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Complement protein expression changes in various conditions of breast cancer: in-silico analyses—experimental research

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Introduction: Breast cancer is the most prevalent cancer diagnosed in females worldwide. The known biomarkers are insufficient to understand the actual prognosis of breast cancer, and identifying new biomarkers is desirable and valuable data to improve the patient's survival. Many inflammatory biomarkers, such as the complement system, can be regarded as prognostic values and as potent inflammatory mediators; complement proteins have a critical role in tumorigenesis. In the current study, the authors aim to investigate complement protein expression changes, particularly complement 3 (C3), complement 7 (C7), complement factor B (CFB), and complement factor D (CFD), in various conditions of breast cancer using in-silico tools.
Methods: The intent data were extracted using webtools, including; Kaplan–Meier plotter, BcGenExMiner, UALCAN, cbioportal, GeneMania, and Enrichr. To select valid data, a P greater than 0.05 was considered.
Results: The current study clarified that 21 complement genes correlated to survival conditions. Also, down or upregulation of extracted genes and breast cancer statuses were identified. Additionally, expression level difference of complement genes in various breast cancer four stages was detected. Ultimately, co-expression genes with complement genes were extracted and networked.
Conclusion: Changes in the expression of complement proteins can strongly correlate to breast cancer's prognosis, status, and survival. Furthermore, considering the vital role of CFD and CFB complement proteins in the alternative pathway in different stages of breast cancer, CFD and CFB can be regarded as reliable prognostic values for diagnosis.

Keywords: bioinformatic, breast cancer, complement, prognosis

Introduction

Breast cancer is the most prevalent cancer diagnosed in females worldwide and accounts for 15% of cancer-related deaths in women[1,2]. Various biomarkers characterize breast cancer prognosis; however, the known biomarkers are insufficient to understand the actual prognosis; hence, identifying new biomarkers is desirable and valuable data to improve the patient's survival[3].

Based on the evidence, many inflammatory biomarkers, such as the complement system, can be regarded as prognostic values[4]. In addition, as potent inflammatory mediators, complement proteins have a critical role in tumorigenesis[5]. Also, the increase of complement regulatory proteins and activation

HIGHLIGHTS

- The current study clarified that 21 complement genes correlated to survival conditions.
- Down or upregulation of extracted genes and breast cancer statuses were identified. Additionally, expression level difference of complement genes in various breast cancer four stages was detected.
- Co-expression genes with complement genes were extracted and networked.
- Changes in the expression of complement proteins can strongly correlate to breast cancer's prognosis, status, and survival.
- Considering the vital role of complement factor D (CFD) and complement factor B (CFB) complement proteins in the alternative pathway in different stages of breast cancer, CFD and CFB can be regarded as reliable prognostic values for diagnosis.

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fragments serve as biomarkers and prognostic indicators for various cancers, such as breast cancer[5]. Additionally, The function of the complement system includes inflammation regulators, facilitating immune mechanisms, and maintaining tissue homeostasis. Moreover, complements have anti-tumor activity through complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC)[5]. Although the complement system was previously thought to have just anti-tumor activity, recent evidence has shown that activation can increase tumor growth in specific conditions[6].

Various complements may be related to the incidence of breast cancer[7]. Complement 3 (C3) is a member of the complement alternative pathway that regulates neutrophil extracellular traps (NET) formation[8]. Evidence indicated that C3 causes tumorigenesis by activating the jak2/stat3 pathway and increases cell division[9]. Complement7 (C7) is another complement member that encodes a serum glycoprotein that forms the membrane attack complex (MAC) accompanying complement components C5b, C6, C8, and C9 as part of the final complement pathway in innate immunity[10]. C7 Dual behavior was reported in malignancies, such that C7 expression decreased in malignancies like ovarian cancer and increased in some other cancers, such as the liver[3]. Moreover, complement factor B (CFB) is a critical component of the complement alternative pathway and has a crucial role in labeling the remaining target particles resulting from the clearance. Recently, CFB was identified as a prognosis biomarker for cancer. Various evidence indicated that while the increase in CFB expression in cancer tissues was higher than in normal tissues, the level of CFB expression directly correlated with the survival rate[11,12]. In addition, complement factor D (CFD) is another complement enzyme that might relate to breast cancer. CFD is a serine protease synthesized by adipocytes, mainly. The enzyme activates the complement alternative pathway and has the "reaction rate" function in the alternative pathway[13,14]. Additionally, CFD plays an essential catalytic role in forming C3 convertase, downstream activation, and function of the pathway[15]. Previous studies have shown the production of CFD by cancer cell lines, such as gastric tumor-derived cells. In addition, Adipose-secreted CFD promotes the proliferation and growth of human breast cancer and worse malignant stem cells' properties in breast tissues. Interestingly, high CFD expression is associated with poor survival in adrenocortical carcinoma, thyroid carcinoma, uveal melanoma, low-grade glioma, and glioblastoma[16].

