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Original Article

Pathological significance of MAD2L1 in breast cancer: an immunohistochemical study and meta analysis

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Abstract: The aberrant expression of mitotic arrest deficient 2-like 1 (MAD2L1) has been found to promote tumor formation by inducing chromosomal instability and aneuploidy in cells. In breast cancer (BRCA), limited studies have been focused on MAD2L1 expression and its impact on tumor progression. Thus, we conducted this study to comprehensively analyze MAD2L1 expression and its clinicopathological significance as well as diagnostic value for BRCA. Immunohistochemistry was performed with the 209 invasive ductal BRCA samples and the corresponding adjacent tissues to investigate MAD2L1 expression in BRCA and its relationship between clinicopathological features of BRCA. Then, the clinicopathological role of MAD2L1 was confirmed by RNA-sequencing or microarray data from the Cancer Genome Atlas (TCGA) and gene expression omnibus (GEO). Particularly, summarized receiver operating characteristic (SROC) curve was plotted to explore the diagnostic capacity of MAD2L1 in BRCA. The results showed that MAD2L1 presented overexpression in BRCA and was significantly associated with higher clinical stage and histological grade of BRCA. A significant correlation was also found between MAD2L1 expression and several tumor indicators including ER, P53, HER-2 and Ki-67. Moreover, area under curve (AUC) value (0.9642) from SROC revealed potential diagnostic value of MAD2L1 for BRCA. In summary, MAD2L1 may be involved in the occurrence and development of BRCA and MAD2L1 detection could improve the diagnosis and prognostic evaluation of BRCA.

Keywords: MAD2L1, breast cancer, IHC, TCGA, GEO, clinicopathological significance

Introduction

According to the GLOBOCAN 2012 International Agency for Research on Cancer database, breast cancer (BRCA) is the most common cancer in females worldwide with extremely high morbidity and mortality. It is the fifth leading cause of deaths in cancers globally [1]. Despite advance has been made in treatment such as pre-operative chemotherapy combined with radiation or hormonal therapy, effective treatment are lacking in BRCA patients with tumor invasion or distant metastasis [2-9]. The pathogenesis of BRCA was believed to be a complicated process involving the interaction of multiple genes and proteins [10-16]. Thus, seeking valid diagnostic and therapeutic biomarker for BRCA might enhance the survival quality of BRCA patients.

Mitotic arrest deficient 2-like 1 (MAD2L1), as a component of spindle checkpoint, plays an essential role in supervising chromosomal segregation during mitosis [17-19]. Dysregulation of MAD2L1 could lead to chromosomal instability and aneuploidy, which might promote formation of human cancers [20]. Overexpression of MAD2L1 has been discovered in several cancers including breast, lung, liver and stomach [21, 22]. In BRCA, limited studies have been conducted on MAD2L1 in BRCA. Zhang et al. reported that MAD2L1 was higher expression in BRCA tissues than in normal tissues and that MAD2L1 associated with malignant progression and poor disease-free survival of BRCA patients [18]. Expression of MAD2L1 has also been investigated in BRCA cell lines by Yuan B et al. and Percy MJ et al. [23, 24]. However, different methods were applied to detect MAD2L1 expression and expression values of MAD2L1 have rarely been compared in tumor tissues and normal tissues in previous studies. Our study was the first to comprehensively evaluate MAD2L1 expression as well as its clinicopatho-

logical significance and diagnostic value in BRCA through immunohistochemistry (IHC), analysis of The Cancer Genome Atlas (TCGA) data and Gene Expression Omnibus (GEO) meta-analysis.

Materials and methods

Patient samples

A total of 209 cases of breast invasive ductal carcinoma and the corresponding adjacent tissues (more than 5 cm from tumor site) in Liuzhou Worker's Hospital from January 2013 to 2015 March were selected. All patients were female aged between 31-81 years with a median age of 50 years. As for histological grade, there were 18 grade I tumor, 97 grade II tumor and 94 grade III tumors. The size of the tumors indicated that there were 57, 131 and 21 cases of tumor with the size of less than 2 cm, between 2-5 cm and more than 5 cm, respectively. Among the 209 cases of breast invasive ductal carcinoma, there were 104, 54, 22, 29 cases of tumor that belonged to the category of N0, N1, N2 and N3, respectively. A total of 204 tumors presented no distant metastasis and five tumor samples had distant metastasis. With regard to the clinical stage, there were 23, 122, 59 and 5 cases of tumor that were classified as stage I, II, III and IV, respectively.

