Pathol. Oncol. Res. (2015) 21:293–300
DOI 10.1007/s12253-014-9820-8

RESEARCH

The Evaluation of WBP2NL-Related Genes Expression in Breast Cancer

Seyedmehdi Nourashrafeddin · Mahmoud Aarabi · Mohammad Hosein Modarressi · Marveh Rahmati · Mohammad Nouri

Received: 18 March 2014 / Accepted: 26 June 2014 / Published online: 25 November 2014

Abstract Breast cancer is the most frequent cause of mortality in women all around the world; therefore, study on molecular aspects of breast cancer is necessary for finding new biomarkers. Recent studies have shown that WW Binding Protein 2 (WBP2) is an important protein for the oncogenic property of cancer. We have previously evaluated the WW Binding Protein 2 N-Terminal Like (WBP2NL) gene expression in cancerous cell line and breast tumor tissues, and reported changes in expression, which could increase tumorigenic cell growth. However, the molecular mechanisms of WBP2NL and its clinical relevance have not been investigated. In this study, the expression of WBP2NL-related genes in the invasive breast carcinoma and normal breast tissues was evaluated for the first time. Analysis of WBP2NL-related genes expression was performed with reverse transcription-PCR and real time-PCR detection method. The target genes studied were as follow: WW domain containing E3 ubiquitin protein ligase 1(WWP1), membrane associated guanylatekinase containing WW and PDZ domain-1 (MAGI1), neural precursor cell expressed developmentally down-regulated 4 (NEDD4), formin binding protein-4 (FNBP4), BCL2-associated athanogene-3 (BAG3), WW domain-containing oxidoreductase (WWOX), yes-associated protein-1 (YAP1), WW domain containing transcription regulator (WWTR1), member RAS oncogene family (RAB2A), and small G protein signaling modulator 3 (SGSM3). The expression of WWP1, BAG3, and WWTR1 was significantly increased in breast cancer. In contrast, the expression of WWOX, YAP1, RAB2A, and SGSM3 was significantly decreased. The MAGI1 and NEDD4 expression was increased, while the expression of FNBP4 was unchanged. These findings lead us to suggest that WBP2NL might play roles as an anti-apoptotic factor or co-activator to promote breast cancer cell survival and proliferation.

Keywords Breast cancer · WBP2NL-related genes · qRT-PCR

S. Nourashrafeddin · M. Nouri (✉)
Women's Reproductive Health Research Center,
Tabriz University of Medical science, Tabriz, Iran
e-mail: nourim@tbzmed.ac.ir

M. Aarabi
Department of Anatomy and Cell Biology, Queen's University,
Kingston, Canada

M. H. Modarressi
Department of Medical Genetics, Faculty of Medicine,
Tehran University of Medical Sciences, Tehran, Iran

M. Rahmati · M. Nouri
Faculty of Medicine, Department of Biochemistry,
Tabriz University of Medical science, Tabriz, Iran

S. Nourashrafeddin
Department of Obstetrics, Gynecology and Reproductive Sciences
and Magee Womens Research Institute, University of Pittsburgh
School of Medicine, Pittsburgh, PA, USA

Introduction

Breast cancer is the most common cause of women death due to cancer in all around the world [1]. Approximately, one out of ten women develops breast cancer during lifetime [2]. Development of breast cancer is associated with altered gene expression profiles [1, 3]; therefore, study on molecular aspects of breast cancer for finding of breast cancer risk genes and new biomarkers for early diagnosis of breast cancer is very important.

Recent studies have shown that WW-domain Binding Protein 2 (WBP2) is essential for the oncogenic property of cancer. The WBP2 has originally identified as a binding partner of yes kinase-associated protein (YAP) [4, 5]. YAP is a transcriptional regulator and component of the Hippo pathway, which is essential for regulation of cell growth, proliferation and tumorigenesis [6, 7]. The WW-domain is a small

structure that makes a binding site for a short PPXY-motif (P, proline, X, any amino acid, Y, tyrosine) within a proline-rich domain [5, 8–10]. The WBP2 binds to YAP through interaction between WW-domains in YAP and PPXY-motifs in WBP2 [11]. The WBP2 protein also interacts with transcriptional co-activator with PDZ-binding motif (TAZ; also known as WWTR1), which is other component of the Hippo pathway and play roles in cell proliferation and tumorigenesis process [12–15]. The interaction of WBP2 with YAP and TAZ activates transcription machinery and increases target genes expression [16]. It has been demonstrated that inhibition of WBP2 expression or prevention of its interaction with YAP1 and WWTR1 suppress tumor cell proliferation, and suggested WBP2 as a target of cancer therapy [16, 17].

