ORIGINAL ARTICLE

Inferring Transcription Factors and microRNAs Associated with Elevated Expression of the Oncogenic B-Cell Lymphoma 11A in Triple Negative Breast Cancer

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Abstract

B-cell lymphoma 11A, a transcriptional repressor, is highly expressed in triple negative breast cancer. The *in vitro* studies and animal models provide initial evidence suggesting that the knockdown of B-cell lymphoma 11A has a therapeutic effect on breast cancer. Defining the regulators driving the high expression of B-cell lymphoma 11A is important to understand its cancer-related functions. Among these regulators, transcription factors and microRNAs are critical for gene expression and associated with expression perturbations. Firstly, we identified the transcription factors that potentially interact with B-cell lymphoma 11A promoter. Based on bioinformatics prediction and multiple Omics datasets, two upregulated transcriptional activators Zinc Finger BED-Type Containing 4 and E2F Transcription Factor 1 in triple negative breast cancer were found to have seven sites within B-cell lymphoma 11A promoter. Secondly, we aimed to determine a putative set of microRNA that can mediate the post-transcriptional repression of B-cell lymphoma 11A. miR-513a-5p, miR-139-5p, miR-1179, miR-140-5p, and miR-542-3p, harboring at least one site of interaction with B-cell lymphoma 11A 3' untranslated region, were found inhibited in triple negative breast cancer. Taken together, the combinatorial regulation by transcription factors and microRNAs provide valuable information for further investigation on controlling the expression level of B-cell lymphoma 11A in triple negative breast cancer.

Keywords

B-cell lymphoma 11A; Breast cancer; Transcription factors; microRNA; Omics; Bioinformatics

Introduction

Breast cancer is the second major cause of death in economically developed and less developed countries. Nearly 1.67 million new cases of breast cancer were diagnosed around the world in 2012 and about 522,000 died of this cancer[1]. Breast cancer is a heterogeneous disease and each of its subtypes can be associated with a unique set of biomarkers. B-cell lymphoma 11A (BCL11A), a multiple C2H2 zinc finger containing transcription factor, was identified to be elevated in breast cancer tissues compared with normal tissues, particularly triple negative breast cancer stem and progenitor cells[2]. Triple negative breast cancer (TNBC) subtype accounts for 10–15% of all breast cancers, however, it is associated with a higher recurrence and poorer survival rates when compared with other subtypes. Disruption of BCL11A expression in TNBC cell lines and in xenograft mouse models significantly reduced the cancer development and progression. Consistent with this, the deletion of BCL11A in a DMBA-induced tumor mice model substantially decreased tumor formation as well as in p53-null mice model for cancer. The transcription factor, BCL11A, appears to be a novel oncogenic target for the treatment of TNBC breast cancer[2]. Moreover, the high expression of BCL11A appears to have a critical role in other aggressive cancers such as B cell chronic lymphocytic leukemia (CLL)[3].

Though the role of BCL11A in the tumorigenesis has been studied, its transcriptional regulation remains unknown. However, its transcriptional regulation of BCL11A has been investigated in other biological contexts such as the regulation of erythropoiesis and fetal hemoglobin level[4]. Kruppel Like Factor 1 (KLF1) is a direct transcriptional activator of BCL11A gene in erythroid progenitor cells[5]. Besides transcription factors, microRNAs (miRNAs) are critical regulators for the post-transcriptional and/or translational level of gene expression. Recent studies reported significant evidence that the deregulated miRNAs regulate the expression of their target genes in cancer[6]. The main role of miRNAs is to inhibit protein expression through interaction with seed complement sites to the 3 untranslated region (3'UTR) of its target messenger RNA (mRNAs), and thereby negatively influence mRNA translation[7]. There is no work that has identified miRNAs able to regulate the expression of BCL11A protein in breast cancer cells.

In this paper we address two central questions concerning the transcriptional and post-transcriptional regulation of BCL11A in breast cancer cells using a combinatorial method of bioinformatics and omic analysis. (1) Whether high expressed transcription factors with the potential to enhance transcription through direct interaction with BCL11A promoter region were present in breast cancer tissues compared with the controls, and (2) whether a decrease in the expression of miRNAs can result in the high expression of BCL11A in breast cancer versus the control cells.

Methods

The Sequence of BCL11A Promoters

The promoter sequences of mouse and human BCL11A were obtained using the GenBank nucleotide sequence database (National Center for Biotechnology Information, Bethesda, MA USA).