IHC

All specimens were fixed by 10% neutral formalin, and then paraffin embedded to be sliced into 4 μm thick sections for immunohistochemical staining. All the experimental procedure was carried out according to the instructions of kit. Antibodies used for the IHC staining were rabbit anti human polyclonal antibody MAD2L1 (purchased from the United States ORIGENE Biotech Corp) and fast enzyme labeled Goat anti mouse/rabbit IgG polymer (purchased from Fuzhou Maixin Biotechnology Development Co., Ltd.). Normal human testis tissue served as the positive control and PBS instead of the first antibody was set as the negative control.

As for the assessment of MAD2L1 immunohistochemical staining, yellow to brown particles in the nucleus and (or) cytoplasm was considered as the positive expression of MAD2L1. Staining intensity was divided into four levels: no positive staining or less than 10% positive cells stand for negative staining (-); 11%-30% positive cells stand for weak positive staining (+); 31%-50% positive cells stand for positive staining (++); more than 51% positive cells stand for strong positive (+++) [6]. More than 1% positively stained invasive carcinoma cells with the positive staining in nucleus was considered as the positive criteria of ER/PR. The standard of HER2 positive staining was as follows: (1) more than 10% invasive carcinoma cells that exhibited strong, complete and homogeneous positive staining in membrane corresponds to positive staining (3+); (2) uncertain results with the further need of FISH experiments or revalidation by immunohistochemistry corresponds to HER2 (2+). IHC results were re-evaluated based on the above criteria. Patients with more than 1% ER/PR positively stained invasive carcinoma cells and HER2 (3+) positive staining was defined as the positive case. Patients with HER2 (2+) staining were classified in accordance with the detection of FISH. Cases with the gene amplification of HER2 was considered as positive staining; otherwise, HER2 (2+) staining cases were removed. Positive staining of Ki-67 was located in nucleus and was divided into two groups of less than 14% or more than 14% positively stained cells. Ten high power fields were randomly selected for each slide and 1000 cells were counted in each high power field. Two experienced pathologists reviewed the results dependently without the information of the samples. When variations in the positively stained cells were more than 10%, the controversial staining results were re-assessed.

Statistical analysis of IHC

All the statistical analysis for IHC was performed by SPSS21.0. The expression of MAD2L1 between cancer and adjacent tissues as well as the relationship between MAD2L1 expression and the clinicopathological parameters of BC was evaluated by χ^2 test and Spearman correlation test. $P<0.05$ was considered as statistically significant.

MAD2L1 expression in BRCA from the Cancer Genome Atlas (TCGA) data

Mining of TCGA data was implemented with Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/), an online tool that delivered interactive and customizable

Table 1. Expression of MAD2L1 in BRCA and cancer adjacent tissues

Group	Number	MAD2L1 staining		χ^2	*P* value
		Negative (%)	Positive (+-+++) (%)		
Cancer tissues	n=209	107 (51.2)	102 (48.8)	10.117	0.00
Cancer-adjacent tissues	n=53	40 (75.5)	13 (24.5)		

Note: χ^2 test was conducted to examine MAD2L1 expression in cancer and cancer-adjacent tissues.