In the current study, the authors aim to investigate complement protein expression changes, particularly C3, C7, CFB, and CFD, in various conditions of breast cancer using in-silico tools. Also, prognostic values for different breast cancer conditions will be provided using obtained data.

Methods

Kaplan–Meier plotter (Kmplotter)

The current research used the kmplotter website (https://kmplot.com/) to evaluate the complement gen-set effect on survival. Kmplotter is a capable web tool to evaluate the relationship between the expression of all genes (ncRNA or protein) and survival in thousands of samples from variated tumor types, such as breast, ovarian, lung, and gastric cancer. The current database uses meta-analyze based data to extract and validate survival biomarkers[17]. The gene set was imported from the mRNA gene chip as the query. The present study considers the P greater than 0.05 to validate the results. This work has been reported in line with the Animals in Research: Reporting In Vivo Experiments (ARRIVE) criteria[18].

BcGenExMiner (v4.8)

BcGenExMiner v4.8 website (http://bcgenex.ico.unicancer.fr/BC-GEM/GEM-Accueil.php) was applied to find the clinicopathology

data set. BcGenExMiner is an online statistical analysis tool for annotated breast cancer transcriptome data. Also, the website offers the possibility of discovering desired gene expression in breast cancer[19]. Found gene set imported as a query from the analysis panel targeted expression analysis, and cancer genome atlas (TCGA) data were selected for analysis. Also, the P greater than 0.05 is considered for data validation.

The CANcer data analysis portal (UALCAN)

In the present study, the UALCAN database (http://ualcan.path.uab.edu/index.html) was utilized to compare complement gene expression data between different stages of breast cancer. UALCAN is an interactive web resource facilitating gene-level queries and Tumor Subgroup Gene Expression data (TSGE). UALCAN also enables researchers to study the expression levels of genes in primary tumor samples compared to normal tissue. The online tool can compare different tumor subgroups defined by pathological cancer stage or tumor grade[20]. From the TCGA panel, the invasive breast was selected as carcinoma, and genes were imported as the query. In order to validate the data, the P greater than 0.05 is considered significant.

Cbioportal

In order to estimate co-expression and mutation data, the cbioportal website (https://www.cbioportal.org/) was operated. cbioportal provides a web resource for exploring, visualizing, and analyzing multidimensional cancer genomic data. The portal transforms molecular profiling data from cancer tissues and cell lines into easily comprehensible genetic, epigenetic, gene expression, and proteomic events. Also, the current database can compare the obtained results with clinical evidence[21]. The P greater than 0.05 is also intended to evaluate the achieved data.

GeneMania

GeneMania database (https://genemania.org/) was used to create the interaction network between the identified genes. GeneMANIA is an innovative website for generating hypotheses about gene function, analyzing gene lists, and prioritizing genes for functional assays. Based on a list of genes query, GeneMANIA analyzes genomic and proteomics data to find genes with similar functions[22].

Enrichr

Finally, the Enrichr database (https://maayanlab.cloud/Enrichr/) was applied to extract various miRNAs and transcription factors of the found genes. The P < 0.05 is considered an "entry" criterion to select the data set. Enrichr currently includes an extensive collection of diverse gene sets for genomic and functional analysis, and the biological data collection is upgraded periodically for further biological discovery[23].

Results

Extraction of survival-related complement genes

Using the Kmplotter web tool, the complement genes correlated to each survival condition involving; overall survival, relapse-free survival, metastasis-free survival, and post-progress survival stage were extracted. Ten genes, including C1r, C1s, C2, C3,

Zabihi et al. Annals of Medicine & Surgery (2024)

Annals of Medicine & Surgery

C4A, C4, C5, C7, CFB, and CFD, are significantly ($P < 0.05$) related to overall survival (OS). Also, 20 genes including C1QB, C1r, C1s, C2, C3, C4A, C4, C5, C6, C7, C8A, C8B, C9, CFB, CFD, CFP, FCN1, FCN2, MASP1, and MASP2 are significantly ($P < 0.05$) related to relapse-free survival (RFS). Additionally, ten genes, including C1r, C1s, C3, C4A, C4, C7, C8B, C8G, CFB, and CFD, correlate to distant metastasis-free survival (DMFS) significantly ($P < 0.05$). Moreover, the single gene includes CFD associated with post-progress survival (PPS). In addition, CFD is related to all four stages with high validity ($P < 0.0001$). Also, C7 is related to overall survival, relapse-free survival, and distant metastasis-free survival stage with high validity ($P < 0.0001$) (Table 1).