MatInspector Prediction Search

MatInspector prediction search (Genomatix GmbH, Munich, Germany) for transcription factor binding sites (TFBSs) is based on a number of parameters including the core and matrix similarities, which are calculated in MatInspector. The core similarity was set to 1 and > 0.95 for the matrix, whereas the optimized matrix threshold was left at the default level[8].

TumourProfile Database

TumourProfile database (Peking University Center for Human Disease Genomics, Beijing, China) (http://tumour.bjmu.edu.cn/) is a database on gene expression profile (GEP) across multiple datasets, mainly microarray transcriptional profiling, of human normal, non-cancerous, cancerous tissues, and cancer cell lines. The *P* values and Pearson's correlation coefficient (r) between tumour profiles were calculated in the database to measure the strength of the correlation between two variables.

BioXpress v1.0

BioXpress (https://hive.biochemistry.gwu.edu/tools/bioxpress/) is a database for curated gene expression in normal and cancer tissues. Based on RNA-seq data provided by The Cancer Genome Atlas, International Cancer Genome Consortium, Expression Atlas and publications, the expression level of genes were calculated[9].

Oncomine™

Oncomine™ (Thermo Fisher Scientific Inc., Waltham, MA USA) (www.oncomine.org) is a curated database containing a large number of cancer transcriptome profiles. The analysis engine is used to measure the differential expression comparing diverse cancer tissues with respective normal tissues as well as cancer subtypes[10].

Human Protein Atlas

Human Protein Atlas (The Knut and Alice Wallenberg Foundation, Stockholm, Sweden) (www.proteinatlas.org/) is a database for human protein expression patterns in normal and disease tissues using a range of experimental procedures, from the classical experiments such as Elisa, Western blot and immunohistochemistry to antibody-based proteomics approaches[11].

Triple-Negative Breast Cancer Database

Triple-Negative Breast Cancer Database (Rajiv Gandhi Centre for Biotechnology, Kerala, India) (http://210.212.254.116/tnbcdb/) is a curated database of the expression profile of miRNA, mRNA, protein and phosphoprotein levels. Changes in expression are measured in comparison with the normal tissues or other subtypes of breast cancers[12].

TargetScan

TargetScan (Whitehead Institute for Biomedical Research, Cambridge, MA USA), (www.targetscan.org/) is *in silico* miRNA target prediction; an algorithm was developed to predict miRNA sites based on the rules and patterns of interaction between mRNAs and miRNAs.

Results

Identification of Potential Transcription Factors Related to the High Expression of BCL11A in Breast Cancers

The BCL11A promoter region was retrieved from National Center for Biotechnology Information database (June, 2016). The length of retrieved promoter is 600 bp (from 500 bp upstream to 100 bp downstream of the transcription start site (TSS)). In order to identify the transcription factor binding sites of BCL11A promoter the sequence was uploaded in MatInspector with core and matrix similarity cut-offs 1 and 0.95, respectively. At least 22 transcription factor families were predicted to occupy at least two interaction sites in BCL11A promoter (Table 1). These families include 33 different transcription factors. Next, we assessed which of these 33 transcription factors can be responsible for the high expression of BCL11A in breast cancer cells. Therefore, each transcription factor was uploaded in three different databases (BioXpress v1.0, Oncomine™, and Human Protein Atlas (June, 2016)) to determine their expression levels in normal breast tissue compared to cancer tissues. Each transcription activator or repressor has a consistent upregulation or downregulation in at least two databases included in our results (Table 2). There are 5 upregulated transcriptional activators (Zinc Finger BED-Type Containing 4 (ZBED4); WT1, Zinc Finger and BTB Domain Containing 7B (ZBTB7B); Zinc Finger Protein 35 (ZNF35); E2F1) and 3 downregulated repressors (Kruppel Like Factor 6 (KLF6); Zinc Finger Protein 300 (ZNF300); Zinc Finger Protein 219 (ZNF219)) in breast cancers. Increased transcriptional activators and inhibited repressors may contribute to the increased expression of BCL11A in breast cancers.

Identification of Potential Transcription Factors Related to the High Expression of BCL11A in TNBC Compared to Non-TNBC

In order to further assess the potential transcription factors involved in the increased BCL11A in triple negative breast cancer, we used the omics dataset from the *Gene Expression Omnibus* (GEO) database (June, 2016) to determine the differential expression of the transcription factors between triple negative and non-triple negative breast cancer. At least 3 transcriptional activators (ZBED4, ZNF35, E2F Transcription Factor 1 (E2F1)) showed a higher level in triple negative than its expression level in non-triple negative breast cancer (Table 3). However, no transcriptional repressors displayed a further decreased level in triple negative breast cancer. The increased level of the transcriptional activators ZBED4, ZNF35, E2F1, may contribute to the higher BCL11A in triple negative breast cancer compared with other breast cancer subtypes.