WW Binding Protein 2 N-Terminal Like (WBP2NL), also known as Postacrosomal sheath WW domain-binding protein (PAWP), is a WW domain-binding protein that has isolated from human normal sperm (Ref. to PMID: 17289678 and PMID: 17988661). WBP2NL is a signaling molecule localized in the postacrosomal sheath (PAS), and transferred into the perinuclear theca (PT) of sperm head during spermatid maturation [18]. It is a candidate sperm borne and oocyte-activating factor (SOAF) that enters the oocyte during fertilization, and induces oocyte activation events [19–21].

According to different databases, the WBP2NL is a testis-specific protein and absolutely absent in other tissue. The WBP2NL protein contains an N-terminal region homologous to WW domain-binding protein-2 (WBP2), and a proline-rich C-terminal half with PPxY motif and 9 YGxPPxG repeats, which allow this protein to interact with other WW domain proteins [18–20].

We have previously shown that WBP2NL contributes in sperm-induced calcium release and thereby activates oocyte [22]. We have also evaluated the WBP2NL gene expression in cancerous cell line and breast tumor tissues and revealed changes in expression that could contribute to increase cancerous cell proliferation [23], and breast cancer tumorigenesis (Unpublished data). However, the mechanisms of WBP2NL in cancerous cell proliferation and its clinical relevance have not been investigated. In order to get more insights into WBP2NL potential role in cellular proliferation and carcinogenesis, we conducted a comprehensive study on the expression of WBP2NL-related genes in the invasive ductal carcinoma (IDC) and normal breast tissues for the first time.

Materials and Methods

Normal and Patient Samples

Tissue samples were obtained from twenty cancerous patient that histologically diagnosed, and ten adjacent noncancerous women (ANCT) as normal control from tumor bank of cancer institute Imam Khomeini hospital affiliated to Tehran University of Medical Sciences under the protocols of medical ethics committee. All patients had been diagnosed on the basis of clinical and laboratory findings and their consent information have been written. The major clinical features and characteristics of included patients are shown in Table 1.

Extraction of RNA and cDNA Synthesis

Total RNA was extracted from frozen normal and tumor samples, and also breast cancer cell lines using TriPure isolating reagent (Roche) according to the manufacturer's instructions with minor modifications. RNA was dissolved in DEPC treated water and its concentration was determined by spectrophotometer (NanoDrop 2000). About 1.0 μg of total RNA of various samples were used for carrying out cDNA synthesis with M-MLV reverse transcription kit (Invitrogen) and random primer (Pharmacia, Sweden).

Analysis of Gene Expression by Reverse Transcription-PCR (RT-PCR)

A pair of specific primers was designed for amplifying 168 bp fragment of human WBP2NL. The PCR amplification of WBP2NL was performed with 30 cycles of denaturation at 94 °C for 30s, annealing at 60 °C for 30s, extension at 72 °C for 30s and a 7 min final extension at 72 °C. We also used a couple of specific primers for amplifying fragments of WBP2NL-related genes (Table 2) for evaluating their expression by RT-PCR. A couple of specific primers of the housekeeping gene HPRT were also used for checking of cDNAs quality. The PCR amplification of HPRT was performed with 35 cycles of denaturation at 94 °C for 30s, annealing at 58 °C for 25 s, extension at 72 °C for 30 s and a 7 min final extension at 72 °C. The total reaction volumes were 25 μl containing 1 μl cDNA, specific primers, PCR set and smart Taq polymerase. The primers were positioned in different exons of gene to avoid false positive because of probable DNA

Table 1 Clinical features of included patients with invasive ductal carcinoma

Subject's characteristics		No. of subjects (n=20)
Age (years)		35–65 (Mean:50)
Tumor grade	I or II (Low grade)	13(65 %)
	III (High grade)	7(35 %)
HER2	Positive	4(20 %)
	Negative	16(80 %)
ER/PR	Positive	15(75 %)
	Negative	5(25 %)

contamination during RNA extraction. The target gene and their primers are shown in Table 2.

Analysis of Gene Expression by Quantitative RT-PCR (qRT-PCR)

A highly sensitive, qRT-PCR method based on the SYBR-Green chemistry was used for the WBP2NL-related genes quantification in normal and malignant breast tissues. The specific primers were used for amplifying fragments of WBP2NL-related genes (Table 2). About 0.5 μl of each specific primers and 1.5 μl of each cDNA sample was added to PCR tubes containing SYBR-Green Master Mix (10 μl) (Qiagen, Berlin, Germany), and sterile water (7.5 μl). Following assay optimization using negative samples (no template control and no RTase), all test samples of cDNA were subjected to amplification (10 min at 95 °C, 45 cycles of 10 s at 95 °C, 20 s at 64 °C and 15 s at 72 °C) using 6500HRM Corbette Real-time PCR instrument. The fold changes in gene expression were calculated by ΔΔCt method. To control variations in the reactions, all PCRs were normalized by HPRT1 amplification. We have repeated all experiments at three independent times. All results are shown as the mean± SEM. Comparisons were made by the t-Test. Statistical vales of $P \leq 0.05$ were considered to be significant.

