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Multiomics analysis identifies EBF1 and MYB as key regulators of PD-1 gene in breast cancer

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ABSTRACT

Programmed cell death protein 1 (PD-1), an immune suppressant controls the effector activity of T lymphocytes through interaction with Programmed death-ligand 1 protein (PD-L1). The T cells become fatigued as a result of this engagement. However, it is still unknown what molecular processes cause T cells to attain a state of exhaustion. Even the PD-1 Transcription Factor's (TFs) molecular roles are unclear. Consequently, to understand the underlying processes of PD-1 expression in T cells, we sought to identify novel PD-1 Transcription Factors (TFs). Using computational techniques, we collected the data of PD-1 TFs and assessed their biological activities, related pathways, and interaction matrix. Two TFs, EBF1 and MYB, that were experimentally found to interact with PD-1 were found in several open databases. The sequence of PD-1, predicted transcription factors of PD-1, and binding strength and affinities of the TFs to PD-1 were all determined using the online tools ENSEMBL, TRAP, and CISTROME. Understanding the binding pattern of PD-1 and its TFs in breast cancer is made easier by the information provided. Associated pathways were discovered using integrated network analysis of PD-1 and its co-expressed genes. Additionally, expression analysis of PD-1 and TFs was conducted using the cBioportal and TIMER 2.0 web tool to investigate any potential correlations. This further demonstrated the strong relationship between EBF1 and MYB, which influences how PD-1 is expressed. The study argues for better comprehension and interpretation of the biological functions and pathways of PD-1, EBF1, and MYB, which would make them more useful as breast cancer biomarkers and therapeutic targets.

Key words: breast cancer, bio-informatics, transcription factors, PD1, EBF1, MYB

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INTRODUCTION

T-cell exhaustion occurs when CD8+ T lymphocytes that are reactive to tumours or viruses are subjected to prolonged antigen stimulation in an inflammatory setting [1]. Poor effector activity decreased cytokine production, and persistent appearance of inhibitory receptors such as PD-1, LAG-3, TIM-3, and CTLA-4 as well as low survival rate are characteristics of exhausted T cells [2]. It has been demonstrated that blocking antibodies against these inhibitory receptors, in particular, CTLA-4 and the PD-1/PD-L1 axis, are highly successful at promoting tumour regression and remission in cancer patients [3]. However, less than half of patients with breast cancer experience long-term responses to checkpoint-blocking therapy, underlining the need for a more comprehensive molecular representation of T-cell scarcity in cancer [1]. The membrane protein known as PD-L1 is a co-inhibitory factor of the immune response expressed on macrophages, dendritic cells, T cells, B cells, and several types of tumour cells. PD-1's association with its ligand, PD-L1, results in self-protection [4]. PD-L1 on breast cancer cells interacts with PD-1 on CD8+ T cells to suppress cytokine release and cause the death of CD8+ T cells. In non-cancerous cells with a strong immunological response, the immune system may attack self-cells, resulting in autoimmune diseases. This process is critical for maintaining peripheral tolerance, avoiding autoimmune disorders, and controlling the progression of chronic inflammatory diseases [5]. However, PD-1 expression in T cells increases quickly in cases of malignancy. Cancer cells defend themselves by producing the co-inhibitory receptor PD-L1, which is identified by PD-1 on CD8+T cells, on their membrane [6]. The interaction between PD-1 and PD-L1 can therefore be prevented in cancer patients if the expression of PD-1 can be downregulated. This will keep the T cells activated, with effective cytokine production and killing the cancer cells. In order to fully grasp the underlying mechanisms of PD-1 expression in CD8+ T cells or T cell depletion in breast cancer, we, therefore, want to identify novel transcription factors of PD-1. The mammalian genome has over 1,500 transcription factors encoded [7]. In order to start or stop transcription through a trans-activation or trans-repression environment, transcription factors can be targeted, that bind DNA helices at specified regulatory sequences [8]. Some transcription factors have a clear carcinogenic association and were once thought to be "undruggable" [9]. A deeper understanding of their precise roles (expression, binding pattern, binding site, and protein/protein interaction) may create new opportunities to use transcription factors as druggable therapeutic targets for CD8+ T cells that have been exhausted in the therapy of breast cancer. These molecules attach to DNA at promoter sites and control the expression

of several genes involved in various biological processes. These transcription factors control some genes that activate or inhibit cytokines and growth factors as well. The exhausted CD8+ T cells in the tumour microenvironment produced less cytokine, resulting in cancer eradication failure. In order to determine the role of these PD-1 transcription factors in decreasing cytokine production by fatigued CD8+ T cells, we investigated the relationship between the expression of PD-1, its transcription factors and cytokines. For the development of innovative therapies, transcription factors are a crucial target due to their intricate function in the expression of PD1 in breast cancer. Notably, a study underway now or in the near future may focus on blocking or inhibiting PD-1 transcription factors in order to control the PD-1 gene, which is involved in the development of cancer. It may provide a therapeutic route for treating breast cancer by inhibiting these TFs with siRNA or transcription factor decoy oligodeoxynucleotides [10].