Correlation between BCL11A and Transcription Factor Expression

We then assessed whether the expression of BCL11A and its potential transcription factors (ZBED4, ZNF35, E2F1) are co-regulated. Therefore, Pearson's correlations were calculated in TumourProfile database. As shown

Table 1. A list of predicted matrix families and their specific transcription factors. The MalInspector software was used and its parameters set the core and matrix similarities to 1 and 0.95. Each family with two or more binding sites was included. The table also includes the position and strand, where each transcription factor can bind to the sequence of BCL11A promoter.

Matrix Family	Detailed Family Information	Matrix	Detailed Matrix Information	Location	Strand	Matrix Similarity
BEDF	BED subclass of zinc-finger proteins	ZBED4	Zinc finger, BED-type containing 4; GC-box binding sites	5 -19 34-48 36-50 138-152 209-223 193-207	+ - - - - -	0.956 0.96 0.957 0.958 0.976 0.96
CIZF	CAS interacting zinc finger protein	ZNF384	Zinc finger protein 384	246-256 428-438 507-517	- - -	0.965 0.96 0.961
E2FF	E2F-myc activator/cell cycle regulator	E2F4	E2F transcription factor 4, p107/p130-binding protein	148-164	-	0.976
		E2F1	E2F transcription factor 1	191-207	-	0.988
EGRF	EGR/nerve growth factor induced protein C & related factors	WT1	Wilms Tumor Suppressor	4-22 31-49	+ -	0.984 1
		EGR2	Egr-2/Krox-20 early growth response gene product	6-24 29-47 42-60	+ - -	0.965 0.963 0.981
		ZBTB7B	Zinc finger and BTB domain containing 7B)	152-170	-	0.967
EVI1	EVI1-myleoid transforming protein	PRDM16	PR/SET domain 16	432-448 545-561	- -	0.958 0.963
HIFF	Hypoxia inducible factor, bHLH/PAS protein family	ARNT	Aryl hydrocarbon receptor nuclear translocator	21-37	-	0.985
		HIF1A	Hypoxia inducible factor 1 alpha subunit	384-400	-	0.978
KLFS	Kruppel like transcription factors	KLF2	Kruppel-like factor 2 (lung) (LKLF)	34-52 47-65 56-74	- - -	0.996 0.996 0.987
		KLF3	Kruppel-like factor 3 (basic)	136-154 153-171 207-225	- - -	0.994 1 0.995
		KLF6	Core promoter-binding protein (CPBP) with 3 Kruppel-type zinc	203-221	-	0.954
MAZF	Myc associated zinc fingers	MAZ	Myc associated zinc finger protein (MAZ)	144-156 156-168	- -	0.97 0.97
MZF1	Myeloid zinc finger 1 factors	MZF1	Myeloid zinc finger protein MZF1	11-21 32-42 142-152 159-169 207-217 213-223 289-299 302-312	+ - - - - - - -	0.996 0.996 0.995 0.974 0.986 0.995 1 0.991
NDPK	Nucleoside diphosphate kinase	NME1	NME/NM23 nucleoside diphosphate kinase 1	153-169 207-223	- -	0.957 0.968
PLAG	Pleomorphic adenoma gene	PLAG1	Pleomorphic adenoma gene 1	8-30 10-32 21-43 23-45 125-147 148-170 167-189 180-202 196-218 414-436	+ + - - - - + - - +	0.959 0.955 0.955 0.959 1 0.967 0.967 0.967 1 1

BCL11A: B-cell lymphoma 11A

Table 1. ***(Continuation)*** A list of predicted matrix families and their specific transcription factors. The MalInspector software was used and its parameters set the core and matrix similarities to 1 and 0.95. Each family with two or more binding sites was included. The table also includes the position and strand, where each transcription factor can bind to the sequence of BCL11A promoter.

