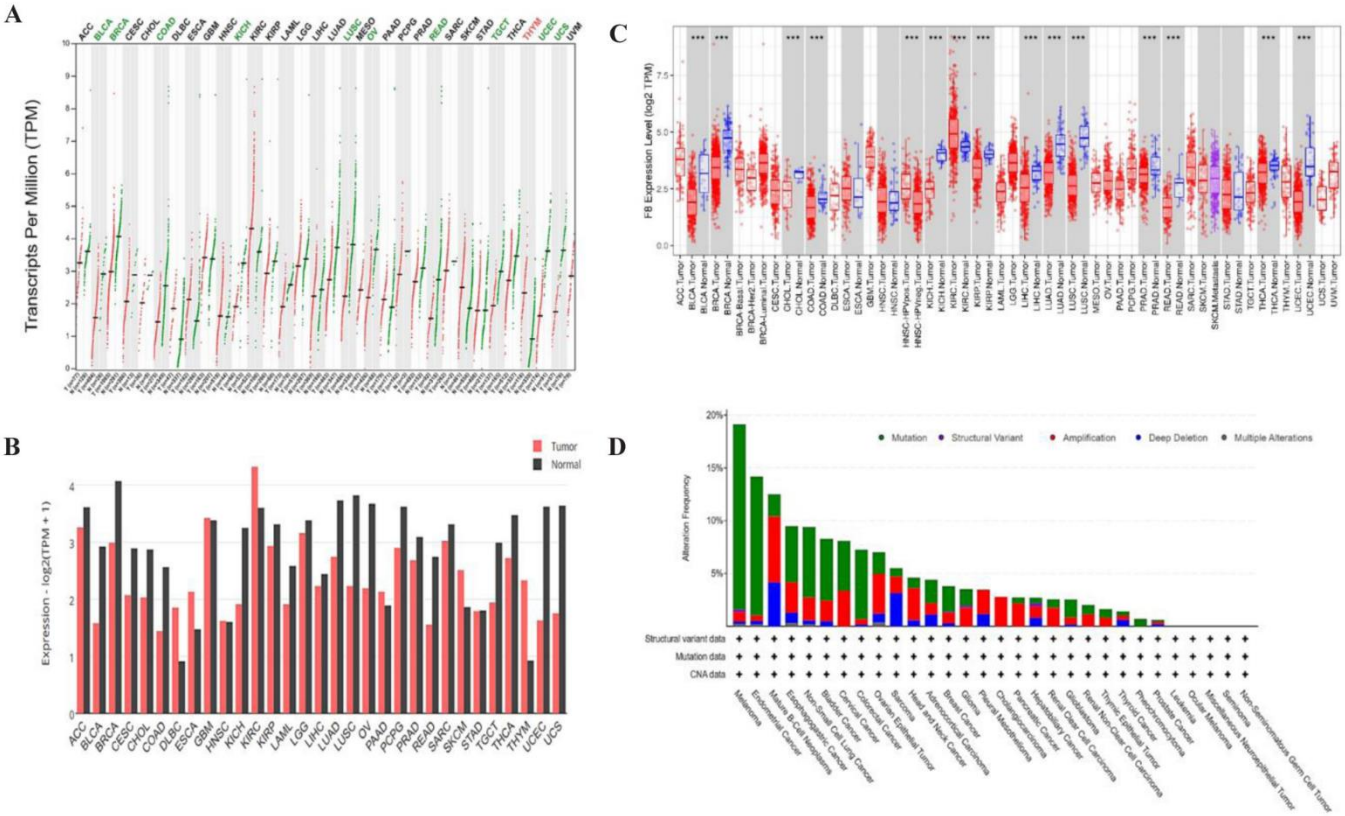

Figure 2. Expression of *F8* in different cancers. A) *F8* expression profile across all tumor samples and paired normal tissues (dot plot) based on GEPIA2 database. B) *F8* expression profile across all tumor samples and paired normal tissues (Bar plot) based on GEPIA2 database. C) *F8* expression levels in different tumor types based on TCGA data based on TIMER database.
*p<0.05, **p<0.01, ***p<0.001. D) The gene alterations of the *F8* gene in different cancers. Data were downloaded from cBioPortal (https://www.cbioportal. org/).

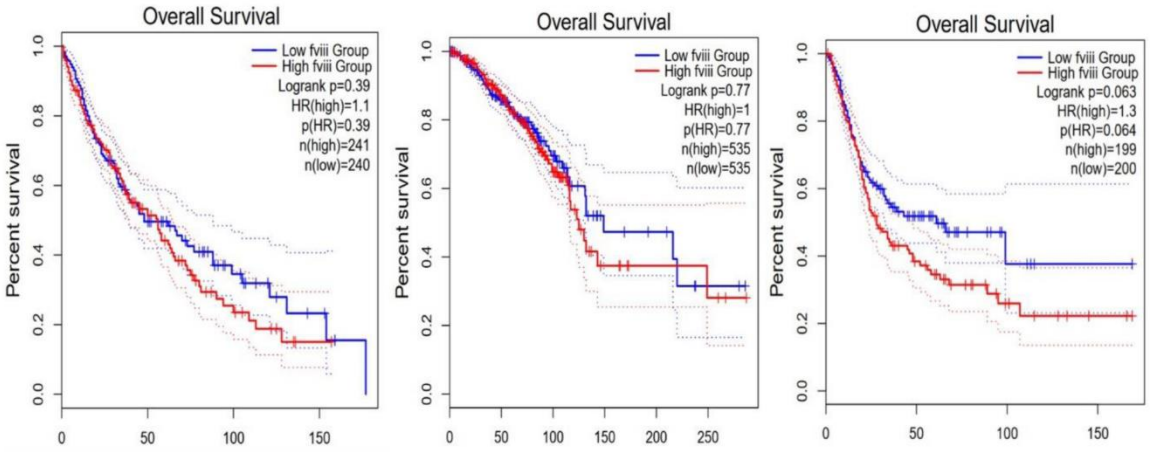

Figure 3. The overall survival curves of patients based on *FVIII* expression through GEPIA2. A) Lung carcinoma B) Breast cancer C) Bladder cancer.