MATERIALS AND METHODS

Predicted Transcription factors

We used the ENSEMBL human database (https://www.ensembl. org/index.html) to download the complete genome sequence of PD-1 [11]. To recognize the TFs that are likely to bind this DNA sequence, the sequence is pasted on the TRAP [12]. This web tool also predicts the binding strength of the predicted TF to the promoter sequences. It calculates the total binding association of each transcription factor to the preferred sequence by "hit-based" ranking using significant p-values ($p<0.05$).

Screening Assay for Transcription Factors

We used LASAGNA search 2.0, a built-in online tool, to further search and display the transcription factor's binding location on the PD-1 gene. For promoter extraction, 1,792 transcription factor variants and 15 species are available in LASAGNA-Search 2.0. [13]. With the use of this web tool, we can screen the transcription factor for the study's target gene, PD-1. We acquired the names of probable transcription factors, the genome's binding sequence, the binding location, strand, binding score, p-value, and E-value after entering the gene symbol *PDCD1* and choosing humans as the species. The chosen gene was bound to the region with the highest binding score ($P<0.01$).

Understanding the binding pattern

Next, to understand the binding pattern of these transcription factors on the PD-1 gene, we used the CISTROME data browser (cistrome.org/db). Cistrome DB keyword searches allow us to find transcription factors of interest [14]. Th is da tabase a ds in identifying the variables that bind in the interval or have a substantial binding overlap with the peaks obtained using the UCSC Browser. It is an excellent resource for transcriptional research.

Potential Transcription Regulatory and cytokine correlation of PD-1

Next, to find out the role of these transcription factors in promoting or inhibiting cytokine production by CD8 + T cells, we used cBioportal and TIMER 2.0 databases. The cBioPortal for Cancer Genomics (http://www.cbioportal.org) is a tool for viewing, interpreting, and visualizing genomics data on the web. The Breast Invasive Carcinoma (TCGA, PanCancer Atlas) cohort's patient data were analyzed using the cBioPortal to determine the patterns of gene co-expression and the relationships between different cytokines, PD-1, and transcription factors [15].

TIMER database analysis

We further assessed how PD-1 expression correlated to the transcription factors with the help of the TIMER 2.0 database. TIMER 2.0 is a sizable database that can assess the amounts of immune infiltration and variations in gene expression in various cancers (https://cistrome.shinyapps.io/timer/). The tumour microenvironment includes tumour-infiltrating leukocytes as a key component of the tumour microenvironment [16,17].

RESULTS

Expression of PD1 on various cells

We looked for the PD-1 expression on diverse cells using information from the Human Protein Atlas database (https://www.proteinatlas.org). Based on data from human tissues and PBMCs from single-cell RNA sequencing (scRNAseq), this segment provides information about single-cell types [18]. Several different cell types express the PD-1 gene, although T lymphocytes express it most strongly. Additionally, it can be found on erythroid, dendritic, NK, and B cells. T cells and plasma cells are particularly enriched in the PD-1 protein's specificity (Figure 1).

Predicted transcription factors of PD-1

The creation of a sequence dataset for analysis is the first step in any genomic investigation. An easy-to-use tool that enables recovery of a wide variety of sequence types is provided by the ENSEMBL database. The gene sequence sight shows all potential exons emphasized and red for all transcripts (splice variants) in one individual gene. The complete genome sequence of PD-1 is downloaded from the ENSEMBL human database for further detailed analysis. The gene *PD-1* is on the Chromosome 2 reverse strand. The DNA sequence within the -2kb upstream promoter of PD-1 is collected and pasted on the TRAP (Transcription factor Affinity Prediction) Web

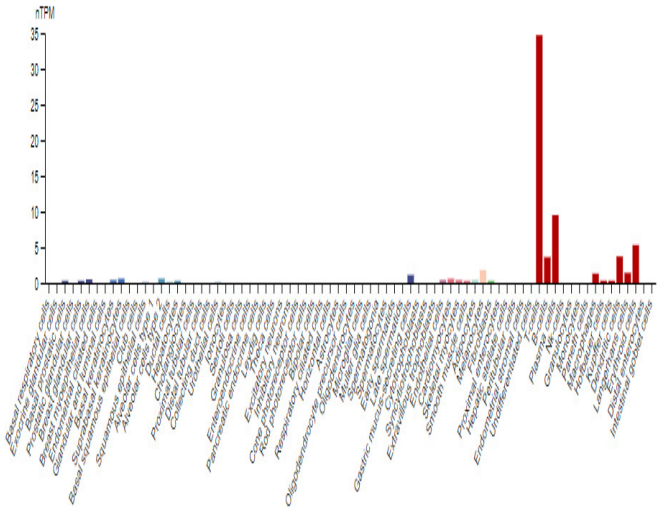

Fig.1. Expression profile of PD-1 mRNA in various types of tissues obtained by data from the Human Protein Atlas

Tools. Comprehending which transcription factor (TF) bind to an assumed cis-regulatory element/sequence is the key to knowing the role of that cis-regulatory sequence. TRAP web tools execute the methods that predict the "affinity" of a TF for a provided sequence, assessing all possible binding sites within the sequence. These methods deliver a quantitative estimation of TF binding strength which may offer a more realistic picture of regulation than individual binding sites. The predicted transcription factors are EBF1, MYB, NFkB1, USF1, MYC, and RORA_1, depending on the binding affinity with a significant p-value <0.05 (Table 1).