Matrix Family	Detailed Family Information	Matrix	Detailed Matrix Information	Location	Strand	Matrix similarity
PURA	Pur-alpha binds both single-stranded and douple-stranded DNA in a sequence-specific manner	PURA	Purine-rich element binding protein A	153-165 207-219 293-305	- - -	0.961 0.984 0.963
SAL2	Spalt-like transcription factor 2	SALL2	Zinc finger protein Spalt-2, sal-like 2, p150(sal2)	39-49 52-62	- -	0.963 0.969
SIX3	Sine oculis homeobox homolog 3	SIX3	SIX3 / SIXdomain (SD) and Homeodomain (HD) transcription factor	564-584 575-595	- +	0.955 0.961
SMAD	Vertebrate SMAD family of transcription factors	SMAD3	Smad3 transcription factor involved in TGF-beta signaling	175-185 354-364	+ -	0.98 1
SP1F	GC-Box factors SP1/GC	SP1	Stimulating protein 1, ubiquitous zinc finger transcription factor	150-166 193-209	- -	0.969 0.997
YY1F	Activator/repressor binding to transcription initiation site	YY2	Transcription factor yin yang 2	422-444	+	0.953
		YY1	Yin and Yang 1 repressor sites	558-580	+	0.966
ZF02	C2H2 zinc finger transcription factors 2	ZNF148	Zinc finger protein 148	2-24 204-226 29-51	- + +	0.959 0.966 0.955
		ZKSCAN3	Zinc finger with KRAB and SCAN domains 3	32-54 35-57 128-150	+ + +	0.973 1 0.953
		ZNF300	KRAB-containing zinc finger protein 300	51-73 126-148 152-174 134-156 195-217 157-179 404-426 190-212	+ + + + + + - +	0.984 0.95 0.954 0.957 0.954 0.987 0.99 0.954
		ZBTB7A	Zinc finger and BTB domain containing 7A	137-159 154-176 192-214	+ + +	0.99 0.986 0.951
		ZNF219	Kruppel-like zinc finger protein 219	207-229	+	0.99
ZF07	C2H2 zinc finger transcription factors 7	ZNF263	Zinc finger protein 263, ZKSCAN12 (zinc finger protein with KRAB and SCAN domains 12)	139-153 156-170 210-224 296-310 299-313	+ + + + +	0.967 0.976 0.967 0.969 0.989
ZF35	Zinc finger protein ZNF35	ZNF35	Human zinc finger protein ZNF35	27-39 550-562	- -	0.962 0.953
ZF5F	ZF5 POZ domain zinc finger	ZBTB14	Zinc finger and BTB domain containing 14	97-111 133-147	- -	0.955 0.951
ZTRE	Zinc transcriptional regulatory element	ZNF658	Zinc finger protein 658	135-151 143-159 152-168 160-176 206-222 214-230	- + - + - +	0.983 0.989 0.984 0.993 0.999 1

BCL11A: B-cell lymphoma 11A

Table 2. The list of differentially expressed transcription factors in breast cancer tissues compared with control tissue. Three databases for gene expression analysis were used, BioXpress v1.0, Oncomine™, and Human Protein Atlas. Activators or repressors are included if these factors have shown at least in two databases an increase or decrease in their expression levels, respectively. TFBS and FC indicate transcription factor binding site and fold change.

Matrix Family	Detailed Family Information	TF	Detailed TF Information	ID	Function	BioXpress v1.0	Oncomine™	Human Protein Atlas	
						BC *vs.* Normal	BC *vs.* Normal	Normal	BC
BEDF	BED subclass of zinc-finger proteins	ZBED4 6 TFBSs	Zinc finger, BED-type containing 4; GC-box binding sites	9889	Transcriptional activator	Up 83.19 % FC: 0.77	NA	++	+++ ++
EGRF	EGR/nerve growth factor induced protein C & related factors	WT1 2 TFBSs	Wilms Tumor Suppressor	7490	Transcriptional activator	Up 84.96% FC: 3.31	Up P-value: 0.018 FC: 1.134	+	+
		ZBTB7B 1 TFBS	Zinc finger and BTB domain containing 7B	51043	Transcriptional activator	Up 82.3 % FC: 0.82	NA	++	+++ ++ +
ZF35	Zinc finger protein ZNF35	ZNF35 2 TFBS	Human zinc finger protein ZNF35	7584	Transcriptional activator	Up 73.45 % FC: 0.54	Up P-value: 1.26E-4 FC: 1.327	++	+
E2FF	E2F-myc activator/cell cycle regulator	E2F1 1 TFBS	E2F transcription factor 1	1869	Transcriptional activator	Up 97.35 % FC: 2.2	Insignificant increase	++	+++ ++ +
KLFS	Kruppel like transcription factors	KLF6 1 TFBS	Core promoter-binding protein (CPBP) with 3 Kruppel-type zinc	1316	Transcriptionalrepressor	Down 87.61% FC: -1.43	Down P-value: 4.20E-8 FC: -3.230	+++	+++ ++ +
ZF02	C2H2 zinc finger transcription factors 2	ZNF300 8 TFBSs	KRAB-containing zinc finger protein 300	91975	Transcriptionalrepressor	Down 70.8% FC: -1.57	Down P-value: 0.033 FC: -1.069	+++	+++ ++
		ZNF219 1 TFBS	Kruppel-like zinc finger protein 219	51222	Transcriptionalrepressor	Down 89.38% FC: -1.55	Down P-value: 6.29E-6 FC: -1.803	++	+++ ++ +