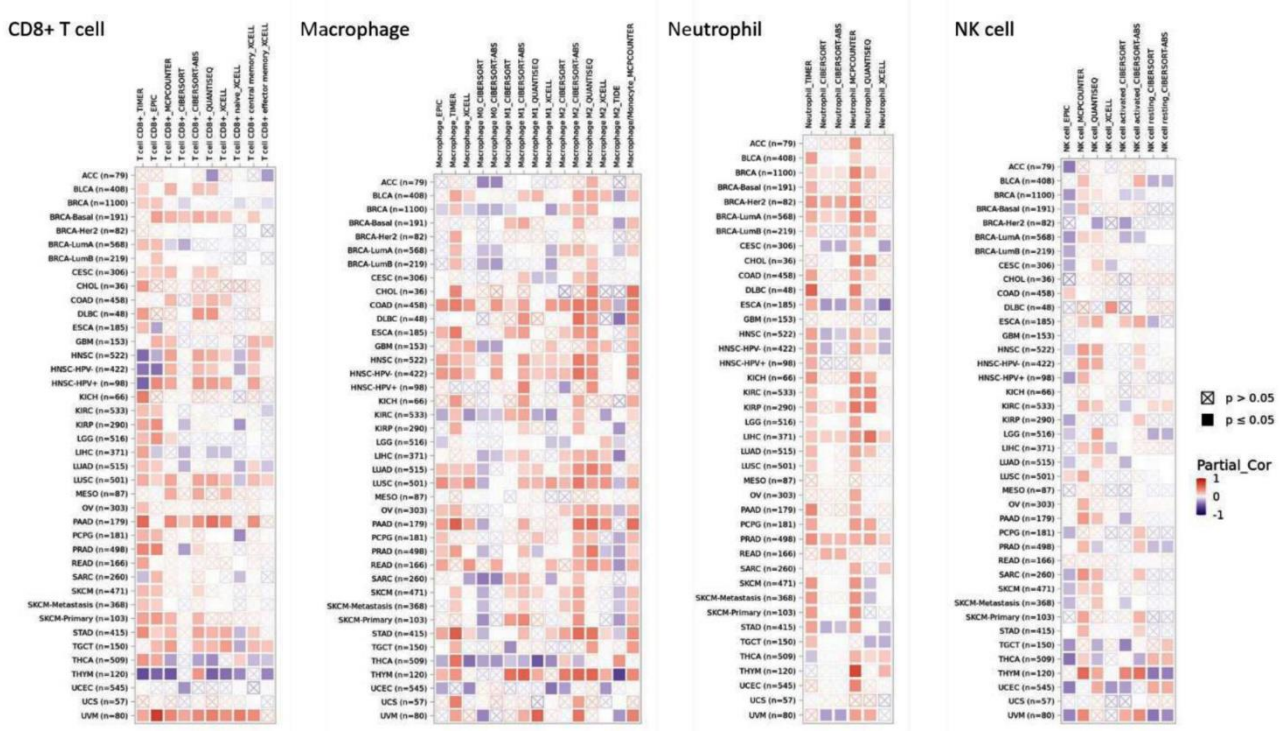

Figure 4. The correlation of *F8* expression and tumor immune infiltration. The correlation between *F8* gene expression and immune infiltration in 40 types of tumors was analyzed by Timer 2.0 software (http://timer.comp-genomics.org/). The CD8 + T cells, macrophages, neutrophils, and NK cells were selected to assess the correlation with F8 expression in tumors by Spearman's test. Red color demonstrates a significantly positive correlation ($p<0.05$), and blue color demonstrates a significantly negative correlation ($p<0.05$). The color depth shows the value of the correlation coefficient.

merous options for exhibiting both discrete genetic events such as mutations and continuous events, including information on the quantity of mRNA or proteins or DNA methylation. The cBioPortal offers access to summarized data on every cancer study featured in the portal, along with performing individual gene searching [59]. We assessed the *F8* gene alterations using the data from the cBio Cancer Genomics Portal (http://cbioportal.org) database. As shown in figure 2D, the mutations and substitutions in the *F8* gene were responsible for most changes in most cancers.

COSMIC: The COSMIC [60] (Catalogue of Somatic Mutations in Cancer) database stores somatic mutation information and related metadata. It is accessible through a series of online websites and offers multiple export choices as well as graphical or tabular views of

the data. Each gene is kept in COSMIC in a static form. Ensembl [61] helped to determine the genomic structure of every gene and its chromosomal placement. The RefSeq project was used to determine the cDNA and protein sequencing [62,63]. The COSMIC database was used to analyze the *F8* mutation distribution in various tissues (Figure 5), which demonstrated that the *F8* mutation rate was different in most tissues and disorders. Pie chart represents that mis-sense substitutions account for the largest amount of mutation types among total samples (46.58%) (Figure 5A). Additionally, the C>T mutation was the most common substitution mutation in the *F8* gene (Figure 5B).

TargetScan and miRDB: TargetScan (https://www.targetscan.org/), and miRDB databases (http://www.mirdb.org/), are two powerful miRNA prediction tools, that were used to predict the miRNAs that target *F8* gene. A total of 76 miRNAs have been identified that target *F8* gene *via* these two databases (Figure 6). Based on the results, *F8* is predicted to be targeted by a variety of miRNAs that are involved in a wide range of cellular functions.