Clinical Relevance Analysis

WBP2NL-related genes have been evaluated for correlation to patient survival using online available meta-analysis tool (http://www.kmplot.com/breast/). The online database was used to assess the effect of 22,277 genes on survival breast cancer patients [24]. This online database has established using gene expression data and survival information of 4,142 patients that downloaded from Gene Expression Omnibus (GEO) (Affymetrix HGU133A and HGU133 plus 2.0 microarrays). Briefly, WBP2NL-related genes were entered into the database to obtain Kaplan-Meier survival plots where the number at risk, hazard ratio (with 95 % confidence intervals), and logrank P value were indicated on the webpage.

Bioinformatics Tools

We used different database such as NCBI (http://www.ncbi.nlm.nih.gov) and STRING (http://string-db.org) for selecting a set of predicted WBP2NL-related genes that share many features in common with the WBP2NL protein [25]. Also the Kyoto Encyclopedia for Genes and Genomes (KEGG) was used for the annotation of protein interaction networks (PATHWAY database). Finally, we have chosen those proteins that have expressed in either testis or breast tissue (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfile).

Results

The Expression of WBP2NL-Related Genes Determined by RT-PCR

The expression of WBP2NL-related genes at molecular level was evaluated by RT-PCR detection method in normal and malignant breast tissues. We evaluated the target genes expression in 10 malignant breast cancer samples from our previous study. In these samples, WBP2NL had been detected by the first round of RT-PCR, and represented high level of WBP2NL gene expression. The expression results of WBP2NL-related genes in normal and malignant breast tissues by RT-PCR detection method have been shown in Fig. 1.

Table 2 Sequences of the designed primers used for analysis of WBP2NL-related genes

Target Gene	Product Size (bp)	Forward (5→3′)	Reverse (5′→3′)
HPRT	190	ACCAGTCAACAGGGGACATAA	CTTCGTGGGGTCCTTTTCACC
WBP2NL	168	GCCATTGAATTTGCCCAGTTG	GGCTCCATAGACAATAACTGAAC
WWP1	166	AAAGGTAACGGTTTCTAGTGCC	GTGGGGTCACATTTACAATCAG
Magi1	150	GCCTTGCACAACCCGATCT	GGCTTGGGTGTCCCATAATAG
Nedd4	175	TACCAGCGTGCAGACAAAAAC	CGGTAAAGGATAGAGAGGGACA
Fnbp4	132	TCACTGGCAGGAGTCGGAAT	CTGTGTGGCAAGATATTGGGG
Bag3	175	GAAGCCGCCCAGACAGATAAA	AAGAGGATGAGCAGTCAGAGG
Wwox	213	TCATCCCCCGCCCGTGTCAT	TGCGTTGGATGTGACCCCGC
YAP1	221	ACCCTCGTTTTGCCATGAAC	TGTGCTGGGATTGATATTCCG
WWTR1	169	GAAGGTGATGAATCAGCCTCTG	GTTCTGAGTCGGGTGGTTCTG
RAB2A	115	GACACAGGTGTTGGTAAATCG	CATCAATCGTTATCATCCGAGCA
Sgsm3	123	TCCGTGTGGACAAGGAAGGT	CATCATGGTTATGGGTGAACTCC

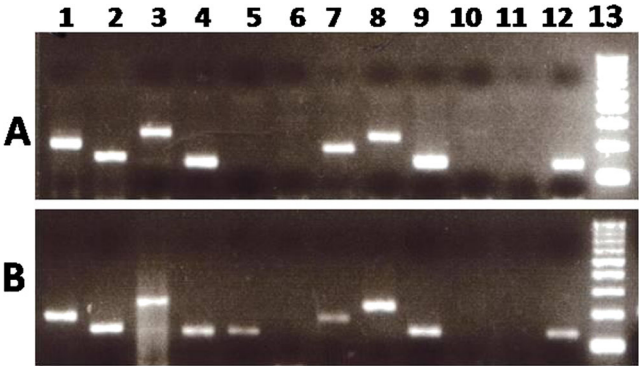

Fig. 1 The WBP2NL-related genes expression was determined by RT-PCR method. The expression analyze of WWP1, MAGI1, NEDD4, FNBP4, BAG3, WWOX, YAP1, WWTR1, RAB2A, and SGSM3 (Lane 1–10, respectively) in normal sample (**a**) and invasive breast carcinoma (**b**). Lane11 is the negative control (H2O), lane 12 is housekeeping gene HPRT, and lane13 is a 100 bp DNA marker

As shown by RT-PCR results, the expression of WWP1, MAGI1, NEDD4, FNBP4, YAP1, WWTR1, and RAB2A was observed in normal breast and malignant breast cancer tissues (Fig. 1a and b, respectively). The RT-PCR result has revealed that expression of WWOX and SGSM3 was absent in both normal breast and malignant breast cancer tissues, whereas, the expression of BAG3 was remarkably observed in malignant breast tissue (Fig. 1b).