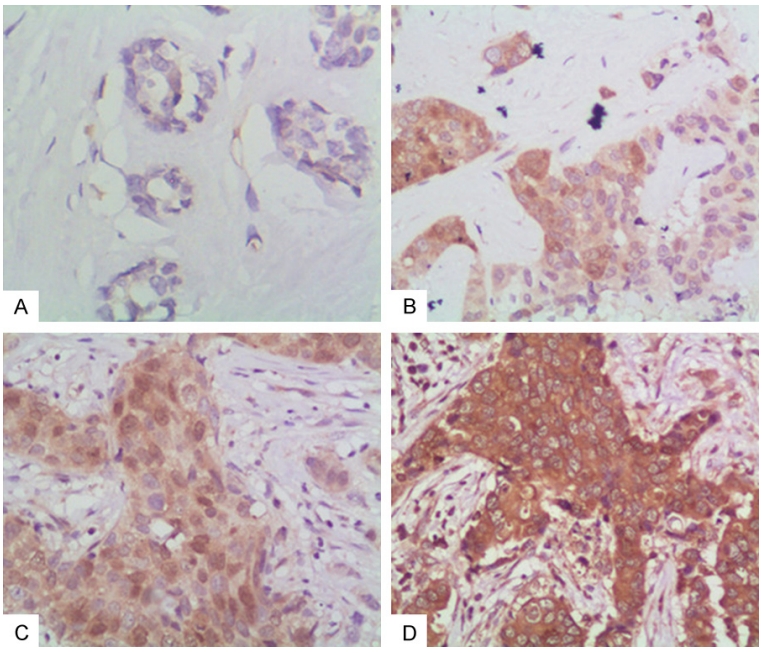

Figure 1. Expression of MAD2L1 in BRCA and adjacent tissues (IHC×200). A: Negative expression of MAD2L1 in adjacent tissues; B: MAD2L1 1+ positive expression in BRCA; C: MAD2L1 2+ positive expression in BRCA; D: MAD2L1 3+ positive expression in BRCA.

functions to facilitate efficient expression analysis of TCGA and GTEx data [25]. We downloaded graphs of MAD2L1 expression in 1084 BRCA tissues and 291 normal tissues as well as MAD2L1 expression in different clinical stages of BRCA from GEPIA to further validate the aberrant MAD2L1 expression in BRCA and its impact on the development of BRCA.

Literature meta-analysis

A systematic searching was performed in databases including PubMed, Chinese VIP, CNKI, WanFang database, Sinomed, Embase, Web of science, Science Direct and Wiley Online Library to identify relevant articles published before April 10, 2017. The following searching strategy was employed in the meta-analysis: ("breast cancer" OR "breast tumor" OR "breast malignancy" OR "breast carcinoma" OR "breast neoplasm") AND (MAD2L1 OR "Mitotic Arrest Deficient 2-like 1"). Language of published articles was restricted to Chinese or English. We also manually searched reference lists of relevant studies for potential eligible studies. Studies were included in the meta-analysis if they complied with the following criteria: (1) Expression value of MAD2L1 between BRCA and normal tissues was provided in the study. (2) Detection of MAD2L1 expression in BRCA and normal tissues were conducted on human-beings. Exclusion of ineligible studies was based on the following criteria: (1) Cell lines were used to evaluate MAD2L1 expression in BRCA and normal tissues. (2) MAD2L1 expression was only examined in BRCA tissues with no normal tissues as controls. (3) Studies that were catalogued as review, meta-analysis, letters, commentaries, conference abstracts. (4) Studies that lacked complete data of MAD2L1 expression in BRCA and normal tissues.

We extracted the following information from the selected studies for further analysis: first author, year of publication, country, area, sample sizes of the cancer and control group, sample types, experiment types, platform, mean (M) and standard deviation (SD) of MAD2L1 expression value in cancer and control group.

Statistical analysis

STATA v.12.0 was utilized to perform all the statistical analysis. Standard mean difference (SMD) and 95% confidential interval (95% CI) of each study was pooled to calculate the overall SMD and 95% CI. The heterogeneity between

Table 2. Relationship between MAD2L1 expression and clinical pathological factor of BRCA

Parameters	MAD2L1 staining		χ^2	*P* value
	Negative (%)	Positive (+-+++) (%)		
Age (years)			2.171	0.141
≤50	51 (46.4)	59 (53.6)		
>50	56 (56.6)	43 (43.4)		
Histological grade			4.852	0.028
I+II	66 (57.9)	48 (42.1)		
III	40 (42.6)	54 (57.4)		
Tumor size (cm)			0.857	0.652
≤2	32 (56.1)	25 (43.9)		
2-5	64 (48.9)	67 (51.1)		
>5	11 (52.4)	10 (47.6)		
Lymph node metastasis (N)			1.936	0.164
No (N0)	58 (56.3)	45 (43.7)		
Yes (N1-N3)	49 (46.7)	56 (53.3)		
Distant metastasis (M)			0.159	1.000
M0	104 (51.0)	100 (49.0)		
M1	3 (60.0)	2 (40.0)		
Clinical stage			2.047	0.152
I+II	79 (54.5)	66 (45.5)		
III+IV	28 (43.8)	36 (56.3)		

Note: χ^2 test was conducted to examine MAD2L1 expression in groups of different clinicopathological parameters of BRCA.