Enrichr: Gene ontology enrichment analysis for the protein interaction network of FVIII was performed using the Enrichr database [64]. Gene ontology is categorized into three separate groups: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). Table 2 shows the results of the GO annotation enrichment analysis related to the interaction network of the *FVIII* gene. The interaction network of the FVIII was remarkably enriched in GO terms, comprising the peptidyl-asparagine modification, regulation of blood coagulation, protein N-linked glycosylation via asparagine, zymogen activation, *etc*. in BP; serine-type

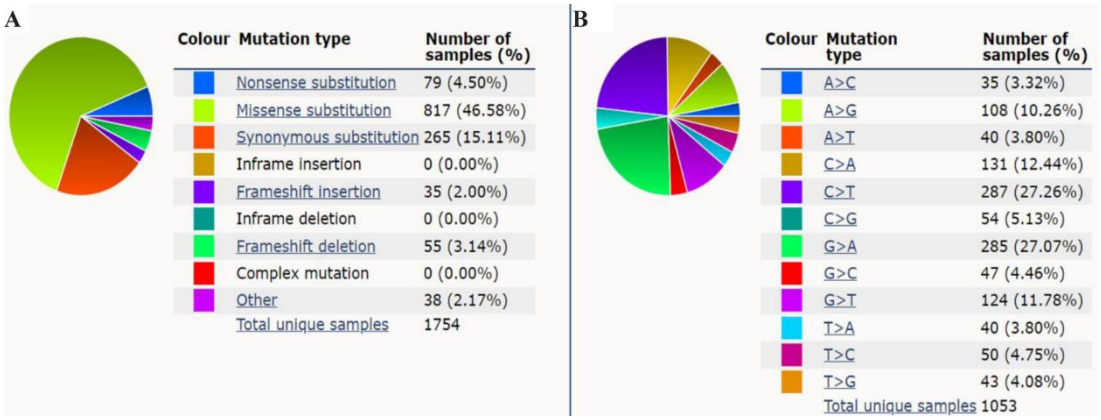

Figure 5. The distribution of different types of mutations for *F8* based on COSMIC database (https://cancer.sanger.ac.uk/cosmic). A) A summary of the types of mutation that have been observed in various samples for *F8* gene. B) A breakdown of the observed substitution mutations.

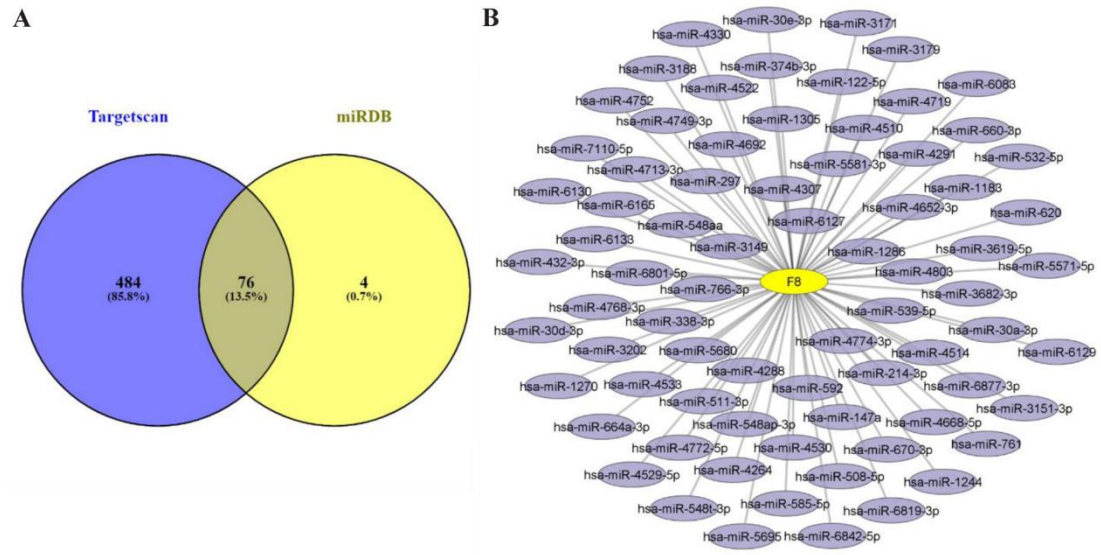

Figure 6. Target miRNA prediction for *F8* gene. A) Prediction of a total of 76 common miRNAs that target *F8* gene in the two databases miRDB, and TargetScan. B) 76 common *F8* gene target miRNAs. The interaction network was constructed by Cytoscape software.

Table 2. Gene ontology enrichment analysis of *F8* interaction network based on Enrichr database

GO Term	Category	Description	p-value	Genes
Biological process				
	(GO:0018196)	Peptidyl-asparagine modification	0.0015	*LMAN1; MCFD2*
	(GO:0030193)	Regulation of blood coagulation	0.0015	*PROC; F2*
	(GO:0018279)	Protein N-linked glycosylation via asparagine	0.0015	*LMAN1; MCFD2*
	(GO:0031638)	Zymogen activation	0.0030	*F9; F11*
	(GO:0006901)	Vesicle coating	0.0037	*LMAN1; MCFD2*
	(GO:0048207)	Vesicle targeting, rough ER to cis-Golgi	0.0037	*LMAN1; MCFD2*
	(GO:0010466)	Negative regulation of peptidase activity	0.0037	*SERPIND1; SERPINC1*
	(GO:0010951)	Negative regulation of endopeptidase activity	0.0037	*SERPIND1; SERPINC1*
	(GO:0048208)	COPII vesicle coating	0.0037	*LMAN1; MCFD2*
	(GO:0006487)	Protein N-linked glycosylation	0.0038	*LMAN1; MCFD2*
Molecular function				
	(GO:0004867)	Serine-type endopeptidase inhibitor activity	0.0012	*SERPIND1; SERPINC1*
	(GO:0004866)	Endopeptidase inhibitor activity	0.0032	*SERPIND1; SERPINC1*
	(GO:0002020)	Protease binding	0.0032	*VWF; SERPINC1*
	(GO:0005537)	Mannose binding	0.0138	*LMAN1*
	(GO:0030414)	Peptidase inhibitor activity	0.0322	*SERPIND1*
	(GO:0061135)	Endopeptidase regulator activity	0.0328	*SERPIND1*
Cellular component				
	(GO:0030134)	COPII-coated ER to Golgi transport vesicle	6.791469109190254E-4	*LMAN1; MCFD2*
	(GO:0031233)	Intrinsic component of external side of plasma membrane	0.0119	*F10*
	(GO:0031093)	Platelet alpha granule lumen	0.0330	*VWF*
	(GO:0030136)	Clathrin-coated vesicle	0.0392	*VWF*
	(GO:0030135)	Coated vesicle	0.0412	*LMAN1*
	(GO:0031091)	Platelet alpha granule	0.0441	*VWF*
	(GO:0005789)	Endoplasmic reticulum membrane	0.0471	*LMAN1; MCFD2*