Clinicopathology data analysis

In the current study, the relation between breast cancer's statuses, including Age, Nodal, Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Triple-negative breast cancer (TNBC), and Basal-like breast cancer (BLBC) and extracted genes were identified. At age status under 51, three genes, including C1r, C1s, and FCN1, are upregulated, and Over 51, C2 and CFB were upregulated ($P < 0.05$). In the Nodal status, C5, C7, and CFD are upregulated in positive conditions, and in negative conditions, C8G and FCN3 are upregulated ($P < 0.05$). In positive ER status, C4A, C4B, C5, C6, C7, CFB, and CFD are upregulated ($P < 0.0001$) and C8A, FCN1and FCN3 are downregulated ($P < 0.05$). In negative condition, C1QA, C1QB, C1QC, C1r, C1s, C2, C3, C8G, C9, and CFP are upregulated ($P < 0.05$) and FCN2 and MASP2 downregulated ($P < 0.0001$). In PR status, in positive condition C4A, C4B, C5, C6, C7, CFD and CFB, are upregulated ($P < 0.0001$) and C8A, FCN1 and FCN3 are downregulated ($P < 0.05$), also. In negative conditions, C1QA, C1QB, C1r, C1s, C2, C8G and C9 are upregulated ($P < 0.05$), and FCN2 and MASP2 are downregulated ($P < 0.0001$).in HER2 status positive conditions, FCN1, MASP, and MASP2 are downregulated ($P < 0.05$). In negative conditions, C3, C5, C6, C7, CFD, and CFP upregulated ($P < 0.05$) and FCN2 is downregulated ($P < 0.05$). In TNBC positive condition, C1QA, C1QB, C1QC, C1r, C1s, C2 and C3 are upregulated ($P < 0.05$) and C6, C7, FCN1, MASP, MBL2, and MASP2 are downregulated ($P < 0.05$). In negative condition C4A, C4B, C5, CFB and CFD are upregulated ($P < 0.0001$) and C8G, C9, CFP, FCN1, and FCN3 are down-regulated ($P < 0.05$). In BLBC positive condition, C1QA, C1QB, C1QC, C1r, C1s, C2 and C3 are upregulated ($P < 0.05$) and C6, C7, C8B, FCN2, and MASP2 are downregulated ($P < 0.0001$). In negative condition C4A, C4B, C5, CFB and CFD are upregulated ($P < 0.0001$),and C8G, C9, CFP, FCN1, and FCN3 are down-regulated ($P < 0.05$) (Table 2).

Complement genes expression comparison

Due to UALCAN data, the expression level difference of complement genes in various breast cancer stages was detected. Fifteen genes, including C1r, C2, C1s, C3, C4A, C5, C6, C7, C8G, CFB, CFD, CFP, FCN3, MASP1, and MASP2, have a difference in expression level between healthy condition and the first stage ($P < 0.05$). Eighteen genes, including C1r, C1s, C2, C3, C4A, C5, C6, C7, C8A, C8G, CFB, CFD, CFP, FCN1, FCN2,

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Table 1

The complement gen-set effect on survival

Criteria	OS			RFS			DMFS			PPS		
	Cases	HR	P	Cases	HR	P	Cases	HR	P	Cases	HR	P
C1QA	1879	0.96	0.68	4929	1.04	0.48	2765	0.97	0.72	458	1.14	0.28
C1QB	1879	1.18	0.091	4929	1.12	0.033	2765	1.04	0.6	458	1.12	0.36
C1QC	943	0.89	0.4	2032	1.01	0.87	958	0.92	0.52	180	1.2	0.32
C1R	1879	0.7	0.00022	4929	0.84	0.00051	2765	0.82	0.012	458	0.96	0.76
C1S	1879	0.66	1.3E-05	4929	0.8	1.1E-05	2765	0.75	2.8E-04	458	0.97	7.8E-01
C2	1879	0.81	0.027	4929	0.86	0.0038	2765	0.97	0.68	458	1.01	0.96
C3	1879	0.67	3.20E-05	4929	0.73	2.10E-09	2765	0.71	1.00E-05	458	0.89	3.20E-01
C4A	1879	0.72	0.00077	4929	0.6	1.00E-16	2765	0.56	2.30E-13	458	0.89	3.10E-01
C4B	1879	0.75	0.0023	4929	0.61	1.00E-16	2765	0.58	4.10E-12	458	0.86	2.00E-01
C5	1879	0.69	9.70E-05	4929	0.78	1.70E-06	2765	0.9	2.00E-01	458	0.98	8.90E-01
C6	1879	0.91	0.31	4929	0.77	2.40E-07	2765	0.96	0.57	458	0.88	0.29
C7	1879	0.68	7.80E-05	4929	0.68	3.10E-14	2765	0.67	2.50E-07	458	0.85	1.80E-01
C8A	1879	0.97	0.73	4929	0.88	0.016	2765	1.11	0.17	458	0.94	0.58
C8B	1879	0.94	5.00E-01	4929	0.86	2.70E-03	2765	1.17	4.70E-02	458	0.97	7.80E-01
C8G	1879	1.1	0.32	4929	1.09	0.1	2765	1.3	0.00081	458	1.17	0.2
C9	1879	1	9.60E-01	4929	0.86	2.80E-03	2765	1.15	8.00E-02	458	1.01	9.40E-01
CFB	1879	0.69	0.00014	4929	0.68	7.10E-14	2765	0.64	2.20E-08	458	0.88	2.70E-01
CFD	1879	0.69	0.0001	4929	0.73	1.70E-09	2765	0.63	4.50E-09	458	0.61	3.00E-05
CFP	1879	0.91	0.34	4929	0.88	0.016	2765	0.94	0.44	458	0.94	0.6
FCN1	1879	1.01	9.30E-01	4929	0.88	1.60E-02	2765	1.13	1.20E-01	458	0.85	1.70E-01
FCN2	1879	1.02	0.87	4929	0.79	8.40E-06	2765	0.99	0.86	458	1.02	0.84
FCN3	1879	1.03	7.90E-01	4929	0.93	1.60E-01	2765	0.99	9.40E-01	458	0.98	8.80E-01
MASP1	943	0.97	0.84	2032	0.68	7.70E-07	958	0.91	0.46	180	1.01	0.95
MASP2	1879	0.96	6.90E-01	4929	0.78	2.00E-06	2765	1.12	1.30E-01	458	1	9.80E-01
MBL2	1879	1.13	0.21	4929	0.9	0.05	2765	1.02	0.8	458	1.13	0.32