The Expression of WBP2NL-Related Genes Determined by qRT-PCR

For quantitative investigation at molecular level, we evaluated the expression of all WBP2NL-related genes in the normal breast and malignant breast tissues by qRT-PCR detection method.

As shown by qRT-PCR results in Fig. 2, a statistical significant difference was observed in WWP1, BAG3, WWOX, YAP1, WWTR1, RAB2A and SGSM3 target genes between normal and breast cancer tissue. The expression of WWP1, BAG3, and WWTR1 were significantly increased in the malignant breast cancer tissue. In contrast, the expression of WWOX, YAP1, RAB2A, and SGSM3 were significantly decreased in the malignant tissue compared with normal tissue (Fig. 2). The qRT-PCR results reveal that there is no statistical significance difference in the expression of Magi1, Nedd4, and Fnbp4, between normal and malignant tissue; although a slight up-regulation in the expression of Magi1 and Nedd4 was detected in the malignant tissue compared with normal tissues, the Fnbp4 expression was unchanged (Fig. 2).

The Analysis of Patient Survival by Online Meta-Analysis Tool

The assessment of clinical relevance was performed in a patient survival analysis using an online database containing the expression of 22,277 genes and 20 years survival information of 1,809 patients [24]. However, this online database has been upgraded to include survival information of 4,142 breast cancer patients (http://www.kmplot.com/analysis/). We used this online data analysis tool to assess the relevance of the expression levels of WBP2NL-related genes on the clinical outcome of breast cancer patients (Table 3). Up-regulation of WWP1, BAG3, and WWTR1 was found to correlate with poor relapse free survival (RFS) for all breast cancer patients (p=0.004, p=0.02 and p=0.009 respectively). For patients with ER+breast tumor, over expression of WWP1 and WWTR1 was also seen as significantly associated with decreased survival (Table 3, p=0.04). As shown in Table 3, down-regulation of WWOX, and RAB2A gene was found to correlate strongly with poor relapse free survival (RFS) for all breast cancer patients ($p=2\times10^{-4}$ and $p=1\times10^{-4}$ respectively). For patients with ER+breast tumor, lower expression of RAB2A was also seen as strongly associated with decreased survival (Table 3, $p=3\times10^{-4}$). As shown in Table 3,

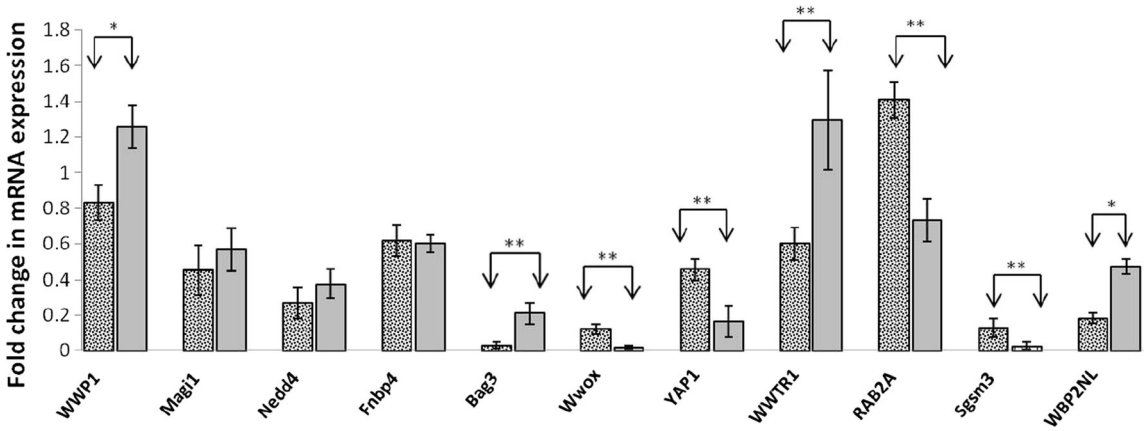

Fig. 2 Normalized expression levels of WBP2NL-related genes in normal and breast cancer tissues were determined using the real-time RT-PCR method. Values are given as mean of three independent experiments ±SD. * P<0.05 and ** P<0.01, a statistically significant difference as compared with normal control

Table 3 The association between WBP2NL-related genes and relapse free survival (RFS)