Table 3. The top significant KEGG terms for the *F8* interaction network based on Enrichr database

KEGG terms	p-value	Genes
Platelet activation	0.001	*VWF; F2*
Coronavirus disease	0.005	*VWF; F2*
ECM-receptor interaction	0.043	*VWF*

endopeptidase inhibitor activity, endopeptidase inhibitor activity, protease binding, mannose binding, peptidase inhibitor activity, *etc*. in MF; COPII-coated ER to Golgi transport vesicle, intrinsic component of the external side of the plasma membrane, platelet alpha granule lumen, clathrin-coated vesicle, platelet alpha granule, *etc.* in CC. Additionally, the KEGG pathway analysis uncovered that platelet activation, Coronavirus disease, and ECM-receptor interaction pathways were the most significant pathways for the *FVIII* gene and its interaction network (Table 3).

Discussion

Approximately 50% of cancer patients and more than 90% of those with metastatic illnesses show hemostatic disorders [65]. Even though elevated VWF and FVIII levels have been detected in cancer patients, the precise mechanism relating the rise in the levels to VTE or survival rates is still up for debate [66]. FVIII normally circulates coupled with VWF and functions as an acute phase reactant protein and an activator in the coagulation cascade. VWF prevents FVIII from proteolytic attacks and premature removal by binding to it [35-37,67-70]. Typically changes in the plasma level of VWF will affect FVIII levels with the same change [71,72]. Increased FVIII levels in cirrhotic patients are most likely caused by increased hepatic VWF production rather than enhanced liver FVIII overexpression activity. Additionally, upregulation of the *FVIII* gene can lead to recurrent VTE, and downregulation of the gene leads to X-linked hemorrhagic disorder HA [19,70,73,74]. FVIII is significantly associated with risk factors of atherosclerosis, including age, diabetes, high BMI, and several coagulative markers [19]. Increased levels of FVIII have been suggested as an independent risk factor for death overall as well as arterial thrombotic diseases including Myocardial Infarction (MI) and stroke [15-18,21,75,76]. There is a much higher elevation in the FVIII levels in African-Americans (AA) than in

patients of European ancestry (EA). Therefore, AAs may be at greater risk of VTE than EAs [77-80].

According to analyses in Jackson Heart Study (JHS), FVIII is significantly associated with age, diabetes, the CRP biomarker, and triglycerides in AAs. Moreover, FVIII is more significantly associated with mortality and less with CVD incident. The reason remains unknown. High levels of FVIII can be due to the downregulation of the miRNAs that typically bind to the *FVIII* gene and suppress it. On the other hand, overexpression of the so-called miRNAs can result in HA phenotypes. Our results have represented a total of 76 miRNAs that could target *F8* gene and might be associated with pathogenesis of several disorders.

There are numerous ways to find and study the miRNA-mediated regulation of the gene expression. Samples from patients and healthy individuals were collected, and following the deep-sequencing process, the expression levels of miRNAs were observed. The MS2-TRAP assay helped with detecting the interactions between *FVIII* gene and miRNAs [26,29,81-92]. The *FVIII* gene can be suppressed by both miR-186-5p and miR-19b-3p. Additionally, it was shown that a severe HA patient had considerably greater levels of miR-19b-3p expression [30]. Furthermore, levels of FVIII mRNA expression in cirrhotic patients were lower than those of healthy individuals. There were no variations in the intracellular distribution of FVIII in the participants. Nevertheless, the bigger arteries in the cirrhotic tissue appeared to overgrow sinusoidal cells that generate FVIII. This could be the reason why *FVIII* expression levels were lower cirrhotic tissues than in healthy counterparts. Also, the amount of data for the link between coagulation abnormalities and cancer has substantially increased in recent years [93,94]. Several coagulative biomarkers have been suggested to have prognostic values in many cancers independently, in the case of VTE [95-98]. In women with recently diagnosed invasive BC, the plasma levels of FVIII strongly correlated with lymph node metastasis and the number of them, and also, with the HER2 status [99]. Moreover, FVIII is correlated with cancer-specific mortality [21].

Furthermore, high VTE occurrence rates have been seen in BLCs, which is associated with the increase in the early biomarkers of coagulation [100,101]. *FVIII* expression was seen to be notably higher than VWF and other coagulative markers. The invasive BLC showed the highest levels of *FVIII* expression when compared to other bladder tumors [41].

Studies have demonstrated that in colorectal cancers, the tumor's staging and differentiation have an impact on the plasma level of FVIII. FVIII levels, however, are not linked to the formation of metastases [43]. It is noteworthy that three years following surgery, patients with lower FVIII levels at the time of the procedure had greater survival chances. In patients with EC after preoperative chemoradiotherapy, FVIII plasma levels are significantly raised. There was no discernible change in FVIII levels between the multimodal group and the patients who only underwent surgery [44]. As mentioned above, VTE is commonly discovered in cancer patients, which adds to the morbidity of the patients. In around 50-80% of HCC patients a portal or hepatic vein invasion is detected, and tumor thrombus is known to indicate a bad prognosis for these individuals. When the tumor spreads and develops a tumor thrombus, it may be fatal [102-106].