Identifying the binding sites of predicted transcription factors on targeted gene

Next, to locate the transcription factor binding locations of EBF1, MYC, MYB, USF1, NFkB1, and RORA 1 at the PD-1 gene's promoter region, LASAGNA-Search 2.0 with cut-off p-values of 0.001was employed. It is a programme for finding transcription factor-binding sites. First, the known variable-length transcription factor binding site of a TF is modified using the LASAGNA algorithm. The -2 kb upstream promoter region near the transcription start point was the only location that was searched. EBF1, MYC, MYB, USF1, NFkB1, and RORA 1 binding site sequences were discovered through research utilizing the bioinformatics tool "LASAGNA-Search 2.0" in the human PD-1 gene (Table 2). The sequence and position of the transcription factors with a significant p-value were identified.

Overview of the binding pattern of the predicted transcription factors on the PD-1 gene

To determine if the human transcription factors EBF1, MYC, MYB, USF1, NFkB1, and RORA_1 binding region have unique binding sites on the PD-1 gene; we used the Cistrome Data Browser. This portal helps to browse public ChIP-seq and DNase-seq datasets to check the factors that regulate our gene of interest, with transcription factors having significant binding overlap with the peak set in the UCSC Browser. The UCSC Genome Browser provides fast and reliable viewing of any requested portion of genomes Motifs based on p-values. The peak enrichment analysis strongly suggested that two transcription factors EBF1 and MYB specifically bind to their target gene through a well-accepted binding sequence and regulate its downstream genes (Figure 2).

The figure suggests the number of the binding site of EBF1 and MYB directly on the promoter region of the PD-1 gene signature and triggers regulation (Figure 3).

Pathway and gene ontology analysis of PD-1, EBF1 and MYB in breast invasive carcinoma

We hold an ontology analysis of 100 co-expressed genes of PD-1, MYB and EBF1 identified with ARCHS4 RNA-seq gene-gene co-expression from the Enrichr database. This allows us to specify the possible signalling pathways for each of these proteins.

The IL-2 signalling route, the CD8/T cell receptor downstream pathway, and PD-1 signalling were the three most popular BioPlanet 2019 papers from PD-1 linked genes (Figure 4).

Tab. 1. Predicted TFs. TFBS over-representation in the PD1 ChIP-Seq binding profiles using TRAP analysis with a significant p-value.

Sl.no	Matrix name	p-value	Sequence	Position (0-based)
1	*EBF1*	0.00896	CCCCCAGGGA	319, 749, 1633
2	*MYB*	0.0198	GACAGTTG	694, 1410
3	*NFKB1*	0.0199	GGGGGTCTCCC	1772
4	*USF1*	0.0347	CACGTGG	1930, 1931
5	*MYC*	0.0433	CCCACGTGGA	1929
6	*RORA_1*	0.0486	AAGAAGGTCA	1239

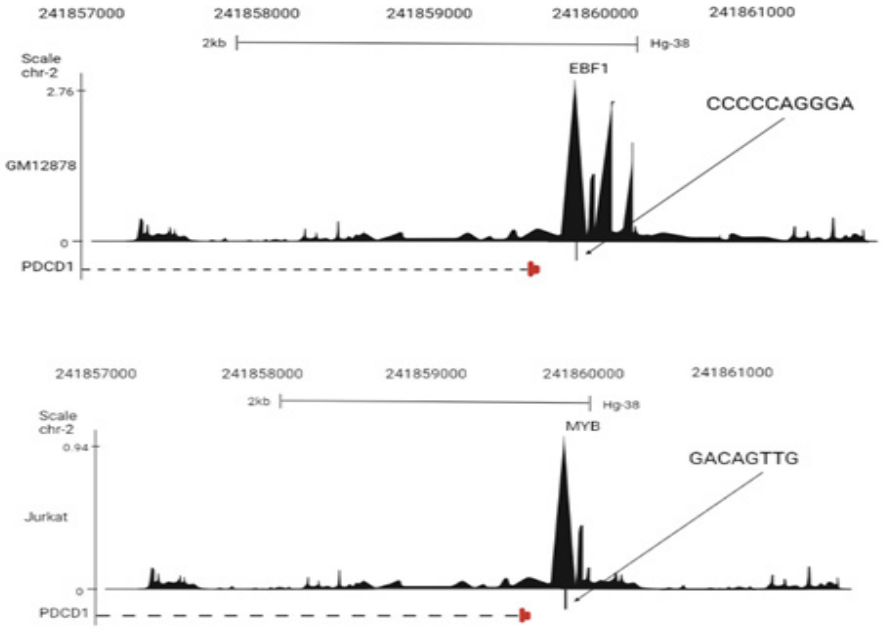

Fig. 2. Binding pattern of TFs. Visualization of EBF1 and MYB binding sites fetched from the UCSC genome browser (version hg38) displaying the locations of TFBS in the promoter regions of PD-1. The peaks represent the critical regions identified from our ChIP-Seq results.