Marker	Gene name	Affymetrix ID	All breast tumor		ER+breast tumor	
			HR*	P value	HR	P value
MAGI1	Membrane associated guanylatekinase containing WW and PDZ domain-1	206144_at	0.6	0	0.81	0.0192
NEDD4	Neural precursor cell expressed developmentally down-regulated 4	213012_at	0.94	0.2728	1.03	0.7183
FNBP4	Formin binding protein-4	212232_at	0	0.76	1.18	0.0637
BAG3	BCL2-associated athanogene-3	217911_s_at	1.14	0.0237	1.14	0.1469
YAP1	Yes-associated protein-1	213342_at	1.1	0.1017	0.78	0.0057
WWP1	WW domain containing E3 ubiquitin protein ligase 1	212637_s_at	1.18	0.0041	1.2	0.0403
		212638_s_at	1.05	0.4195	1.05	0.5986
WWOX	WW domain-containing oxidoreductase	221147_x_at	0.8	0.0002	0.95	0.5304
		210695_s_at	0	0.67	1.07	0.4281
		219077_s_at	1.03	0.6423	1.16	0.1042
WWTR1	WW domain containing transcription regulator	202134_s_at	1.11	0.0684	0.93	0.4481
		202132_at	0.3105	0.5399	0.84	0.0452
		202133_at	1.16	0.0098	0.88	0.1371
RAB2A	Member RAS oncogene family	208730_x_at	0.79	0.0001	0.9	0.1952
		208731_at	1.45	0	1.38	0.0003
		208732_at	1.11	0.0706	1.11	0.262
		208733_at	0.95	0.4315	1.14	0.1438
		208734_x_at	1.46	0	1.13	0.1648
		221960_s_at	0.93	0.2516	0.96	0.6272
SGSM3	Small G protein signaling modulator 3	203014_x_at	0.76	0	0.85	0.0727
		214779_s_at	0.91	0.0976	1.03	0.7523
		215519_x_at	0	0.74	0.83	0.0372

HR hazard ratio

for ER+patients with reduced expression of YAP1 and SGSM3 predict worse outcome in survival (p=0.005 and p= 0.03 respectively).

Discussion

It has been demonstrated that WBP2 acts as a co-activator of estrogen receptor (ER) in breast cancer and is involved in the regulation of ER target genes [26]. Recent studies were also shown the mechanism of WBP-2 in regulating of ER target gene expression as co-activator of ER. [27, 28].

We have previously evaluated the expression of WBP2NL gene in cancerous cell line and breast tumor tissues and reported changes in expression that could contribute to increase cancerous cell proliferation [23], and breast cancer tumorigenesis (Unpublished data). In the present study, we evaluated a wide range of genes expression related to WBP2NL in both breast cancers and normal breasts.

As mentioned in PCR results, the expression of WWTR1 (TAZ), WWP1 and BAG3 was significantly up-regulated in breast cancer rather than normal tissue. Recent evidence obtained from human cancers demonstrates that WWTR1 is over-expressed in cancers [14, 15], and it promotes lung cancer cell growth and inhibits apoptosis [15, 29, 30]. There is evidence that shows WWTR1 plays a role in the invasion and tumorigenesis of breast cancer cells [31], and interaction of its WW domain with WBP2 is important for the oncogenic property of WWTR1 [13]. WWP1, with four WW domains recognizing substrates with PY (PPXY) motifs, promotes degradation of substrates by ubiquitination. Previous studies have shown that WWP1 is overexpressed in prostate cancer [32] and positive estrogen receptor-α (ER-α) breast cancers, and it promotes breast cell survival and proliferation [33]. The over-expression of WWTR1 in breast cells promotes breast cell proliferation through protecting of Krüppel-like factor 5 (KLF5), a transcription factor containing PY motif, from degradation by WWP1 [34]. WWTR1 interacts with KLF5 through the WW domain of WWTR1 and the PY motif of KLF5, which is the binding site for WWP1 [34]. BAG3 is a member of the Bcl-2 family with a WW domain and a PY motif, and thereby it modulates different biological processes, such as anti-apoptotic activity. It has been indicated that BAG3 is increased in some tumors, and its down-regulation suppress cancerous cell growth [35]. It has been demonstrated

that BAG3 modulates proteasome activity, and increases cell survival by suppression of apoptosis [36]. Recent study has revealed that BAG3 expression promotes breast cancer cell line (MDA-MB231) survival [37].

In present study, we demonstrated that the expression of WWOX, SGSM3, YAP1, and RAB2A was significantly down-regulated in breast cancer tissue compared with normal tissue. These results are compatible with previous studies that have published independently. It has been reported that WWOX acts as a tumor suppressor, and plays a role in apoptosis, and it has been lost in cervical cancer [38]. It has been indicated that breast cancer patients with high expression of WWOX have good prognosis to treatment [39]. The SGSM family members mediate the small G protein RAB-mediated signal transduction and vesicular transport pathway [40]. Before study revealed that SGSM3 may play a role in NF2-mediated growth suppression of cells by interaction with Merlin, a component of the Hippo signaling pathway [41]. YAP1 is a major transcriptional regulator in the Hippo signaling pathway with both co-activator and co-repressor functions. It has been reported that the expression of YAP1 in cancer depends on cell type [42]. As an oncogene, the overexpression of YAP1 has found in several cancers [42]; however, knockdown of YAP1 in a subset of breast cancer increased tumor growth [43], which suggests its tumor suppression role. The RAB family members are membrane-bound proteins that involve in vesicular fusion and organelle biogenesis [44, 45], and abnormality in RAB function lead to cancer [46]. RAB2A is required for vesicular transport from the Golgi to the nuclear envelop in spermatids during acrosomal biogenesis [47].