According to current studies, patients with lung cancer had shown considerably higher plasma levels of many coagulative factors, including VWF and FVIII regardless of the presence of distant metastases. Additionally, increased levels of coagulative biomarkers have been associated with poor prognosis for lung cancer [51]. Most importantly, in an attempt to assess association between FVIII and cancer, Walker *et al*, conducted a research to investigate the possible direct expression and secretion of FVIII by cancer cells. The goal of their research was to assess the expression and synthesis of FVIII in cancer, using BLC as the model system. They demonstrated that *FVIII* expression is elevated in BLC compared with normal bladder tissue, and it is released by BLC cells. Moreover, they showed that this can be extended to other cancer cell lines with a pattern independent of VWF and different relevant players in the coagulation cascade, thus providing criteria of a potential independent function for FVIII in cancer-related pathophysiology [11].

Conclusion

In the present study, we have analyzed the expression of *F8* in various tumors and adjacent normal tissues using publicly available expression databases. *F8* was differentially expressed between normal and tumor tissues in different cancers. Furthermore, *F8* expression levels were significantly related to levels of immune cell infiltration. These results suggest that *F8* has a significant function in the regulation of immune cell infiltration in several tumors, with particularly high impact on macrophages, neutrophils, CD8+T cells, and NK cells infiltration. Therefore, these results indicate that *F8* contributes to the immune response in various tumors and could be a novel prognostic biomarker.

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Conflict of Interest

The authors declare they have no competing interests.

References

1. Khalilian S, Motovali-Bashi M, Rezaie H. Factor VIII: Perspectives on immunogenicity and tolerogenic strategies for Hemophilia A patients. Int J Mol Cell Med 2020;9(1):33-50.

2. Rezaei H, Motovali-Bashi M, Khalilian S. Identification of Novel miRNAs in the F8 Gene Via Bioinformatics Tools. Iran J Biotechnol 2021 Apr;19(2):e2700.
3. Rezaei H, Motovali-Bashi M, Khalilian S. MicroRNA prediction in the FVIII gene locus: A step towards hemophilia A control. Gene Cell Tissue 2020;7:e103096.
4. Abdulqader AMR, Mohammed AI, Rachid S, Ghoraishizadeh P, Mahmood SN. Identification of the intron 22 and intron 1 inversions of the factor VIII gene in Iraqi Kurdish patients with hemophilia A. Clin Appl Thromb Hemost 2020 Jan-Dec;26:1076029619888293.
5. Nasirnejad Sola F, Morovvati S, Sabetghadam Moghadam M, Entezari M. Mutation detection and inhibitor risk in Iranian patients with Hemophilia A: Six novel mutations. Clin Case Rep 2020 Dec;8(12):2976-85.
6. Jalali-Qomi S, Motovali-Bashi M, Rezaei H, Khalilian S. Experimental validation of a predicted microRNA within human FVIII gene. Mol Biol Res Commun 2021 Jun;10 (2):45-53.
7. Yamamoto K, de Waard V, Fearns C, Loskutoff DJ. Tissue distribution and regulation of murine von Willebrand factor gene expression in vivo. Blood 1998;92 (8):2791-801.
8. Wion KL, Kelly D, Summerfield JA, Tuddenham EG, Lawn RM. Distribution of factor VIII mRNA and antigen in human liver and other tissues. Nature 1985;317 (6039):726-9.
9. Do H, Healey JF, Waller EK, Lollar P. Expression of factor VIII by murine liver sinusoidal endothelial cells. J Biol Chem 1999;274(28):19587-92.
10. Hollestelle MJ, Thinnes T, Crain K, Stiko A, Kruijt JK, van Berkel TJ, et al. Tissue distribution of factor VIII gene expression in vivo–a closer look. Thromb Haemost 2001;86(09):855-61.
11. Walker GE, Merlin S, Zanolini D, Vandoni A, Volpe A, Gaidano G, et al. Factor VIII as a potential player in cancer pathophysiology. J Thromb Haemost 2022 Mar; 20(3):648-60.
12. Rubio VEC, Pérez-Segura P, Muñoz A, Farré AL, Ruiz LC, Lorente JA. High plasma levels of soluble P-Selectin and Factor VIII predict venous thromboembolism in non-small cell lung cancer patients: The Thrombo-Nsclc risk score. Thromb Res 2020;196:349-54.
13. Moik F, Posch F, Grilz E, Scheithauer W, Pabinger I, Prager G, et al. Haemostatic biomarkers for prognosis and prediction of therapy response in patients with metastatic colorectal cancer. Thromb Res 2020;187:9-17.
14. Folsom AR, Delaney JA, Lutsey PL, Zakai NA, Jenny NS, Polak JF, et al. Associations of factor VIIIc, D-dimer, and plasmin–antiplasmin with incident cardiovascular disease and all-cause mortality. Am J Hematol 2009;84(6):349-53.
15. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation 1997;96(4):1102-8.
16. Folsom AR, Rosamond WD, Shahar E, Cooper LS, Aleksic N, Nieto FJ, et al. Prospective study of markers of hemostatic function with risk of ischemic stroke. Circulation 1999;100(7):736-42.
17. Smith F, Lee A, Fowkes F, Price J, Rumley A, Lowe G. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. Arterioscler Thromb Vasc Biol 1997;17(11):3321-5.
18. Meade T, Cooper J, Stirling Y, Howarth D, Ruddock V, Miller G. Factor VIII, ABO blood group and the incidence of ischaemic heart disease. Br J Haematol 1994;88 (3):601-7.
19. Kamphuisen PW, Eikenboom JC, Bertina RM. Elevated factor VIII levels and the risk of thrombosis. Arterioscler Thromb Vasc Biol 2001;21(5):731-8.
20. Raffield LM, Lu AT, Szeto MD, Little A, Grinde KE, Shaw J, et al. Coagulation factor VIII: Relationship to cardiovascular disease risk and whole genome sequence and epigenome-wide analysis in African Americans. J Thromb Haemost 2020;18(6):1335-47.
21. Yap E, Timp J, Flinterman L, van Hylckama Vlieg A, Rosendaal F, Cannegieter S, et al. Elevated levels of factor VIII and subsequent risk of all-cause mortality: results from the MEGA follow-up study. J Thromb Haemost 2015;13(10):1833-42.
22. El-Maarri O, Herbiniaux U, Graw J, Schröder J, Terzic A, Watzka M, et al. Analysis of mRNA in hemophilia A patients with undetectable mutations reveals normal splicing in the factor VIII gene. J Thromb Haemost 2005; 3(2):332-9.
23. Johnsen JM, Fletcher SN, Huston H, Roberge S, Martin BK, Kircher M, et al. Novel approach to genetic analysis and results in 3000 hemophilia patients enrolled in the my life, our future initiative. Blood Adv 2017;1(13):824-34.
24. Jankowska KI, McGill J, Pezeshkpoor B, Oldenburg J, Sauna ZE, Atreya CD. Further evidence that microRNAs can play a role in Hemophilia A disease manifestation: F8 gene downregulation by miR-19b-3p and miR-186-5p. Front Cell Dev Biol 2020;8:669.
25. Jankowska KI, McGill J, Pezeshkpoor B, Oldenburg J, Atreya CD, Sauna ZE. Clinical manifestation of hemophilia A in the absence of mutations in the F8 gene that encodes FVIII: role of microRNAs. Transfusion 2020;60 (2):401-13.
26. Graw J, Brackmann H-H, Oldenburg J, Schneppenheim R, Spannagl M, Schwaab R. Haemophilia A: from mutation analysis to new therapies. Nat Rev Genet 2005;6(6): 488-501.
27. Oldenburg J, Pezeshkpoor B, Pavlova A. Historical review on genetic analysis in hemophilia A. Semin Thromb Hemost 2014 Nov;40(8):895-902
28. Nienhuis AW, Nathwani AC, Davidoff AM. Gene therapy for hemophilia. Mol Ther 2017;25(5):1163-7.
29. Benson G, Auerswald G, Dolan G, Duffy A, Hermans C, Ljung R, et al. Diagnosis and care of patients with mild haemophilia: practical recommendations for clinical management. Blood Transfus 2018;16(6):535-44.