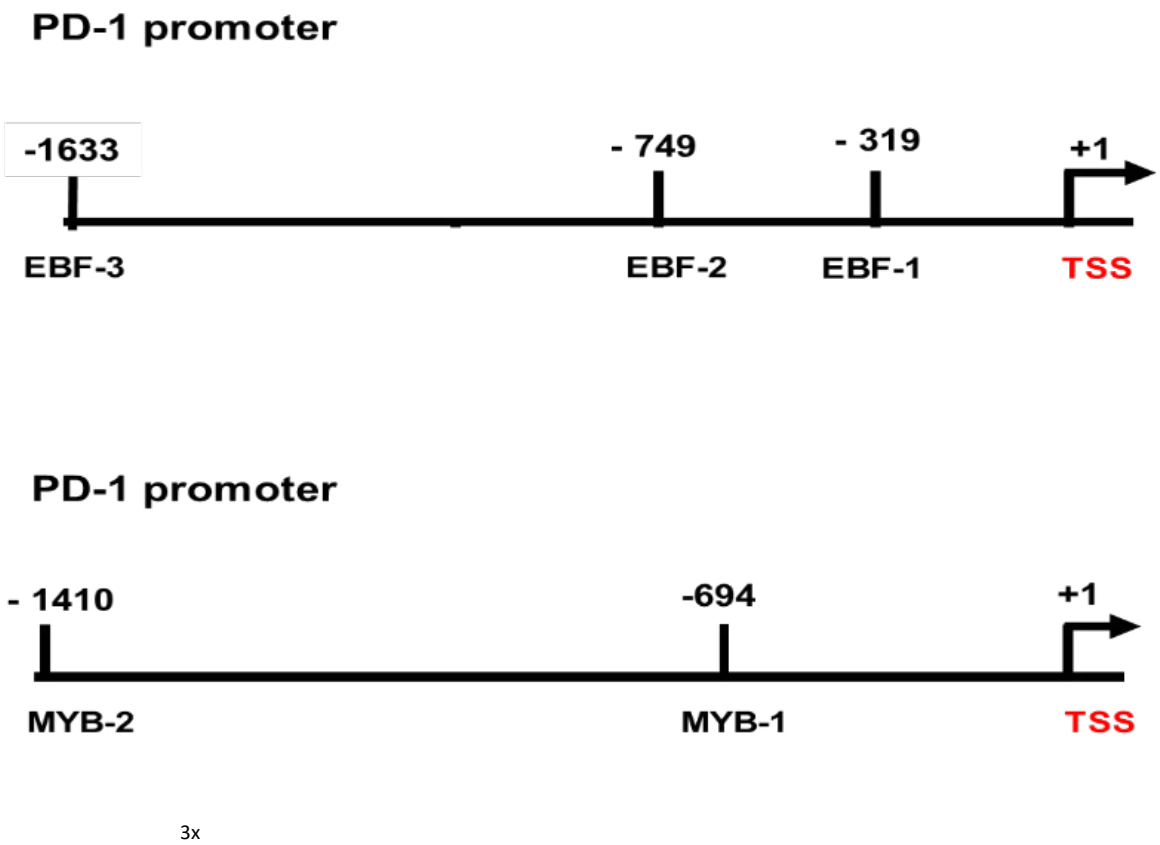

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Fig.3. Examining the binding sites for EBF1 and MYB. In the promoter regions of the human PD-1 gene, the locations of the in silico expected binding sites are schematically shown.

Tab.2. Transcription factors of PD-1 as per Chea 2022 from ARCHS4 RNA-seq gene-gene co-expression matrix.

Term	P-value	Combined Score
STAT3 20064451 ChIP-Seq CD4+T Mouse	5.59E-16	319.8282143
MYB 26560356 Chip-Seq TH2 Human	1.92E-13	173.1269118
FOXP3 17237761 ChIP-ChIP TREG Mouse	1.59E-10	506.5187613
MECOM 23826213 ChIP-Seq KASUMI Mouse	9.34E-10	99.25695613
STAT4 19710469 ChIP-ChIP TH1 Mouse	9.73E-10	116.6550755
FOXO1 25302145 ChIP-Seq T-LYMPHOCYTE Mouse	2.62E-09	89.65243369
GATA3 27048872 Chip-Seq THYMUS Human	3.36E-09	87.43497758
MAF 26560356 Chip-Seq TH1 Human	4.07E-09	85.75723288
SMRT 22465074 ChIP-Seq MACROPHAGES Mouse	4.03E-08	70.62721983
RUNX1 20887958 ChIP-Seq HPC-7 Mouse	5.27E-08	87.99232728

The MYB may be the transcription factor that controls PD-1, according to the Chea 2016 transcription factor targets dataset (Tables 2 and 3). According to the top KEGG pathway for PD-1 and the linked genes, PD-1 has a crucial role in the differentiation of Th17 cells and Th1/Th2 cells (Figure 4).