Table 3. Relationship between MAD2L1 expression and ER, PR, P53, HER-2, Ki-67

Variables	MAD2L1 staining		χ^2	*P* value
	Negative (-) (%)	Positive (+-+++) (%)		
ER			5.786	0.016
Negative	33 (40.7)	48 (59.3)		
Positive	74 (57.8)	54 (42.2)		
PR			2.691	0.101
Negative	44 (45.4)	53 (54.6)		
Positive	63 (56.8)	48 (43.2)		
P53			5.745	0.017
Negative	70 (58.3)	50 (41.7)		
Positive	37 (41.6)	52 (58.4)		
HER-2			16.647	<0.001
Negative	86 (61.0)	55 (39.0)		
Positive	21 (30.9)	47 (69.1)		
Ki-67			11.531	0.001
Negative	65 (63.1)	38 (36.9)		
Positive	42 (39.6)	64 (60.4)		

Note: ER: estrogen receptor; PR: progesterone receptor; HER-2: human epidermal growth factor receptor 2; χ^2 test was conducted to assess the relationship between MAD2L1 expression and ER, PR, P53, HER-2, Ki-67.

all the included studies was assessed by Cochran Q test and Higgins I^2 statistic. When I^2 was more than 50% or P was less than 0.05, significant heterogeneity existed in studies and random-effect model was chosen for SMD pooling; otherwise, fixed-effect model was employed [26-28]. If there was significant heterogeneity between studies, subgroup analysis and sensitivity analysis were performed to explore the potential source of heterogeneity. Subgroup analysis was carried out by groups of area and experiment types. Sensitivity analysis was used to evaluate the influence of a single study on the overall pooling result by omitting one study at a time. Additionally, we estimated publication bias through Begg's and Egger's test [29, 30].

GEO meta-analysis

GEO archived high-through put molecular data and served as a powerful tool to mine gene expression data in human cancers [31]. In this study, we referred to GEO database (https://www.ncbi.nlm.nih.gov/gds/) with the key searching word: "breast cancer" to select qualified microarray chips that contained MAD2L1 expression in BRCA and normal tissues. Eligible GSE data chips should meet the following criteria: (1) Genome-wide RNA expression data was evaluated on human breast cancer tissue and normal breast tissue. (2) Platforms of the microarray chips were Affymetrix. After the final GSE datasets were determined, the following data was extracted: GSE number, author, race, platform, last update date, sample sizes of the cancer and control group, mean (M) and standard deviation (SD) of MAD2L1 expression value in cancer and control group. Statistical analysis for GEO meta-analysis was carried out in the same way as literature meta-analysis.

MedCalc was applied to generate receiver operating characteristic (ROC) curves for all the included GSE datasets with the purpose of obtaining sensitivity and specificity value of MAD2L1 expression to distinguish BRCA tissues from normal tissues in each GSE dataset.