According to our results, there was no significant difference in MAGI1, NEDD4 and FNBP4 expression between normal and cancerous breast tissues. Although the expression of Nedd4 and Magi1 were a little increased in breast cancer compared with normal sample, the expression of FNBP4 in normal tissues was particularly the same. These findings are partially compatible with before studies that have been published independently. NEDD4 plays a role in the ubiquitination of targeted substrates that leading to their degradation by lysosomes, and it acts as an oncoprotein by ubiquitination and degradation of the tumor suppressor proteins [48]. It has been reported that over-expression of NEDD4 facilitate the tumorigenesis of lung cancer [49]. FNBP4 is a member of the intersectin (ITSN) family that interacts with formin domains and corresponds in the maintenance of membrane curvature [50]. Although formin domains regulates cytoskeletal dynamics during cell division and migration, but exact function of FNBP4 remains undetermined [51]. MAGI1 plays an important role in stabilization of cell-cell junctions and suppression of metastasis. Anyway, recent study has revealed that MAGI1 expression levels positively inhibits hepatocellular carcinoma cell migration and invasion via regulating PTEN [52].

These findings in patients with breast cancer confirmed our previous studies that showed WBP2NL increase tumorigenesis and proliferation of cancerous cell. This conclusion is also supported by a patient survival analysis to correlate the predicted WBP2-NL-related genes expression and relapse free survival for 4,142 breast cancer patients (www.kmplot.com) where increased level of WWP1, BAG3, and WWTR1 genes, and reduced level of WWOX, and RAB2A genes is predictive of worse outcome generally for all breast cancer patients, and particularly for ER+patients (Table 3).

In conclusion, these evidences, taken together with our previous results, lead us to suggest that WBP2NL might play roles as an anti-apoptotic factor or co-activator to promote breast cancer cell survival and proliferation. Overall, our studies provide new insight into the biological function of WBP2NL and its role in the pathogenesis of breast cancer. Further studies are necessary to clarify the exact mechanisms that regulate WBP2NL expression, and the effects of WBP2NL activation in breast cancer.

Acknowledgments This research supported by a grant from Women's Reproductive Health Research Center of Tabriz University of Medical Sciences, and we appreciate Department of Medical Genetic, Faculty of Medicine, Tehran University of Medical Sciences for technical supports.

References

1. Patsialou A, Wang Y, Lin J, Whitney K, Goswami S, Kenny PA, Condeelis JS (2012) Selective gene-expression profiling of migratory tumor cells in vivo predictsclinical outcome in breast cancer patients. Breast Cancer Res 14(5):R139
2. Key TJ, Verkasalo PK, Banks E (2001) Epidemiology of breast cancer. The Lancet Oncology 2(3):133–140
3. Nishidate T, Katagiri T, Lin ML, Mano Y, Miki Y, Kasumi F, Yoshimoto M, Tsunoda T, Hirata K, Nakamura Y (2004) Genome-wide gene-expression profiles of breast-cancer cells purified with laser microbeam microdissection: identification of genes associated with progression and metastasis. Int J Oncol 25(4):797–819
4. Chen HI, Sudol M (1995) The WW domain of Yes-associated protein binds a proline-rich ligand that differs from the consensus established for Src homology 3-binding modules. Proc Natl Acad Sci U S A 92(17):7819–23
5. Sudol M, Chen HI, Bougeret C, Einbond A, Bork P (1995) Characterization of a novel protein-binding module—the WW domain. FEBS Lett 369:67–71
6. Komuro A, Nagai M, Navin NE, Sudol M (2003) WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. J Biol Chem 278:33334–33341
7. Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, Deng CX, Brugge JS, Haber DA (2006) Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci USA 103:12405–12410
8. Sudol M (1996) Structure and function of the WW domain. Prog Biophys Mol Biol 65:113–132
9. Sudol M, Hunter T (2000) NeW wrinkles for an old domain. Cell 103(7):1001–4