The top BioPlanet 2019 findings derived from EBF1-linked genes were primarily concerned with beta integrin cell surface contacts and TGF beta control of the extracellular matrix (Figure 4). Additionally, the best findings in M SigDB Hallmark 2020 demonstrate that EBF1 participates in myogenesis, adipogenesis, the epithelial-mesenchymal transition, and apoptosis. EBF1 plays a crucial role in the PI3K-AKT signalling pathway, focal adhesion, melanoma, cardiomyopathy, and pathways in cancer, as well as the Ras signalling route and the MAPK signalling pathway, according to the KEGG and WIKI pathways (Figure 4). More notably, the MGI Mammalian Phenotype level 4 2021 states that the MYB significantly contributes to decreased T-Cell and matured B-Cell numbers, hypoplasia of the thymus, higher incidence of lymphomas and decreased CD-4 positive and thymocyte numbers (Figure 4).

Correlation of PD1, EBF1 and MYB with Cytokines from cBioPortal and TIMER database

Small proteins called cytokines play a key role in regulating the activity and development of cancer cells [19]. We investigated the correlation of PD1, EBF1 and MYB with cytokines in breast cancer pathogenesis and their association with disease activity. cBioPortal Co-expression tool and TIMER 2.0 was used to get the gene expression pattern and correlation of various cytokines (TNF, IFNG, cBioPortal Co-expression tool and TIMER 2.0 was used to get the gene expression pattern and correlation of various cytokines like TNF, IFNG and IL2 with PD-1, EBF1 and MYB across the patients in Breast Invasive Carcinoma (TCGA, PanCancer Atlas) cohort.

Fig. 4. Signaling pathways obtained from the set of co-expressed genes with (A, B) PD1, (C, D) EBF1, and (E)MYB.

1, EBF1 and MYB across the patients in Breast Invasive Carcinoma (TCGA, PanCancer Atlas) cohort. We found genes IFNG, IL2, TNF, IL10, TGFB1, TNFAIP2, TNFAIP3, and TNFAIP8 are correlated positively with PD-1. The IL-2, We found genes IFNG, IL2 and TNF are correlated positively with PD-1. genes are positively correlated with EBF1. And interestingly with MYB, the genes IFNG, IL2, TNF, And interestingly with MYB, the genes IFNG, IL2, TNF,were found to be negatively correlated were found to be negatively correlated (Figure 5-6).

Correlation and expression of PD1, EBF1 and MYB as per TIMER 2.0

Using the TIMER 2.0 database, we investigated the level of PD-1 expression in several breast cancer subtypes as well as

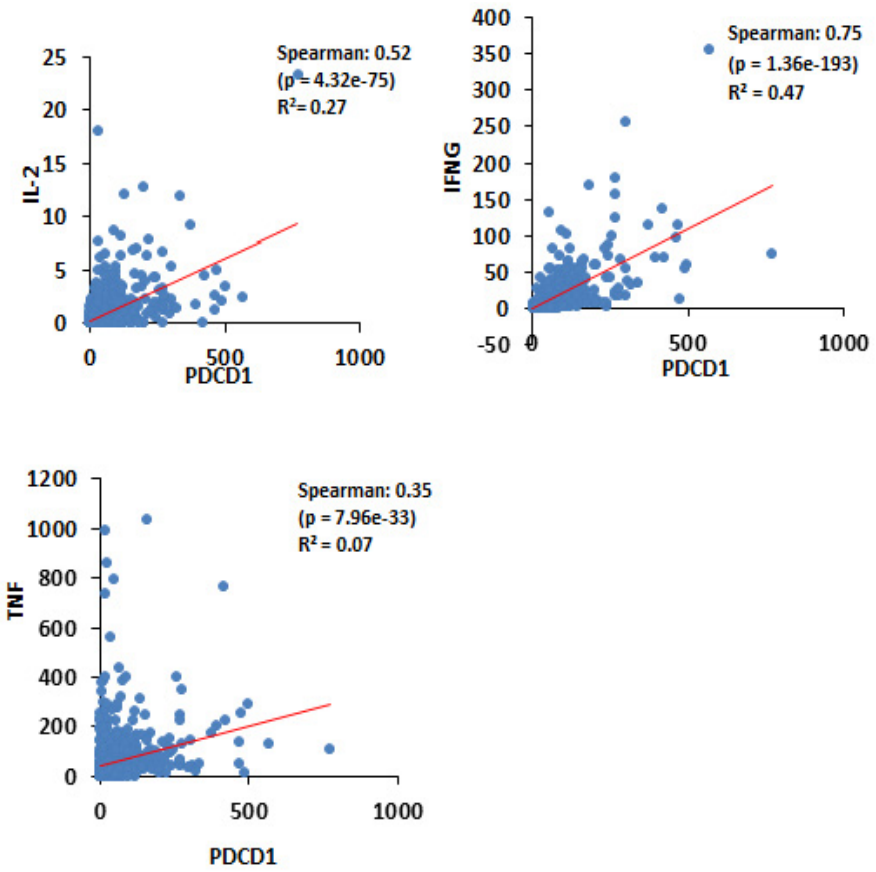

Fig.5. Correlation plots with Cytokines**.** Correlation plots for co-occurrence and correlation of mRNA for the genes PD1 with IL2, IFNG and TNF from cBioportal.