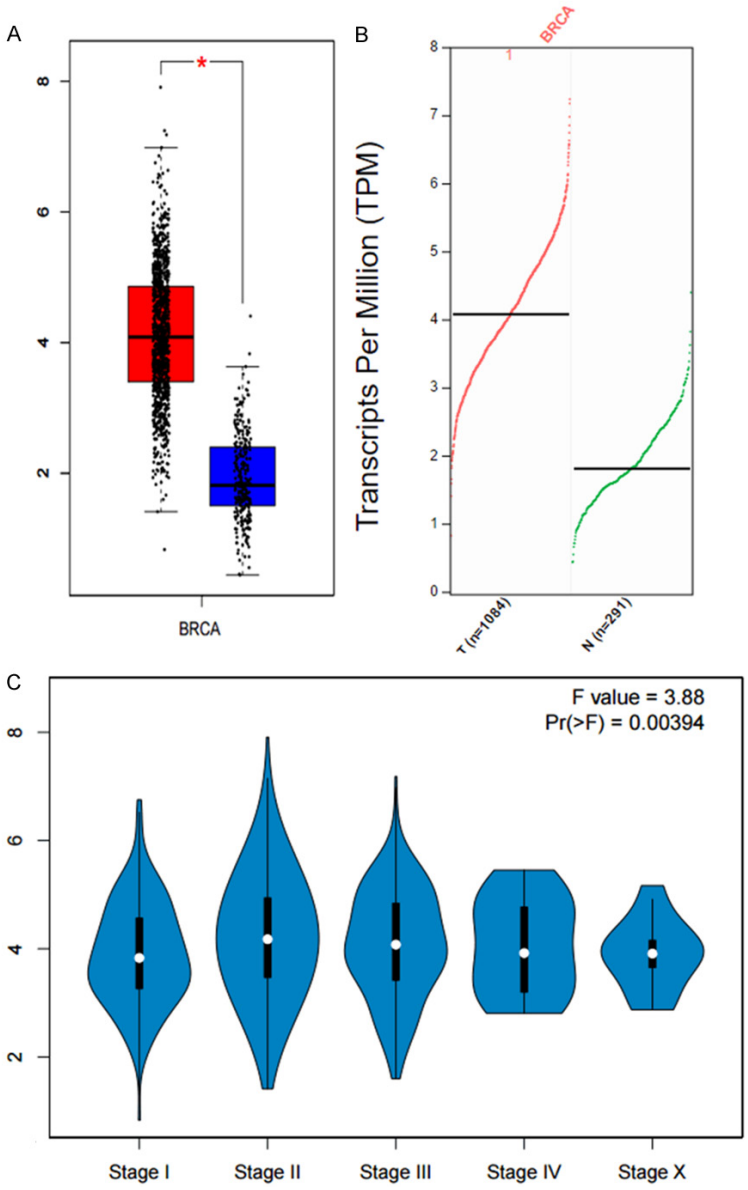

Figure 2. MAD2L1 expression in BRCA from GEPIA. MAD2L1 Expression patterns and transcripts per million (TPM) of MAD2L1 RNA in 1084 BRCA tissues (T) and 291 normal tissues (N) were displayed in (A and B), respectively. MAD2L1 expression was remarkably higher in BRCA than in normal tissues. (C) MAD2L1 expression varied significantly in different clinical stages of BRCA, MAD2L1 showed significantly higher level of expression in advanced clinical stages of BRCA (stage II-V) (F value =3.88, P=0.00394).

According to sensitivity and specificity for the diagnostic capacity of MAD2L1 in all the included GSE datasets, we calculated the corresponding true positivity (TP), false positivity (FP), false negativity (FN) and true negativity (TN) for sensitivity and specificity value in each GSE dataset. Finally, summarized receiver operating characteristic (SROC) curve was drawn by MetaDiSc v.1.4 based on TP, FP, FN and TN of each study.

Results

MAD2L1 expression in BRCA and para-carcinoma tissues

Among the 209 BC tissues, MAD2L1 showed positive staining in 102 cases (48.8% of all BC tissues), which was composed of eight weak positive stained cases, 66 positively stained cases and 28 strongly positive stained cases. The remaining 107 tissues were observed to present negative MAD2L1 staining. In para-carcinoma tissues, MAD2L1 achieved a positive expression rate of 24.9% (13 in 53 tissues). The above results indicated higher MAD2L1 expression in BC tissues than in adjacent tissues with a statistical significance (P<0.05) (**Table 1**; **Figure 1**).

Relationship between MAD2L1 expression in BRCA and the clinicopathological parameters of BRCA

The expression of MAD2L1 in breast cancer was associated with histological grade (P< 0.05), while no significant relationship was established between MAD2L1 expression and age, tumor size, lymph node metastasis, distant metastasis and clinical stage of BC patients (P>0.05) (**Table 2**).

Relationship between MAD2L1 expression in BC and common index for immunohistochemical detection of BC

MAD2L1 expression in BC was significantly correlated with the expression of ER, P53, HER-2 and Ki-67 in BC (P<0.05) (**Table 3**).