30. Sarachana T, Dahiya N, Simhadri VL, Pandey GS, Saini S, Guelcher C, et al. Small ncRNA expression-profiling of blood from hemophilia A patients identifies miR-1246 as a potential regulator of factor 8 gene. PLoS One 2015; 10(7):e0132433.
31. Meng F. Hsa-miR-5581-3p and Hsa-miR-542-3p target the F8 gene in hemophilia A without F8 mutations. Mediterr J Hematol Infect Dis 2021;13(1):e2021041.
32. Mei B, Chen Y, Chen J, Pan CQ, Murphy JE. Expression of human coagulation factor VIII in a human hybrid cell line, HKB11. Mol Biotechnol 2006;34(2):165-78.
33. Hollestelle MJ, Geertzen HG, Straatsburg IH, van Gulik TM, van Mourik JA. Factor VIII expression in liver disease. Thromb Haemost 2004;91(02):267-75.
34. Colman RW. Hemostasis and thrombosis: basic principles and clinical practice: Lippincott Williams & Wilkins; 2006. 1827 p.
35. Lenting PJ, Van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. Blood 1998;92(11):3983-96.
36. Kaufman RJ, Pipe SW, Tagliavacca L, Swaroop M, Moussalli M. Biosynthesis, assembly and secretion of coagulation factor VIII. Blood Coagul Fibrinolysis 1997; 8:S3-14.
37. Saenko EL, Ananyeva NM, Tuddenham EG, Kemball-Cook G. Factor VIII–novel insights into form and function. Br J Haematol 2002;119(2):323-31.
38. Mandoj C, Pizzuti L, Sergi D, Sperduti I, Mazzotta M, Di Lauro L, et al. Observational study of coagulation activation in early breast cancer: development of a prognostic model based on data from the real world setting. J Transl Med 2018;16(1):129.
39. Khorana AA, Francis CW. Risk prediction of cancer-associated thrombosis: appraising the first decade and developing the future. Thromb Res 2018;164:S70-S6.
40. Zareba P, Duivenvoorden W, Pinthus JH. Thromboembolism in patients with bladder cancer: incidence, risk factors and prevention. Bladder Cancer 2018;4(2):139-47.
41. Walker GE, Merlin S, Zanolini D, Vandoni A, Volpe A, Gaidano G, et al. Factor VIII as a potential player in cancer pathophysiology. J Thromb Haemost 2022;20(3): 648-60.
42. Gujam FJ, Going JJ, Mohammed Z, Orange C, Edwards J, McMillan DC. Immunohistochemical detection improves the prognostic value of lymphatic and blood vessel invasion in primary ductal breast cancer. BMC Cancer 2014;14(1):1-11.
43. Schellerer VS, Mueller-Bergh L, Merkel S, Zimmermann R, Weiss DR, Schildberg C, et al. Is coagulation factor VIII a useful marker for colorectal carcinoma? Int J Biol Markers 2012 2012/01/01;27(1):20-6.
44. Byrne M, O'Donnell J, White B, Kennedy J, Reynolds J. Differential response of factor VIII and protein C expression following multimodal therapy for esophageal carcinoma. Journal of Clinical Oncology 2007;25(18_ suppl):15106.
45. Zhuang M, Xin G, Wei Z, Li S, Xing Z, Ji C, et al. Dihydrodiosgenin inhibits endothelial cell-derived factor VIII and platelet-mediated hepatocellular carcinoma metastasis. Cancer Manag Res 2019;11:4871-82.
46. He Z, Chen H, Li G, Zhu H, Gao Y, Zhang L, et al. Diosgenin inhibits the migration of human breast cancer MDA-MB-231 cells by suppressing Vav2 activity. Phytomedicine 2014;21(6):871-6.
47. Hao-Peng Y, Lei Y, Jiang W-W, Qian L, Jun-Ping K, Bo-Yang Y. Diosgenin inhibits tumor necrosis factor-induced tissue factor activity and expression in THP-1 cells via down-regulation of the NF-κB, Akt, and MAPK signaling pathways. Chin J Nat Med 2013;11(6):608-15.
48. Ma H-D, Deng Y-R, Tian Z, Lian Z-X. Traditional Chinese medicine and immune regulation. Clin Rev Allergy Immunol 2013;44(3):229-41.
49. Zheng H, Wei Z, Xin G, Ji C, Wen L, Xia Q, et al. Preventive effect of a novel diosgenin derivative on arterial and venous thrombosis in vivo. Bioorg Med Chem Lett 2016;26(14):3364-9.
50. Wei Z, Xin G, Wang H, Zheng H, Ji C, Gu J, et al. The diosgenin prodrug nanoparticles with pH-responsive as a drug delivery system uniquely prevents thrombosis without increased bleeding risk. Nanomedicine 2018;14(3): 673-84.
51. Liu X, Chen X, Yang J, Guo R. Association of ABO blood groups with von Willebrand factor, factor VIII and ADAMTS-13 in patients with lung cancer. Oncol Lett 2017;14(3):3787-94.
52. Tas F, Kilic L, Serilmez M, Keskin S, Sen F, Duranyildiz D. Clinical and prognostic significance of coagulation assays in lung cancer. Respir Med 2013;107(3):451-7.
53. Guadagni F, Ferroni P, Basili S, Facciolo F, Carlini S, Crecco M, et al. Correlation between tumor necrosis factor-alpha and D-dimer levels in non-small cell lung cancer patients. Lung Cancer 2004;44(3):303-10.
54. Oleksowicz L, Bhagwati N, DeLeon-Fernandez M. Deficient activity of von Willebrand's factor-cleaving protease in patients with disseminated malignancies. Cancer Res 1999;59(9):2244-50.
55. Gabazza EC, Taguchi O, Yamakami T, Machishi M, Ibata H, Suzuki S. Evaluating prethrombotic state in lung cancer using molecular markers. Chest 1993;103(1):196-200.
56. Ünsal E, Atalay F, Atikcan S, Yilmaz A. Prognostic signif https://pubmed.ncbi.nlm.nih.gov/14971870/icance of hemostatic parameters in patients with lung cancer. Respir Med 2004;98(2):93-8.
57. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 2019;47 (W1):W556-W60.
58. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017;77 (21):e108-e10.
59. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer

genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6(269):pl1.

60. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res 2019;47(D1):D941-D7.
61. Birney E, Andrews TD, Bevan P, Caccamo M, Chen Y, Clarke L, et al. An overview of Ensembl. Genome research. 2004;14(5):925-8.
62. Wheeler DL, Church DM, Edgar R, Federhen S, Helmberg W, Madden TL, et al. Database resources of the National Center for Biotechnology Information: update. Nucleic Acids Res 2004;32(suppl_1):D35-D40.
63. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, et al. The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. Br J Cancer 2004;91(2):355-8.
64. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016 Jul 8;44(W1):W90-7.
65. Goad KE, Gralnick HR. Coagulation disorders in cancer. Hematol Oncol Clin North Am 1996;10(2):457-84.
66. Comerford C, Glavey S, Quinn J, O'Sullivan JM. The role of VWF/FVIII in thrombosis and cancer progression in multiple myeloma and other hematological malignancies. J Thromb Haemost 2022;20(8):1766-77.
67. Pépin M, Kleinjan A, Hajage D, Büller H, Di Nisio M, Kamphuisen P, et al. ADAMTS-13 and von Willebrand factor predict venous thromboembolism in patients with cancer. J Thromb Haemost 2016;14(2):306-15.
68. Terraube V, Marx I, Denis CV. Role of von Willebrand factor in tumor metastasis. Thromb Res 2007;120:S64-S70.
69. Yang X, Sun H-j, Li Z-r, Zhang H, Yang W-j, Ni B, et al. Gastric cancer-associated enhancement of von Willebrand factor is regulated by vascular endothelial growth factor and related to disease severity. BMC Cancer 2015; 15(1):1-11.
70. Bannow BS, Recht M, Négrier C, Hermans C, Berntorp E, Eichler H, et al. Factor VIII: Long-established role in haemophilia A and emerging evidence beyond haemostasis. Blood Rev 2019;35:43-50.
71. Noe DA. A mathematical model of coagulation factor VIII kinetics. Haemostasis 1996;26(6):289-303.
72. Fijnvandraat K, Peters M, Ten Cate JW. Inter-individual variation in half-life of infused recombinant factor VIII is related to pre-infusion von Willebrand factor antigen levels. Br J Haematol 1995;91(2):474-6.
73. Jenkins PV, Rawley O, Smith OP, O'Donnell JS. Elevated factor VIII levels and risk of venous thrombosis. British J Haematol 2012;157(6):653-63.
74. Risch L, Huber AR, Schmugge M. Diagnosis and treatment of heparin-induced thrombocytopenia in neonates and children. Thromb Res 2006;118(1):123-35.
75. Zakai N, Katz R, Jenny N, Psaty B, Reiner A, Schwartz S, 8, et al. Inflammation and hemostasis biomarkers and cardiovascular risk in the elderly: the Cardiovascular Health Study. J Thromb Haemost 2007;5(6):1128-35.
76. Rumley A, Lowe G, Sweetnam P, Yarnell J, Ford R. Factor VIII, von Willebrand factor and the risk of major ischaemic heart disease in the Caerphilly Heart Study. Br J Haematol 1999;105(1):110-6.
77. Conlan MG, Folsom AR, Finch A, Davis C, Sorlie P, Marcucci G, et al. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. Thromb Haemost 1993;70(09):380-5.
78. Lutsey P, Cushman M, Steffen L, Green D, Barr R, Herrington D, et al. Plasma hemostatic factors and endothelial markers in four racial/ethnic groups: the MESA study. J Thromb Haemost 2006;4(12):2629-35.
79. Roberts LN, Patel RK, Chitongo P, Bonner L, Arya R. African–Caribbean ethnicity is associated with a hypercoagulable state as measured by thrombin generation. Blood Coagul Fibrinolysis 2013;24(1):40-9.
80. Patel RK, Ford E, Thumpston J, Arya R. Risk factors for venous thrombosis in the black population. Thromb Haemost 2003;90(11):835-8.
81. Krek A, Grün D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. Nat Genet 2005;37(5):495-500.
82. Grimson A, Farh KK-H, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell 2007;27(1):91-105.
83. Calin GA, Liu C-G, Sevignani C, Ferracin M, Felli N, Dumitru CD, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA 2004;101(32):11755-60.
84. Goff LA, Davila J, Swerdel MR, Moore JC, Cohen RI, Wu H, et al. Ago2 immunoprecipitation identifies predicted microRNAs in human embryonic stem cells and neural precursors. PloS One 2009;4(9):e7192.
85. Guerau-de-Arellano M, Alder H, Ozer HG, Lovett-Racke A, Racke MK. miRNA profiling for biomarker discovery in multiple sclerosis: from microarray to deep sequencing. J Neuroimmunol 2012;248(1-2):32-9.
86. Yoon J-H, Srikantan S, Gorospe M. MS2-TRAP (MS2-tagged RNA affinity purification): tagging RNA to identify associated miRNAs. Methods 2012;58(2):81-7.
87. Ahmadi H, Ahmadi A, Azimzadeh-Jamalkandi S, Shoorehdeli MA, Salehzadeh-Yazdi A, Bidkhori G, et al. HomoTarget: a new algorithm for prediction of microRNA targets in Homo sapiens. Genomics 2013;101(2): 94-100.
88. Zheng H, Fu R, Wang J-T, Liu Q, Chen H, Jiang S-W. Advances in the techniques for the prediction of microRNA targets. Int J Mol Sci 2013;14(4):8179-87.
89. Agarwal V, Bell GW, Nam J-W, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife 2015;4:e05005.
90. Dusl M, Senderek J, Müller JS, Vogel JG, Pertl A, Stucka R, et al. A 3′-UTR mutation creates a microRNA target site in the GFPT1 gene of patients with congenital