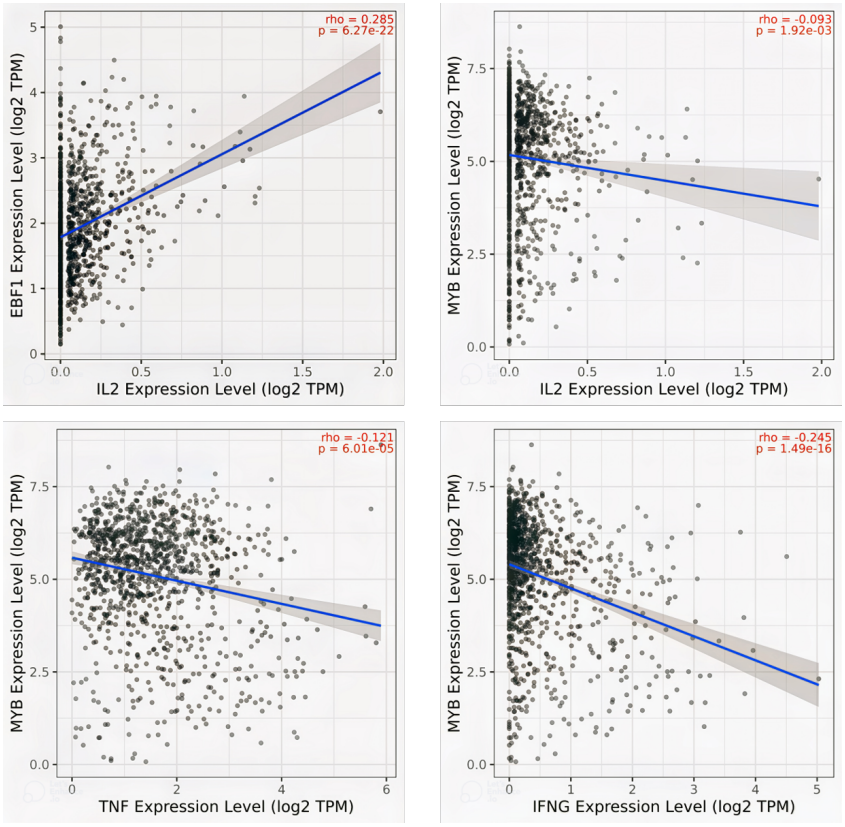

Fig. 6. Correlation plots with Cytokines**.** Correlation plots for co-occurrence and correlation of mRNA for the genes EBF1 with IL-2 and MYB with IL2, IFNG and TNF from TIMER2.0.