TFBS: Transcription factor binding site; FC: Fold change

Table 3. The average of differential expression intensities of the transcriptional factors in triple negative breast cancer compared with non-triple negative breast cancer. AV and ARS indicate the average value and the average rank score, respectively.

TF	Detailed TF Information	Non-Triple Negative Breast Cancer		Triple Negative Breast Cancer		Sample Size	GEO Profile ID
		AV	ARS	AV	ARS		
ZBED4	Zinc finger and BTB domain containing 7B	303.50	67.36	344.55	71.8	19	ID: 77907276
ZNF35	Human zinc finger protein ZNF35	193.17	58.29	231.46	56.2	19	ID: 77907803
E2F1	E2F transcription factor 1	109.23	38.07	134.96	41.8	19	ID: 77906428

in (Fig.1), there are significant levels of co-regulation between BCL11A and ZBED4 (*r value* = 0.196, *P* value = 4.3E-28) or BCL11A and E2F1 (*r value* = 0.17, *P* value = 7.84E-22) while the expression profile of BCL11A and ZNF35 in breast cancer has shown insignificant correlation. These correlated genes ZBED4 and E2F1with BCL11A are likely to be functionally linked and contribute to regulate a common biological pathway.

High Conservation of ZBED4 and E2F1 Binding Sites between Orthologous Promoters

In order to examine conservation of transcription factor binding sites between human and mouse, we aligned both species BCL11A promoter sequences (~600bp) and found ~56% of the sequence are not aligned. However, at least a site for ZBED4 and another site for E2F1 are highly conserved between mouse and human (Fig. 2).