10. Macias MJ, Wiesner S, Sudol M (2002) WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. FEBS Lett 513(1):30–7
11. McDonald CB, McIntosh SK, Mikles DC, Bhat V, Deegan BJ, Seldeen KL, Saeed AM, Buffa L, Sudol M, Nawaz Z, Farooq A (2011) Biophysical analysis of binding of WW domains of the YAP2 transcriptional regulator to PPXY motifs within WBP1 and WBP2 adaptors. Biochemistry 50:9616–9627
12. Wang K, Degerny C, Xu M, Yang XJ (2009) YAP, TAZ, and Yorkie: a conserved family of signal-responsive transcriptional coregulators in animal development and human disease. Biochem Cell Biol 87(1): 77–91
13. Chan SW, Lim C, Huang C, Chong YF, Gunaratne HJ, Hogue KA, Blackstock WP, Harvey KF, Hong W (2011) WW domain-mediated interaction with Wbp2 is important for the oncogenic property of TAZ. Oncogene 30:600–610
14. de Cristofaro T, Di Palma T, Ferraro A, Corrado A, Lucci V, Franco R, Fusco A, Zannini M (2011) TAZ/ WWTR1 is overexpressed in papillary thyroid carcinoma. Eur J Cancer 47:926–33
15. 15- Wang L, Chen Z, Wang Y, Chang D, Su L, Guo Y, Liu C (2013) WWTR1 promotes cell proliferation and inhibits apoptosis through cyclin A and CTGF regulation in non-small cell lung cancer. Tumour Biol. [Epub ahead of print]
16. Sudol M (2010) Newcomers to the WW domain-mediated network of the hippo tumor suppressor pathway. Genes Cancer 1(11):1115–8
17. Zhang X, Milton CC, Poon CL, Hong W, Harvey KF (2011) Wbp2 cooperates with yorkie to drive tissue growth downstream of the Salvador-warts-hippo pathway. Cell Death Differ 18(8):1346–55
18. Wu AT, Sutovsky P, Xu W, Vander Spoel AC, Platt FM, Oko R (2007) The postacrosomal assembly of sperm head protein, PAWP, is independent of acrosome formation and dependent on microtubular manchette transport. Dev Biol 312(2):471–83
19. Sutovsky P, Manandhar G, Wu A, Oko R (2003) Interactions of sperm perinuclear theca with the oocyte: implications for oocyte activation, anti-polyspermy defense, and assisted reproduction. Microsc Res Tech 61(4):362–78
20. Wu AT, Sutovsky P, Manandhar G, Xu W, Katayama M, Day BN, Park KW, Yi YJ, Xi YW, Prather RS, Oko R (2007) PAWP, A sperm specific ww-domain binding protein, promotes meiotic resumption and pronuclear development during fertilization. J Biol Chem 282(16):12164–75
21. Oko R, Sutovsky P (2009) Biogenesis of sperm perinuclear theca and its role in sperm functional competence and fertilization. J Reprod Immunol 83(1–2):2–7
22. Aarabi M, Qin Z, Xu W, Mewburn J, Oko R (2010) Sperm-borne protein, PAWP, initiates zygotic development in Xenopus laevis by eliciting intracellular calcium release. Mol Reprod Dev 77(3):249–56
23. Nourashrafeddin S, Aarabi M, Miryounesi M, Ebrahimzadeh-Vesal R, Zarghami N, Modarressi MH, Nouri M (2014) Expression analysis of PAWP during mouse embryonic stem cell-based spermatogenesis in vitro. Vitro Cell Dev Biol Anim 50(5):475–81
24. Győrffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q, Szallasi Z (2010) An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 123:725–731
25. von Mering C, Jensen LJ, Kuhn M, Chaffron S, Doerks T, Kruger B, Snel B, Bork P (2007) STRING 7 - recent developments in the integration and prediction of protein interactions. Nucleic Acids Res 35:D358–D362
26. Dhananjayan SC, Ramamoorthy S, Khan OY, Ismail A, Sun J, Slingerland J, O'Malley BW, Nawaz Z (2006) WW domain binding protein-2, an E6-assoiated protein interacting protein, acts as a coactivator of estrogen and progesterone recep- tors. Mol Endocrinol 20: 2343–54
27. Lim SK, Orhant-Prioux M, Toy W, Tan KY, Lim YP (2011) Tyrosine phosphorylation of transcriptional coactivator WW-domain binding protein 2 regulates estrogen receptor α function in breast cancer via the Wnt pathway. FASEB J 25(9):3004–18
28. Buffa L, Saeed AM, Nawaz Z (2013) Molecular mechanism of WW-domain binding protein-2 coactivation function in estrogen receptor signaling. IUBMB Life 65(1):76–84
29. Xie M, Zhang L, He CS, Hou JH, Lin SX, Hu ZH, Xu F, Zhao HY (2012) Prognostic significance of TAZ expression in resected non-small cell lung cancer. J Thorac Oncol 7(5):799–807
30. Zhou Z, Hao Y, Liu N, Raptis L, Tsao MS, Yang X (2011) TAZ is a novel oncogene in non-small cell lung cancer. Oncogene 30(18):2181–6
31. Chan SW, Lim CJ, Guo K, Ng CP, Lee I, Hunziker W, Zeng Q, Hong W (2008) A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. Cancer Res 68(8):2592–8
32. Chen C, Sun X, Guo P, Dong XY, Sethi P, Zhou W et al (2007) Ubiquitin E3 ligase WWP1 as an oncogenic factor in human prostate cancer. Oncogene 26:2386–2394
33. Li Y, Zhou Z, Alimandi M, Chen C (2009) WW domain containing E3 ubiquitin protein ligase 1 targets the full-length ErbB4 for ubiquitin-mediated degradation in breast cancer. Oncogene 28(33): 2948–58
34. Zhao D, Zhi X, Zhou Z, Chen C (2012) TAZ antagonizes the WWP1-mediated KLF5 degradation and promotes breast cell proliferation and tumorigenesis. Carcinogenesis 33(1):59–67
35. Rosati A, Graziano V, De Laurenzi V, Pascale M, Turco MC (2011) BAG3: a multifaceted protein that regulates major cell pathways. Cell Death Dis 2:e141
36. Chen Y, Yang LN, Cheng L, Tu S, Guo SJ, Le HY, Xiong Q, Mo R, Li CY, Jeong JS, Jiang L, Blackshaw S, Bi LJ, Zhu H, Tao SC, Ge F (2013) BAG3 interactome analysis reveals a new role in modulating proteasome activity. Mol Cell Proteomics 12(10):2804–19
37. Boiani M, Daniel C, Liu X, Hogarty MD, Marnett LJ (2013) The stress protein BAG3 stabilizes Mcl-1 protein and promotes survival of cancer cells and resistance to antagonist ABT-737. J Biol Chem 288(10):6980–90
38. Qu J, Lu W, Li B, Lu C, Wan X (2013) WWOX induces apoptosis and inhibits proliferation in cervical cancer and cell lines. Int J Mol Med 31(5):1139–47
39. Göthlin Eremo A, Wegman P, Stål O, Nordenskjöld B, Fornander T, Wingren S (2013) Wwox expression may predict benefit from adjuvant tamoxifen in randomized breast cancer patients. Oncol Rep 29(4):1467–74
40. Yang H, Sasaki T, Minoshima S, Shimizu N (2007) Identification of three novel proteins (SGSM1, 2, 3) which modulate small G protein (RAP and RAB)-mediated signaling pathway. Genomics 90(2):249–60
41. Chia WJ, Tang BL (2009) Emerging roles for Rab family GTPases in human cancer. Biochim Biophys Acta 1795:110–6
42. Diep CH, Zucker KM, Hostetter G, Watanabe A, Hu C, Munoz RM, Von Hoff DD, Han H (2012) Down-regulation of yes associated protein 1 expression reduces cell proliferation and clonogenicity of pancreatic cancer cells. PLoS One 7(3):e32783
43. Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T, Gangeswaran R, Manson-Bishop C, Smith P, Danovi SA, Pardo O, Crook T, Mein CA, Lemoine NR, Jones LJ, Basu S (2008) Yes-associated protein (YAP) functions as a tumor suppressor in breast. Cell Death Differ 15:1752–1759
44. Ma S, Huang J, Xie Y, Yi N (2012) Identification of breast cancer prognosis markers using integrative sparse boosting. Methods Inf Med 51(2):152–61
45. Stenmark H (2009) Rab GTPases as coordinators of vesicle traffic. Nature Rev Mol Cell Biol 10:513–525
46. Mosesson Y, Mills GB, Yarden Y (2008) Derailed endocytosis: an emerging feature of cancer. Nature Rev Cancer 8(11):835–850
47. Mountjoy JR, Xu W, McLeod D, Hyndman D, Oko R (2008) RAB2A: a major subacrosomal protein of bovine spermatozoa implicated in acrosomal biogenesis. Biol Reprod 79(2):223–32