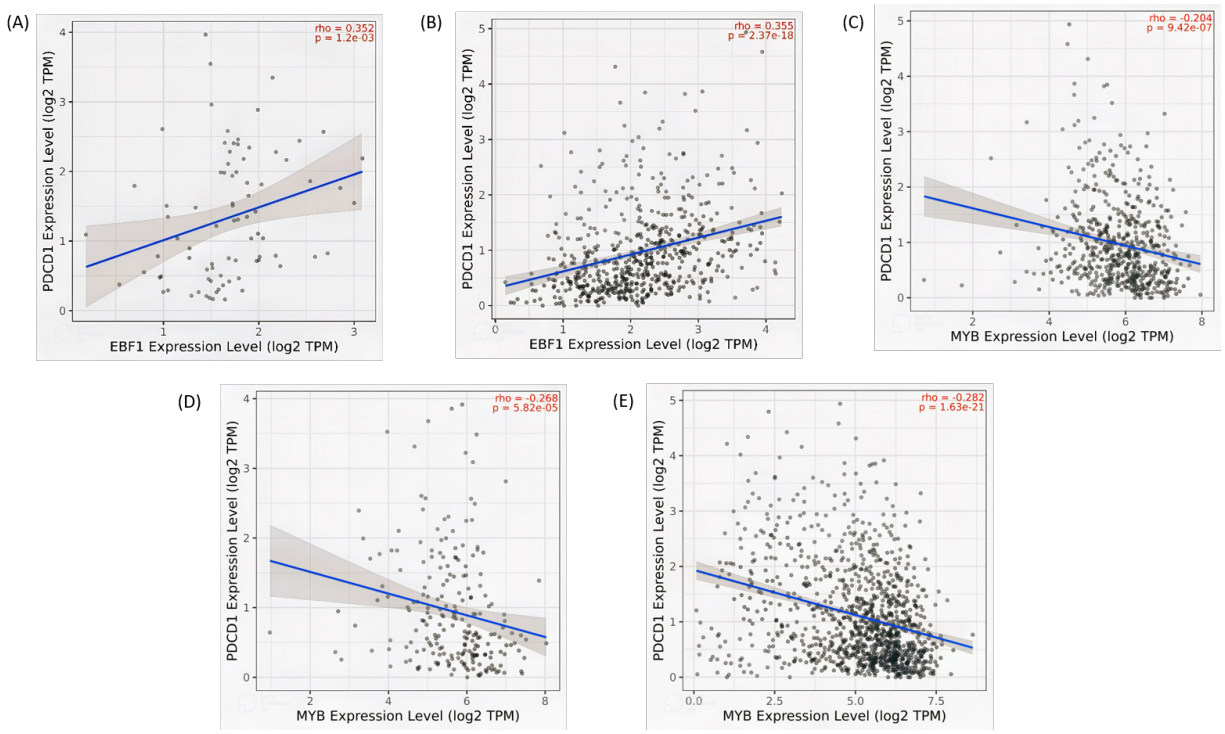

Fig.7. Correlation of PD1, EBF1 and MYB in TIMER **2.0.** A. Favorable correlation of PD-1 and EBF1 in HER2 breast cancer. B. Positive correlation of PD-1 and EBF1 in Luminal A breast cancer. C. Negative correlation of PD-1 and MYB in Luminal A breast cancer. D. Adverse correlation of PD-1 and MYB in Luminal B breast cancer. E. Negative correlation of PD-1 and MYB in breast cancer.

its relationships with EBF1 and MYB. It is revealed that PD1 and EBF1 are positively correlated in HER2 breast cancer and luminal breast cancer. The PD1 and MYB are negatively correlated in Luminal A breast cancer, Luminal B breast cancer and Breast invasive Carcinoma (Figure 7).

DISCUSSION

Growing evidence has revealed that the inhibitory receptor PD-1 plays a vital role in the exhaustion of T cells, tumorigenesis, and advancement of BRCA. According to certain research, PD-1 expression is elevated in the invasive CD8+ T cells of BRCA patients, indicating that PD-1 may have a role in the regulation of tumour growth and the outcomes of immunotherapy. Nevertheless, the prognostic value of PD-1 in the exhaustion of CD8+ T cells in BRCA has yet to be fully explained. Here to determine the regulatory areas in CD8+ tumor-infiltrating T cells that are related to exhaustion in breast cancer patients, we used computational methods to analyze some unexplored transcription factors that regulate the PD-1 gene in CD8+ T cells. The majority of the evidence indicates that PD-1 inhibits T cell activation, multiplication, and cytokine generation, while there is some evidence that the protein also provides T cells with encouraging signals [20]. The transcriptional control of these exhaustion mechanisms is yet unclear. Here, we demonstrate how the transcription factors EBF1 and MYB controlled the presence of the inhibitory receptor PD-1expression, causing CD8+ T cells to become exhausted. Using freely accessible bioinformatics tools, we conducted a comprehensive multi-omics investigation of the EBF1 and MYB expression patterns in breast cancer. EBF1 expression was elevated in all cases, although MYB expression was downregulated, which is an interesting finding. We picked the significant pathways induced by PD-1, EBF1, and MYB and their topmost co-expressed genes. In most cases, PD-1 and its co-expressed genes are connected to the signalling routes associated with cancer development, the IL-2 signalling pathway, CD8/T cell receptor downstream pathway, and PD-1 signalling. Interestingly the Chea 2016 transcription factor targets dataset also disclosed that the MYB could be the regulating transcription factor of PD-1. The top KEGG pathway of PD-1 has an influential role in Th17, Th1 and Th2 cell differentiation. It is known that the Th17 and Th17-prompted CD8+ T cells typically yield preventive and therapeutic antitumor immunity [21]. And the contact between the costimulatory molecule PD-1 and its ligand PD-L1 is known to regulate Th17 cells [22]. Th1 cells making IFN-gamma, IL-2, and TNF-beta trigger macrophages and initiate cell-mediated defence. Th1 cells, vital for antitumor and anti-infection immunity, are subjected to the challenge of PD-L1-mediated conversion to a Treg phenotype [23]. Th2 cells producing IL-4, IL-5, IL-10, and IL-13 are accountable for vigorous antibody production [24]. Some studies imply that Th1 and Th2 cells help CD8 T-cell activation, and memory acquisition and induce effector qualities of CD8+ T cells [25]. To selectively regulate Th2-associated inflammatory responses, PD-1 signalling is activated [26]. Moreover, PD-1 and its correlated genes are involved in IL-2/STAT5 signalling, inflammatory response, and IL-6/JAK/STAT3 signalling. IL2 and STAT5 together induce Tregs and governs Treg biology [27].

More than 40% of breast tumours, among other cancer types, activate STAT3. The binding of IL-6 and IL-11 to their receptors triggers signalling through the IL-6/JAK/STAT3 pathway, which promotes the growth of breast cancer [28]. It is understood that the interplay of PD-1 and PD-L1 is a decisive factor in regulating T cell signalling pathways, thus preventing T lymphocyte proliferative and effector functions.