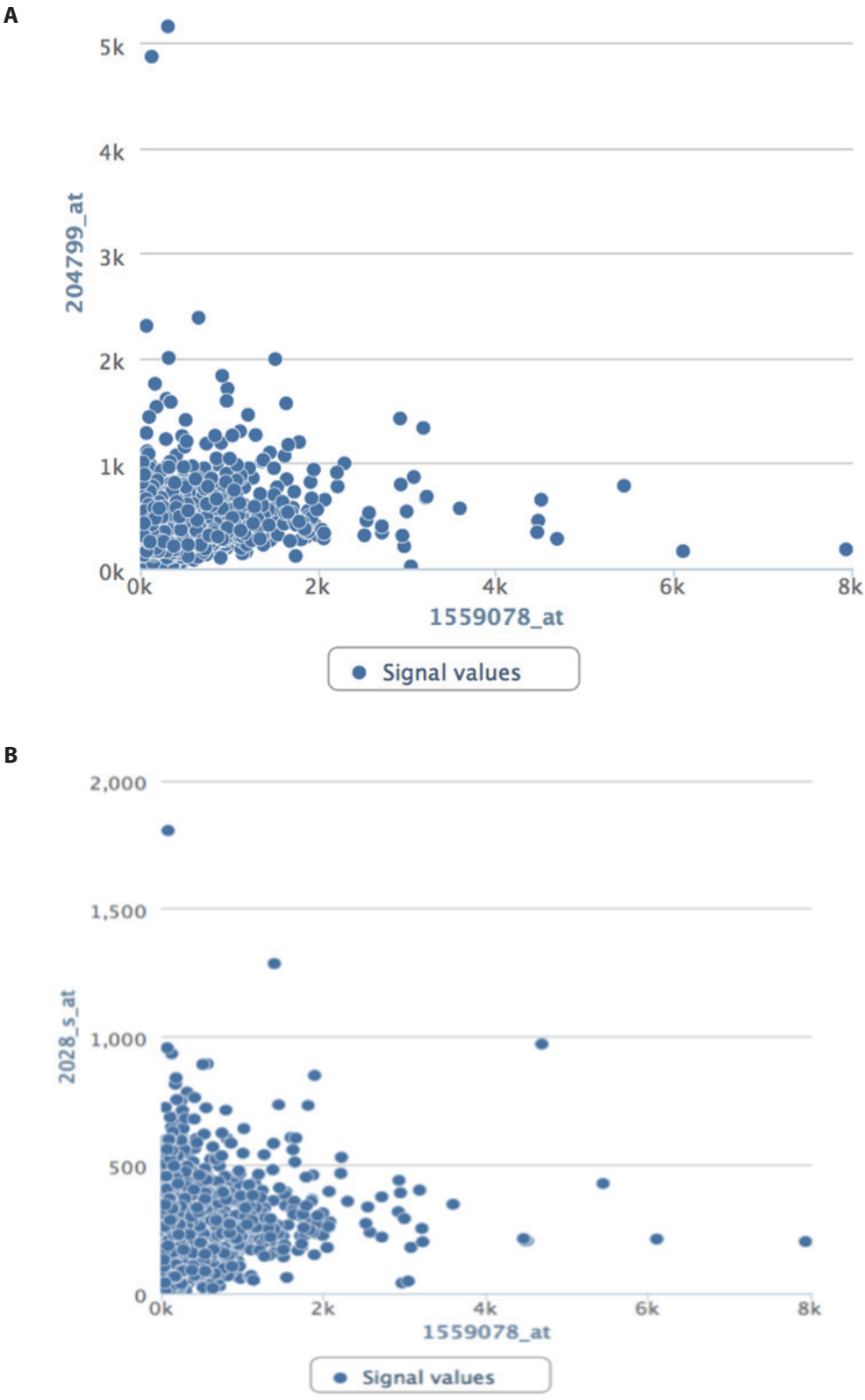

Figure 1. TBCL11A and transcription factors are significantly correlated and co-expressed in breast cancer tissues. The correlation between probe sets for (A) ZBED4 or (B) E2F1 and BCL11A is shown.

Figure 2. Sequence alignment of BCL11A promoters showing 44% conservation between the mouse and human.

Identification of potential miRNAs related to the high expression of BCL11A in TNBC

miRNAs can affect gene expression, which target BCL11A mRNAs and suppress its translation into protein. It is possible that the increased level of BCL11A gene is likely attributed to downregulated miRNAs bearing interaction sequences on the 3'UTR for BCL11A mRNAs. In order to determine the list of those candidate miRNAs, we obtained the profile of miRNA expression from Triple-Negative Breast Cancer Database (TNBCDb) viewer. The profile compares the non-metastatic or metastatic triple negative breast cancer with normal adjacent breast tissues. Among 108 downregulated miRNAs in TNBC, five of them were identified as able to recognize BCL11A mRNAs by using at least 6-8 complement nucleotide (Table 4). According to TargetScanHuman (Whitehead Institute for Biomedical Research, Cambridge, MA USA), Release 7.1, miR-513a-5p and miR-139-5p have two target sites; whereas, miR-1179, miR-140-5p, and miR-542-3p have one site within BCL11A 3'UTRs.

Discussion

Breast cancer is the second leading cause of death among females worldwide[1]. Genome-wide expression studies helped to determine the heterogeneity and complexity of breast cancer. Breast cancer subtypes: i) ER+/luminal, ii) HER2+ (HER2-enriched), iii) basal-like or TNBC, and iv) normal-like have not only distinct expression profiles but also different response patterns to treatments impacting the clinical outcomes[13,14]. Among them, TNBC shows the most aggressive biological behavior and highest recurrence rate[15,16]. Due to a lack of therapeutic target for TNBC, it remains a significant research challenge. Thus, more understanding for the mechanism underlying the development, maintenance, and metastatic process of TNBC is highly required. Interestingly, increased expression of BCL11A has been reported in B cell lymphoid malignancies as well as breast cancers, in particular triple negative breast cancer. This alternation of BCL11A gene expression is related to the cancer pathogenesis[2]. Transcription factors and miRNAs are important regulators of gene expression at the transcriptional and post-transcriptional levels, respectively. The promoter region of BCL11A remains to be characterized, however, the present work found transcription factors such as KLF6, ZNF300, ZNF219, ZBED4 and E2F1 as potential inducers of BCL11A transcription in cancer cells. Studies on KLF6, ZNF300, ZNF219, ZBED4 and E2F1 revealed that the binding sites of these transcription factors in human promoters can positively affect the BCL11A transcription[17-21]. In agreement with the role of BCL11A in breast cancer, KLF6 was reported to have significant effect on breast cancer metastasis and associated with poor clinical outcomes[22]. The functions of ZNF300, ZNF219, ZBED4 and E2F1 have not been studied in breast cancer yet, however, the overexpression of ZNF300 resulted in enhanced uncontrolled proliferation and metastasis of HeLa cancer cells *in vitro* and *in vivo*[23]. Furthermore, E2F1 is suggested to behave as an oncoprotein being an important regulator of cell cycle progression and growth signaling in a tissue-specific manner[24].The higher BCL11A level in triple negative breast cancer tissues correlated with an increase in the expression levels of ZBED4 and E2F1 in this cancer subtype versus others. More validation experiments are essential to confirm whether these transcriptional activators are associated with BCL11A expression in breast cancer and triple negative disease. Besides the transcriptional regulation, BCL11A can be regulated post-transcriptionally by miRNAs. We therefore hypothesized a relationship between the