DMFS, distant metastasis-free survival; HR, hazard ratio; OS, overall survival; PPS, post-progress survival; RFS, relapse-free survival.

Table 2

The relation between BC's statuses and complement genes

Criteria	Age ≤51	Age >51	Nodal −	Nodal +	ER (IHC) −	ER (IHC) +	PR (IHC) −	PR (IHC) +	HER2 (IHC) −	HER2 (IHC) +	TNBC Not	TNBC TNBC	BLBC Not	BLBC BLBC
C1QA														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↑	—	↑	—	—	—	—	↑	—	↑
P value	0.3013		0.3803		<0.0001*		0.0094*		0.4653		0.0031*		0.0006*	
C1QB														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↑	—	↑	—	—	—	—	↑	—	↑
P value	0.2677		0.6759		0.0006*		0.0390*		0.8444		0.0137*		0.0083*	
C1QC														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↑	—	—	—	—	—	—	↑	—	↑
P value	0.3967		0.4639		0.0008*		0.0587		0.9112		0.0129*		0.0029*	
C1r														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	↑	—	—	—	↑	—	↑	—	—	—	—	↑	—	↑
P value	0.0006*		0.9274		<0.0001*		0.0095*		0.3984		<0.0001*		<0.0001*	
C1s														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	↑	—	—	—	↑	—	↑	—	—	—	—	↑	—	↑
P value	0.0003*		0.6365		<0.0001*		0.0242*		0.7054		0.0002*		<0.0001*	
C2														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	↑	—	—	↑	—	↑	—	—	—	—	↑	—	↑
P value	0.0151*		0.1334		<0.0001*		0.0274*		0.1558		0.0013*		<0.0001*	
C3														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↑	—	—	—	↑	—	—	↑	—	↑
P value	0.0942		0.7101		0.0024*		0.4771		0.0101*		0.0114*		<0.0001*	
C4A														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	—	↑	—	↑	—	—	↑	—	↑	—
P value	0.2603		0.0894		<0.0001*		<0.0001*		0.6347		<0.0001*		<0.0001*	
C4B														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	—	↑	—	↑	—	—	↑	—	↑	—
P value	0.1703		0.0639		<0.0001*		<0.0001*		0.3738		<0.0001*		<0.0001*	
C5														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	↑	—	↑	—	↑	↑	—	↑	—	↑	—
P value	0.3388		0.0173*		<0.0001*		<0.0001*		0.0016*		<0.0001*		<0.0001*	
C6														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	—	↑	—	↑	↑	—	—	↓	—	↓
P value	0.6909		0.3389		<0.0001*		<0.0001*		<0.0001*		0.0085		<0.0001*	

Table 2

(*Continued*)