EBF1 also plays a vital role in the TGF-beta control of the extracellular matrix, epithelial-mesenchymal transition, PI3K-AKT signalling circuit, Ras signalling route, and MAPK signalling pathway, according to the ENRICHR database. According to earlier findings, TGF-1 causes T cell suppression by upregulating PD-1 in the TME [29]. Cancer cells aid in the loss of epithelial characteristics and the gain of mesenchymal phenotype throughout the epithelial-to-mesenchymal transition phase. Such change encourages the migration and invasion of cancer cells [30]. Interestingly, breast cancer is known to activate the PI3K/Akt/mTOR and Ras pathways [30-32]. Another study shows that over-expression of EBF1 in colon cancer cell lines inhibits cell viability and survival, which results in cell cycle arrest, and decreases cell growth. According to these findings, EBF1 may be a new tumour suppressor in colon cancer [33]. Similar to this, over-expression of EBF1 in CD8+ T cells may inhibit cell development and cause apoptosis in these cells. Additionally, databases revealed that overexpressing EBF1 reduces cell growth and triggers cell death via the p53/p21 pathway [34]. Gene ontology analysis for MYB was fascinating. It has a role in menopause, breast cancer, decreased T cell and matured B cell number, thymus hypoplasia, increased lymphoma incidence, decreased cd4positive and thymocyte number, and increased central memory cd8 positive T cells. According to earlier research, TCF1, CD62L, and MYB are co-expressed by CD8+ T cells during chronic infection. The entire CD8+ T cell response disintegrates when these cells lose MYB [35]. MYB is required to survive the developing thymocytes, the proliferation of double-positive thymocytes and mature T cells, and the differentiation of single-positive CD4/CD8 T cells [36]. Transversely, in colon cancer, it was seen that overexpression of MYB is associated with enhanced Bcl-xL protein levels predicting poor prognosis and contributing to enhanced tumorigenicity [37]. c-Myb pathway overactivation is related to disorders in cellular homeostasis, cell proliferation, and malignancy in adult T-cell leukaemia cells. Knockdown of total MYB yielded adult T-cell leukaemia cell death [38, 39]. These findings imply that Myb proteins play a crucial new role in regulating cell survival during carcinogenesis. Correlation analysis with cytokines (IL-2, IFN- γ, TNF-α, IL10, TGFBs, TNFAs) revealed that largely cytokines are positively correlated with PD-1 and EBF1 and negatively correlated with MYB. Previous analyses have shown that circulating pro-inflammatory cytokines, TNF, IL-6, and anti-inflammatory cytokine, IL-10, are increased in breast cancer and chronic disorders [40,41]. Additionally, TNFAIPs which are produced from TNF, help tumour cells grow and spread through the NF-κB signalling pathway [42]. The TCGA analysis indicated that the PD 1 expression and cytokine signature (TNFAIPs, TGFBs) exhibited a positive correlation. Then when we analyzed the correlation and expression of PD-1 with the TFs EBF1 and MYB as per TIMER 2.0, it provided a shred of clear evidence that PD1 and EBF1 are positively correlated to each other, whereas MYB expression is negatively related to PD-1. Hence, the data suggest that EBF1 is a TF that positively regulates PD-1 and cytokine production, and MYB negatively regulates PD-1 and cytokine production.

CONCLUSION

This work is based on the hypothesis that immunological infiltrating CD8+ T cells from breast cancer have high levels of PD-1 inhibitory receptor expression. In this multi-omics investigation, we made use of a variety of bioinformatics methods to demonstrate the effectiveness of two transcription factors, EBF1 and MYB, of the gene PD-1 as novel therapeutic biomarkers for CD8+ T cell exhaustion in breast cancer. We carried out a methodical multi-omics analysis to look at how the PD-1 gene's transcription factors, pathway and gene ontology analysis, expression level, and impact on different signalling pathways linked to the emergence of breast cancer. Additionally, examined were the relationships between PD-1 and its TFs, their expression patterns, and the association between TFs and cytokine production. The research presented here examined two new transcription factors of the checkpoint inhibitor PD-1, EBF1 and MYB. EBF1 expression is markedly elevated in CD8+ T cells from breast cancer, and it positively correlates with PD-1. MYB expression is significantly downregulated in CD8+ T cells from breast cancer patients, despite the fact that it is inversely linked with PD-1. According to several databases, these two transcription factors (TFs) are engaged in a variety of cancer signalling pathways that control the release of cytokines and the exhaustion of CD8+ T lymphocytes. Given these results, it is important to consider the importance of EBF1 and MYB expression on CD8+ T cells. It would be ideal to have a more comprehensive molecular understanding of the oncological significance and functional role of EBF1 and MYB in breast cancer.

ABBREVIATIONS

CTLA4: Cytotoxic T-lymphocyte-associated protein 4; EMT: Epithelial-mesenchy maltransition; IFN: Interferon; IL: Interleukin; LAG-3: Lymphocyte-activation gene 3; NK: PD-1:Programmeddeath-1; PDL1: Programmed death ligand-1; Th1: Type I T helper cell; TIM-3: T-cell immunoglobulin and mucin-domain containing-3; TNF: Tumor necrosis factor; Tregs: Regulatory T cells; TFs: transcription factor's; TRAP: transcription factor affinity prediction; TCGA: The Cancer Genome Atlas; TIMER 2.0: Tumor IMmune Estimation Resource; NK: Natural killer cells; DNA: Deoxyribonucleic acid; EBF1: Early B-Cell Factor 1; USF1: Upstream transcription factor 1; NFkB1: Nuclear factor kappa B subunit1;ChIP:Chromatin Immunoprecipitation; TFBS: Transcription factor binding site; KEGG: Kyoto Encyclopedia of Genes and Genomes; TNFAIP: Tumor necrosis factor-alpha-induced proteins.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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