myasthenic syndrome. Hum Mol Genet 2015;24(12): 3418-26.

91. Laganà A. Computational prediction of microRNA targets. Adv Exp Med Biol 2015:887:231-52.
92. He B-S, Qu J, Chen M. Prediction of potential disease-associated microRNAs by composite network based inference. Sci Rep 2018;8(1):15813.
93. Wun T, White RH. Venous thromboembolism (VTE) in patients with cancer: epidemiology and risk factors. Cancer Invest 2009;27(sup1):63-74.
94. Lauw MN, van Doormaal FF, Middeldorp S, Buller HR. Cancer and venous thrombosis: current comprehensions and future perspectives. Semin Thromb Hemost 2013 Jul;39(5):507-14.
95. Altiay G, Ciftci A, Demir M, Kocak Z, Sut N, Tabakoglu E, et al. High plasma D-dimer level is associated with decreased survival in patients with lung cancer. Clinl Oncol (R Coll Radiol) 2007;19(7):494-8.
96. Oya M, Akiyama Y, Okuyama T, Ishikawa H. High preoperative plasma D-dimer level is associated with advanced tumor stage and short survival after curative resection in patients with colorectal cancer. Jpn J Clin Oncol 2001;31(8):388-94.
97. Sakurai M, Satoh T, Matsumoto K, Michikami H, Nakamura Y, Nakao S, et al. High pretreatment plasma D-dimer levels are associated with poor prognosis in patients with ovarian cancer independently of venous thromboembolism and tumor extension. Int J Gynecol Cancer 2015;25(4):593-8.
98. Marfia G, Navone SE, Fanizzi C, Tabano S, Pesenti C, Abdel Hadi L, et al. Prognostic value of preoperative von Willebrand factor plasma levels in patients with Glioblastoma. Cancer Med 2016;5(8):1783-90.
99. Yigit E, Gönüllü G, Yücel İ, Turgut M, Erdem D, Çakar B. Relation between hemostatic parameters and prognostic/predictive factors in breast cancer. Eur J Intern Med 2008;19(8):602-7.
100. Alevizopoulos A, Tyritzis S, Leotsakos I, Anastasopoulou I, Pournaras C, Kotsis P, et al. Role of coagulation factors in urological malignancy: a prospective, controlled study on prostate, renal and bladder cancer. Int J Urol 2017;24(2):130-6.
101. Falanga A, Russo L, Milesi V, Vignoli A. Mechanisms and risk factors of thrombosis in cancer. Crit Rev Oncol Hematol 2017;118:79-83.
102. Ingle PV, Samsudin SZ, Chan PQ, Ng MK, Heng LX, Yap SC, et al. Development and novel therapeutics in hepatocellular carcinoma: a review. Ther Clin Risk Manag 2016;12:445-55.
103. Yang Y, Sun X, Chi C, Liu Y, Lin C, Xie D, et al. Upregulation of long noncoding RNA LINC00152 promotes proliferation and metastasis of esophageal squamous cell carcinoma. Cancer Manag Res 2019;11:4643-54.
104. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso MDC, Sala M, et al. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. Hepatology 1999;29(1):62-7.
105. Chen X-P, Qiu F-Z, Wu Z-D, Zhang Z-W, Huang Z-Y, Chen Y-F, et al. Effects of location and extension of portal vein tumor thrombus on long-term outcomes of surgical treatment for hepatocellular carcinoma. Ann Surg Oncol 2006;13(7):940-6.
106. Minagawa M, Makuuchi M, Takayama T, Ohtomo K. Selection criteria for hepatectomy in patients with hepatocellular carcinoma and portal vein tumor thrombus. Ann Surg 2001;233(3):379.