Criteria	Age ≤51	Age >51	Nodal status −	Nodal status +	ER (IHC) −	ER (IHC) +	PR (IHC) −	PR (IHC) +	HER2 (IHC) −	HER2 (IHC) +	TNBC Not	TNBC TNBC	BLBC Not	BLBC BLBC
C7														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	↑	—	↑	—	↑	↑	—	—	↓	—	↓
P value	0.4338		0.0082*		<0.0001*		<0.0001*		0.0013*		0.0009		<0.0001*	
C8A														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	—	↓	—	↓	—	—	—	—	—	—
P value	0.7204		0.2060		0.0029*		0.0053*		0.0826		0.3331		0.1577	
C8B														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	—	—	—	—	—	—	—	—	—	↓
P value	0.9973		0.7967		0.0793		0.0844		0.1292		0.7782		<0.0001*	
C8G														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	↑	—	↑	—	↑	—	—	—	↓	—	↓	—
P value	0.3020		0.0045*		<0.0001*		<0.0001*		0.2771		<0.0001*		<0.0001*	
C9														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↑	—	↑	—	—	—	↓	—	↓	—
P value	0.8794		0.2680		0.0024*		0.0022*		0.8032		0.0378*		0.0291*	
Criteria	Age ≤51	Age >51	Nodal status −	Nodal status +	ER (IHC) −	ER (IHC) +	PR (IHC) −	PR (IHC) +	HER2 (IHC) −	HER2 (IHC) +	TNBC Not	TNBC TNBC	BLBC Not	BLBC BLBC
CFB														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	↑	—	—	—	↑	—	↑	—	—	↑	—	↑	—
P value	0.0394*		0.1370		<0.0001*		<0.0001*		0.6570		<0.0001*		<0.0001*	
CFD														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	↑	—	↑	—	↑	↑	—	↑	—	↑	—
P value	0.1995		0.0016*		<0.0001*		<0.0001*		0.0371*		<0.0001*		<0.0001*	
CFP														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↑	—	—	—	↑	—	↓	—	↓	—
P value	0.0593		0.9995		0.0347*		0.1519		0.0002*		0.0071		0.0207*	
FCN1														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	↑	—	—	—	—	↓	—	↓	—	↓	↓	—	↓	—
P value	0.0487		0.3065		<0.0001*		0.0002*		0.0154		<0.0001*		<0.0001*	
FCN2														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168
mRNA	—	—	—	—	↓	—	↓	—	↓	—	—	↓	—	↓
P value	0.2981		0.7101		<0.0001*		<0.0001*		0.0459*		<0.0001*		<0.0001*	
FCN3														
NO.	344	689	402	479	228	756	325	656	532	155	810	111	864	168

5156

Zabihi et al. Annals of Medicine & Surgery (2024)

Annals of Medicine & Surgery

	mRNA	—	←	→	→	—	→	→
	P value	0.1032	0.0468*	<0.0001*	<0.0001*	0.3148	<0.0001*	<0.0001*
MASP1	NO.	344 / 689	402 / 479	228 / 756	325 / 656	532 / 155	810 / 111	864 / 168
	mRNA	—	—	—	—	→	—	—
	P value	0.3332	0.9768	0.1352	0.2281	0.0002*	0.0719	0.1067
MASP2	NO.	344 / 689	402 / 479	228 / 756	325 / 656	532 / 155	810 / 111	864 / 168
	mRNA	—	—	→	→	→	→	→
	P value	0.4550	0.5909	<0.0001*	<0.0001*	<0.0001*	0.0004*	<0.0001*
MBL2	NO.	344 / 689	402 / 479	228 / 756	325 / 656	532 / 155	810 / 111	864 / 168
	mRNA	—	—	—	—	—	→	—
	P value	0.1608	0.8013	0.8417	0.3198	0.2750	0.0127*	0.9338

*$P < 0.05$

BLBC, basal-like breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; TNBC, triple-negative breast cancer.

FCN3, MASP1, and MASP2 have a difference in expression level between healthy conditions and the second stage ($P < 0.05$). Eighteen genes, including C1r, C1s, C2, C3, C4A, C5, C6, C7, C8G, C9, CFB, CFD, CFP, FCN1, FCN2, FCN3, MASP1, and MASP2 have a difference in expression level between healthy conditions and the third stage ($P < 0.05$) also. Thirteen genes, including C1r, C1s, C3, C5, C6, C7, CFB, CFD, CFP, FCN1, FCN2, MASP1, and MASP2 have a difference in expression level between healthy conditions and the fourth stage ($P < 0.05$) also. Four genes, including C3, C4A, C8G, and CFP, differ in expression level between the first and second stages ($P < 0.05$). Two genes, C3 and CFP, differ in expression level during the first and third stages ($P < 0.05$). Three genes, including C3, C6, and FCN1, differ in expression level between the first and fourth stages ($P < 0.05$). C5 has an expression level difference between the second and third stages ($P < 0.05$). FCN1 has an expression level difference between the second and fourth stages ($P < 0.05$). Finally. CFD and FCN1 have an expression level difference between the third and fourth stages.