Spearman correlation test: Analysis of Spearman correlation test revealed that MAD2L1 expression in BC was positively correlated with histological grade (r=0.158, P=0.028), HER-2 expression (r=0.216, P=0.000), Ki-67 expression (r=0.233, P=0.001) and P53 expres-

Table 4. Basic information of the included datasets

GSE number	Author	Race	Platform	Last update date	Cancer N	Cancer M	Cancer SD	Control N	Control M	Control SD
GSE15852	Ivyna, Bong	Asian	GPL96	Jul 01 2016	73	10.00845447	0.651095305	43	9.829742319	0.565808176
GSE25407	Jean, J, Latimer	American	GPL570	Apr 09 2017	5	10.4508514	0.112134244	5	10.6136129	0.153729025
GSE10810	Vicente, Pedraza	European	GPL570	Apr 09 2017	31	7.071309952	1.043193234	27	4.923809465	0.428516693
GSE20711	Sarah, Dedeurwaerder	North American	GPL570	Apr 09 2017	88	7.704285875	1.001455992	2	5.599624	1.054467331
GSE10797	Theresa, Casey	American	GPL571	Apr 05 2017	56	3.830242268	1.403286121	10	3.8921222	1.080166707
GSE10780	Dung-Tsa, Chen	American	GPL570	Apr 09 2017	42	7.315071952	1.16400513	143	5.370366028	0.553808354
GSE5764	Gulisa, Turashvili	European	GPL570	Apr 09 2017	10	6.890201844	1.936432741	20	6.022723176	1.497544582
GSE7904	Andrea, Richardson	American	GPL570	Apr 09 2017	43	8.972624561	0.773823246	7	7.080406811	0.394178345
GSE42568	Colin, Clarke	European	GPL570	Apr 09 2017	104	7.814128048	1.31947288	17	6.005553294	1.250201568
GSE61304	Surya, P, Yenamandra	Asian	GPL570	Apr 09 2017	58	7.417258517	0.712955633	4	6.220965	0.047957571
GSE65194	Thierry, Dubois	European	GPL570	Apr 09 2017	153	9.189512895	1.594949305	11	4.553580727	0.715525081

Note: N: number; M: mean; SD: standard deviation.

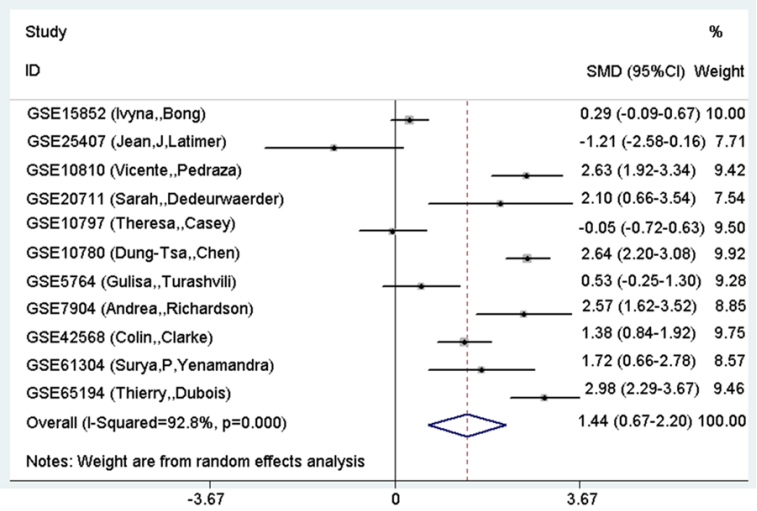

Figure 3. The forest plot of GEO meta-analysis. The pooled SMD of 1.44 (0.67-2.20) with great heterogeneity (I^2=92.8%, P<0.001) suggested that MAD2L1 expression was significantly higher in BRCA tissues than in normal tissues.

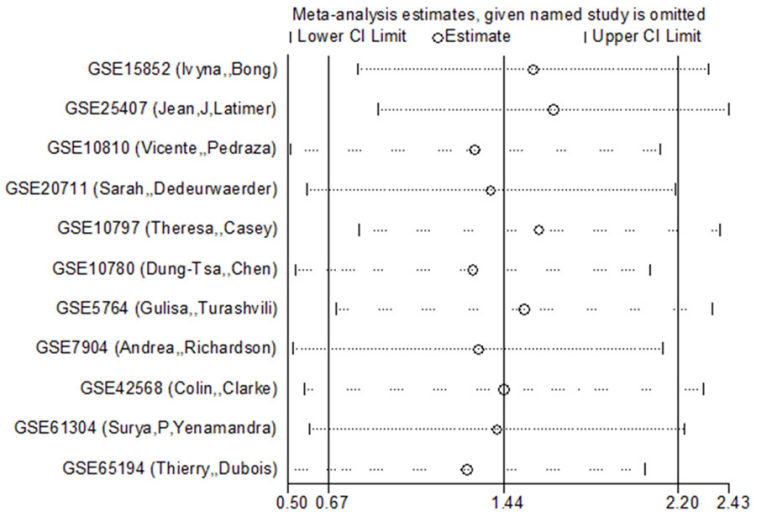

Figure 4. The sensitivity analysis. The sensitivity analysis revealed that no study exerted significant influence on the overall pooling result.