48. Wang X, Trotman LC, TKoppie T, Alimonti A, Chen Z, Gao Z, Wang J, Erdjument-Bromage H, Tempst P, Cordon Cardo C, Pandolfi PP, Jiang X (2007) NEDD4-1 is a proto-oncogenic ubiquitin ligase for PTEN. Cell 128(1):129–39
49. Amodio N, Scrima M, Palaia L, Salman AN, Quintiero A, Franco R, Botti G, Pirozzi P, Rocco G, De Rosa N, Viglietto G (2010) Oncogenic role of the E3 ubiquitin ligase NEDD4–1, a PTEN negative regulator, in non-small-cell lung carcinomas. Am J Pathol 177(5):2622–34
50. Wong KA, Wilson J, Russo A, Wang L, Okur MN, Wang X, Martin NP, Scappini E, Carnegie GK, O'Bryan JP (2012) Intersectin (ITSN) family of scaffolds function as molecular hubs in protein interaction networks. PLoS One 7(4):e36023
51. Kondo Y, Koshimizu E, Megarbane A, Hamanoue H, Okada I, Nishiyama K, Kodera H, Miyatake S, Tsurusaki Y, Nakashima M, Doi H, Miyake N, Saitsu H, Matsumoto N (2013) Whole-exome sequencing identified a homozygous FNBP4 mutation in a family with a condition similar to microphthalmia with limb anomalies. Am J Med Genet A 161A(7):1543–6
52. Zhang G, Wang Z (2011) MAGI1 inhibits cancer cell migration and invasion of hepatocellular carcinoma via regulating PTEN. Zhong Nan Da Xue Xue Bao Yi Xue Ban 36(5):381–5