Mutation rate

Also, using Cbioportal mutation rate was extracted. Mutation rates (highest to lowest) include C9 (6%), C1s (5%), C3 (5%), CFB (5%), MASP1 (5%), C1r (4%), C2 (4%), C5 (4%), C6 (4%), C8A (4%), C8B (4%), C8G (4%), C1QA (3%), C1QC (3%), C7 (3%), MBL2 (3%), C1QB (2.9%), MASP2 (2.9%), CFD (2.8%), FCN2 (2.6%), FCN3 (2.6%), CFP (2%), FCN1 (2%), C4A (1.6%), C4B (1.5%) (Fig. 1).

Co-expression data

Using the Cbioportal website, other co-expression genes with complement genes were extracted. By using the "Calculate and draw custom Venn diagrams website" (https://bioinformatics.psb.ugent.be/webtools/Venn/), shared genes were identified[24]. The string web tool also created the interaction network among extracted genes (Fig. 2)[24,25].

Gene expression network

The gene interaction was predicted by GeneMania online functional illustrator (Fig. 3). In the inner circle were the complement genes, while in the outer circle were the predicted co-expressed genes. Their functions focused on complement activation, humoral immune response, and regulation of humoral immune response.

Discussion

The current study extracted 21 complement genes that correlated to survival conditions. Also, down or upregulation of extracted genes and breast cancer statuses were identified. Additionally, expression level difference of complement genes in various breast cancer four stages was detected. Ultimately, co-expression genes with complement genes were extracted and networked.

According to the findings, ten genes, including; C1r, C1s, C3, C4A, C4, C5, C7, CFB, and CFD, have a highly validated correlation to at least three survival conditions. Zhao et al.[26] demonstrated that; C1r, C1s, C4A, C3, C4, C5, C7, CFB, and CFD mRNAs are expressed in lung and breast cancers. In addition, recent evidence has shown that reduced expression of C1s, C1r, CFB, and C3 is related to lymph node metastases and poor

Zabihi et al. Annals of Medicine & Surgery (2024)