Table 4. A list of downregulated miRNAs in TNBC, which has at least one target site within BCL11A 3'UTRs using Triple-Negative Breast Cancer Database and TargetScanHuman Release 7.1, respectively. FC indicates fold change. ER is estrogen receptor.

miRNAs	Control Sample	FC	Total Potential miRNA Binding Sites
miR-139-5p	Non-TNBC, Her2+,ER+PR+, Normal breast tissue	-3 -3 -3 -2.9	2
miR-513a-5p	Normal adjacent tissue	-3.11	2
miR-542-3p	Matched adjacent normal Normal breast tissue	-2.69 -11.6	1
miR-1179	Normal breast tissue	-3.8	1
miR-140-5p	Normal adjacent tissue	-2.15	1

BCL11A: B-cell lymphoma 11A

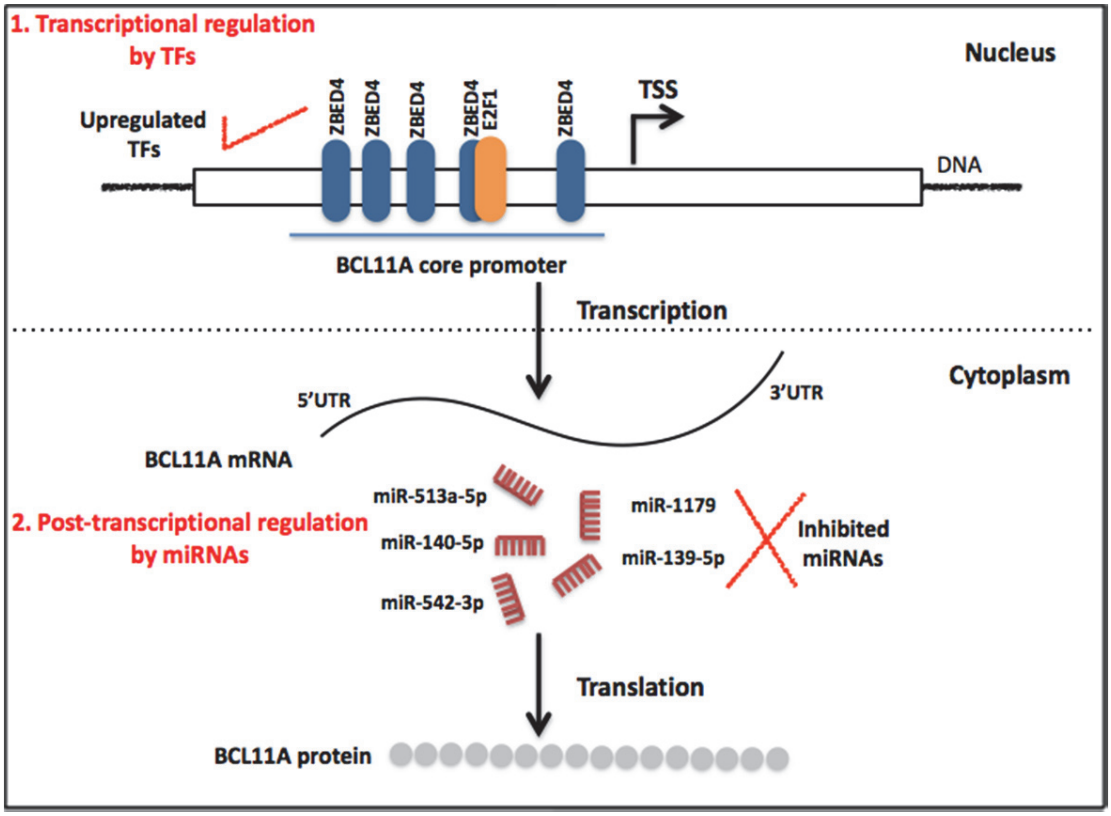

Figure 3. Schematic representation of the suggested mechanism of the regulation of BCL11A expression in triple negative breast cancer. First, the transcriptional regulation is controlled by ZBED4 and E2F1. ZBED4 and E2F1 are known to induce the gene transcription through direct binding to promoter sequences. Second is the post-transcriptional regulation through the inhibition of several miRNAs that are potentially able to interact with BCL11A mRNAs, degrading them and/or blocking their translation. Both levels of regulation can positively influence the expression of BCL11A in triple negative breast cancer cells.

downregulation of miRNAs and increase of BCL11A expression in the triple negative breast cancer. To date, there is no miRNA that has been experimentally validated for targeting BCL11A. Here we predicted that miR-1179, miR-140-5p, miR-542-3p, miR-513a-5p and miR-139-5p, might act as oncosuppressive miRNAs in triple negative breast cancer, through direct targeting of BCL11A. For instance, miR-205 expression downregulation mediates Zinc Finger E-Box Binding Homeobox 1 (ZEB1) and Zinc Finger E-Box Binding Homeobox 2 (ZEB2) gene upregulation. However, the negative regulation in epithelial–mesenchymal transition (EMT) and cancer progression are a consequence of miR-205 targeting ZEB1 and ZEB2 in triple negative breast cancer[25]. As another example, increased