Annals of Medicine & Surgery
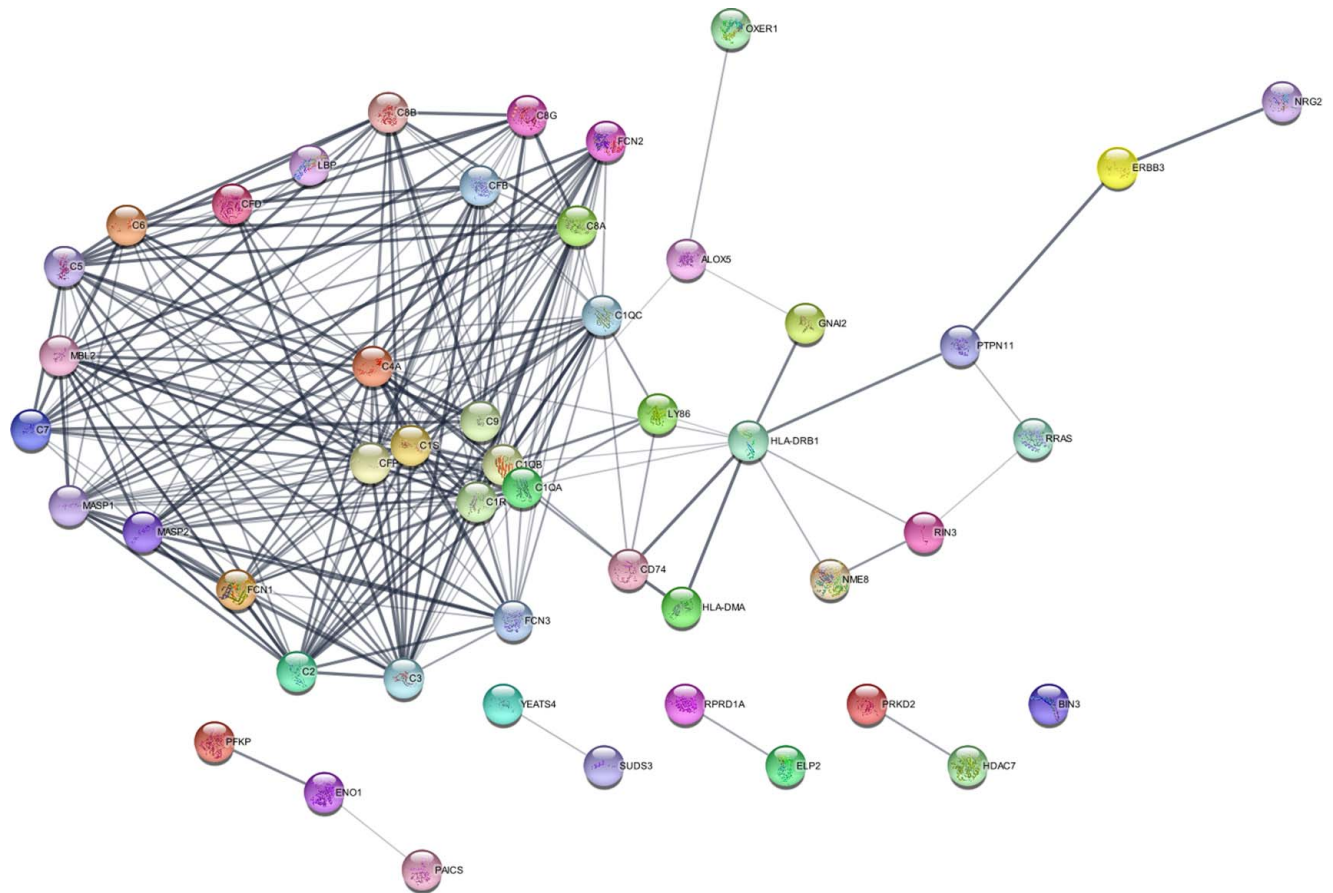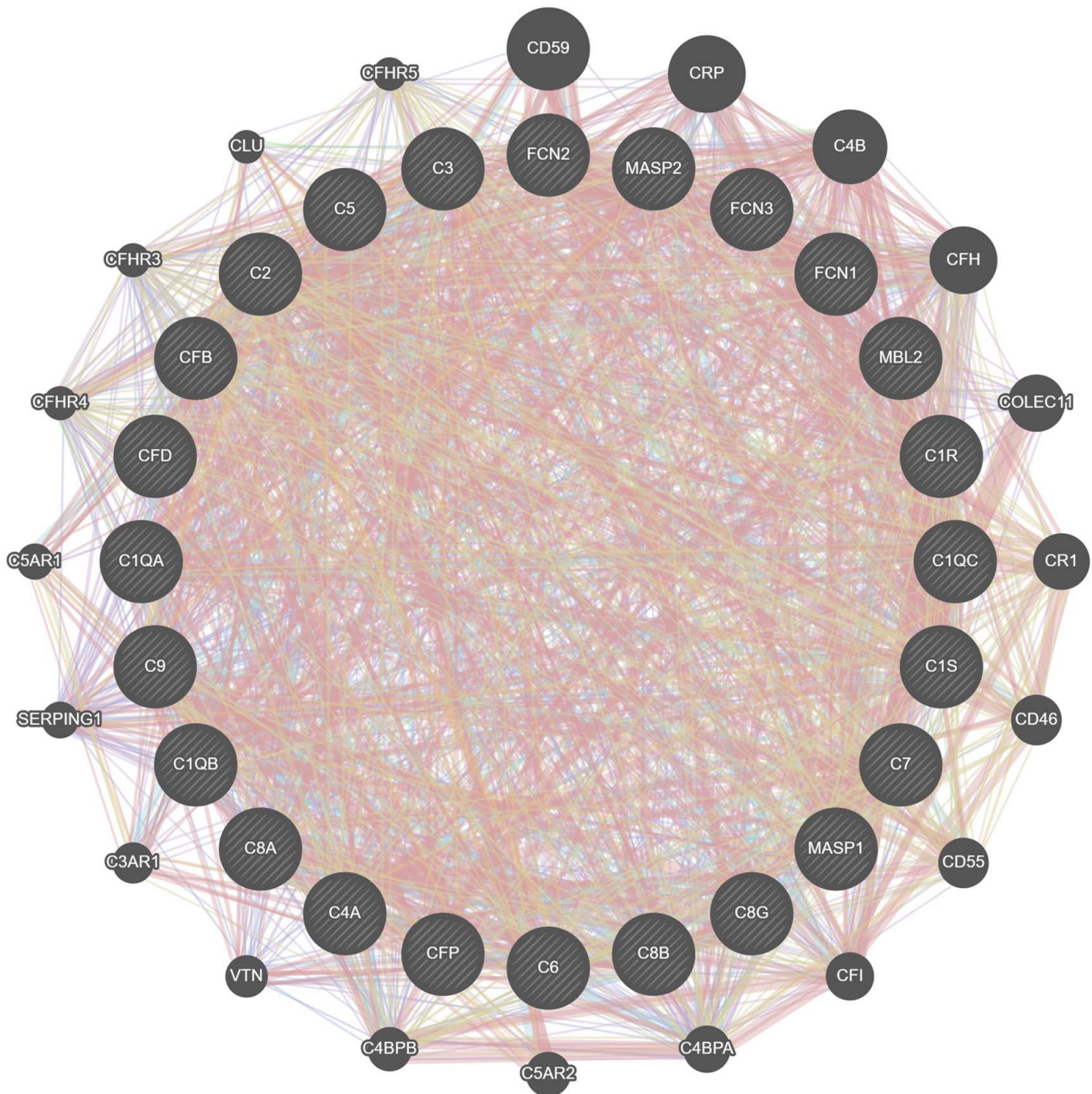

Figure 1. Complement mutation rate 1.

Figure 2. Complement co-expression network, illustrated by String.

Figure 3. Complement co-expression network and main genes interaction network, illustrated by GeneMania.

prognosis in breast cancer[27]. Mamoor *et al.*[28] showed that C1r down-regulation correlates with poor survival. Interestingly, current data showed that C1s and C1r have significant upregulation in five breast cancer statuses and significant differential expressions in four breast cancer stages versus healthy conditions, and C1s have a significant mutation rate (5%) among the other genes. Also, evidence has shown that increases in serum levels of C3 and C4 are related to cancer survival[29,30]. Additionally, the current data has shown that C3 and C4 have significant differential expressions in four breast cancer stages versus healthy conditions, and C3 has a significant mutation rate (5%) among the other genes. Moreover, a study in china demonstrated that C3

genetic alteration is related to breast cancer prevalence among east Asian females[31]. C5, as a proinflammatory factor, plays a central role in the activation complement cascade[32]; evidence revealed that C3, C4, and C5 might increase tumor survival due to immunosuppression[33]. Further, the current data points that C5 has upregulation in five breast cancer statuses and differential expressions in breast cancer stages. Likewise, C6 has a vital role in tumor growth inhibition and apoptosis induction by downstream Akt/Erk inhibition in HER2 statuses[34]. C7 plays a dual role in breast cancer prognosis[3], and based on the present data, C7 has significant differential expressions in breast cancer stages. Although C7 is upregulated in Nodal status, ER, PR, and HER2

Zabihi et al. Annals of Medicine & Surgery (2024)

Annals of Medicine & Surgery

statuses, this complement is downregulated in TNBC and BLBC. CFB and CFD are complements expressed in progesterone/estrogen-related tumors such as Endometriosis-Associated Ovarian Cancer[35,36]. Interestingly, the highly validated upregulation of CFB and CFD in ER and PR statuses was observed in the current study. According to the high validity of the mentioned complement genes, the present gene set can be regarded as a prognostic value for breast cancer; however, more research is required to prove this hypothesis.

According to "Enrichr" data, the present gene set (main and co-expressed network) is the Regulator of the process, including immune effector, humoral immune response, and complement activation. Additionally, the current set involved cell and sub-cell essential functional structures such as; integral components of the plasma membrane and collagen – containing extracellular matrix (ECM). The immune effector is a component of an immune response carried out by the immune system[37]. Based on the evidence, breast cancer can escape the immune effector process via tolerance induction and triggers immunosuppressive pathways[38]. Accordingly, modifying and evaluating the present set could be impressive in breast cancer treatment by diagnosing and preventing immune escape. Various evidence indicates changes in membrane structures, such as glycoproteins and receptors, during breast cancer[38,39]. Furthermore, While the collagen-containing extracellular matrix positively correlates with tumor size, this structure has an inverse relation with ER and PR receptor statuses[40]. Also, the collagen-containing extracellular matrix is essential in cancer metastasis[41]. Current findings revealed that the plasma concentration of the extracted genes is correlated with different cancer statuses, which include changes in the expression of membrane or ECM structures such as receptors and collagen V; However, the interplay between both changes (membrane structures, glycoproteins, and ECM) during breast cancer is still poorly understood and requires more research.

Conclusion

According to the achieved data, Changes in the expression of complement proteins can be strongly correlated to the prognosis, status, and survival of breast cancer. Furthermore, considering the vital role of CFD and CFB complement proteins in the alternative pathway in different stages of breast cancer, CFD and CFB can be regarded as reliable prognostic values for diagnosis. However, more experimental studies, mainly cohort and clinical trials, are required to clarify better the role of competent in the classic and alternative pathways in breast cancer prognosis.

Ethical approval

This article does not contain any studies with human or animal subjects performed by any authors and does not require ethical approval and consent.

Consent

Not applicable.

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Author contribution

Study concept and design by all authors; Data acquisition by all authors; Data interpretation by all authors; drafting the manuscript by all authors; Revision of the manuscript by all authors; the final version of the manuscript is approved by all authors.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Research registration unique identifying number (UIN)

We could not register our manuscript in the Research Registry UIN: www.researchregistry.com due to internet access restrictions and international sanctions. we live in Iran. We hardly even meet the basic needs of our daily life. We do not receive any funding for our research and we cannot pay for our research. Please excuse us from registering this manuscript in the Research Registry UIN: www.researchregistry.com.

Guarantor

Mohammad Akhoondian.

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Provenance and peer review

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