focal adhesion kinase (FAK) expression is a result of a decrease in miR-7 in tumors compared with normal breast tissue. There is an inverse correlation between miR-7 level and metastasis in human breast cancer tissues[26]. Therefore, these data confirm that oncogene expression upregulation can result from the downregulation of specific miRNAs. Taken together, the increased level of BCL11A expression is mediated by upregulation of transcription factors at the transcriptional level and downregulation of miRNAs at the post-transcriptional level in negative breast cancer (Fig. 3).

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التعرف على الجينات المسؤولة عن زيادة تعبير جين B-Cell Lymphoma 11A في سرطان الثدي ثلاثي السلبية

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المستخلص. يعتبر جين BCL11A بمثابة قامع نسخي ذا تعبير عالي في سرطان الثدي ثلاثي السلبية. وفرت الدراسات على الخلايا وحيوانات التجارب الأدلة الأولية التي تشير إلى أن قمع BCL11A له تأثير علاجي على هذا السرطان لذلك اكتشاف العناصر المنظمة والمسؤولة عن زيادة تعبيرBCL11A سيكون مهما في فهم وظائفه المتعلقة بعلاج السرطان. تعتبر بروتينات النسخ ((Transcription factors وجزيئات (microRNAs) من اهم العناصر المنظمة للتعبير الجيني. ولذلك اهتم الجزء الأول من العمل بتحديد بروتينات النسخ التي يمكن ان تتفاعل مع تسلسل النيوكليوتيدات المنظمة لجين BCL11A والمسمى بروموتر وتؤثر على عمليه نسخه. باستخدام المعلومات الحيوية وقواعد البيانات المتعددة، وجدنا عاملان نسخ محفزه للتعبير (E2F1, ZBED4) قادره على الارتباط بسبع مواقع في بروموتر جين BCL11A. عنى الجزء الثاني من الدراسة ب microRNAs ذات الأثر التثبطي ما بعد النسخ، وقد وجدنا انخفاض في مستوى عدد منها في خلايا سرطان الثدي ثلاثي السلبية مثل miR-513a-5p miR-139-5p, miR-1179, miR-140-5p, miR-542-3p والتي لديها القدرة على الارتباط الجزيئي بموقع او اكثر مع BCL11A mRNAs معطله عمليه الترجمة للبروتين. استخدام المعلومات الحيوية وقواعد البيانات المتعددة ساهمت في تزويدنا بمعلومات قيمة عن بروتينات النسخ وجزيئات microRNAs التي يمكن ان تكون مسؤوله عن مستوى التعبير العالي لجين BCL11A في خلايا سرطان الثدي ثلاثي السلبية.