sion (r=0.159, P=0.017) as well as negatively correlated with ER expression (r=-0.170, P=0.016).

MAD2L1 expression in BRCA from TCGA data

As shown in **Figure 2A** MAD2L1 expression was overexpressed in BRCA tissues, which was further verified by the remarkably higher number of MAD2L1 mRNA transcripts in 1084 BRCA tissues than in 291 normal tissues (**Figure 2B**). Moreover, MAD2L1 expression displayed significant difference between various clinical stages of BRCA. Compared with stage I, MAD2L1 showed significantly higher level of expression in advanced clinical stages of BRCA (stage II-V) (F value =3.88, P=0.00394) (**Figure 2C**).

GEO meta-analysis

As a result of literature selection, no study was eligible for literature meta-analysis; therefore, we failed to conduct literature meta-analysis. With respect to microarray chip searching, a total of 11 GSE datasets with 663 BRCA samples and 289 normal samples matched the inclusion criteria and were included for GEO meta-analysis. Basic information of all the qualified GSE datasets were listed in **Table 4**. An overall SMD of 1.44 with a 95% CI of 0.67-2.20 from the forest plot in **Figure 3** revealed that MAD2L1 presented higher expression in BRCA than in normal tissues. Since great heterogeneity existed in GSE datasets (I^2=92.8%, P<0.001), random-effect model was employed to calculated the aggregated SMD with 95% CI. To find out the source of heterogeneity, sensitivity analysis and subgroup analysis were performed. After excluding each study at a time to yield a SMD with 95% CI of the remaining studies, we observed from the sensitivity analysis that no GSE dataset caused significant influence on the whole cohort of studies (**Figure 4**). Then we carried out subgroup analysis by dividing the GSE datasets into three subgroups of race: Asian, American and European. Unfortunately, even subgroup analysis failed to explain the origin of heterogeneity satisfactorily. Value of 95% CI of SMD in the subgroups of Asian and American contained 0, which suggested that the results were statistically insignificant

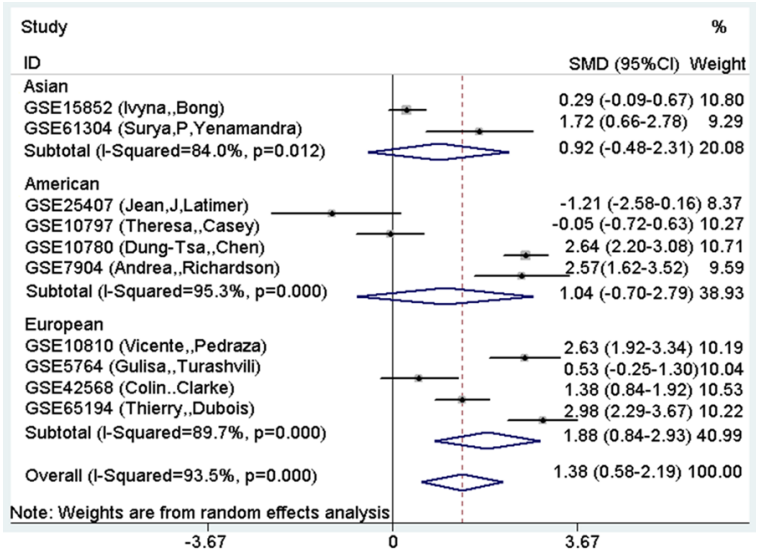

Figure 5. The subgroup analysis. Subgroup analysis was performed according to race, SMD with 95% CI in the subgroup of Asian, American and European were 0.92 (-0.48-2.31), 1.04 (-0.70-2.79) and 1.88 (0.84-2.93), respectively. The results were statistically insignificant.

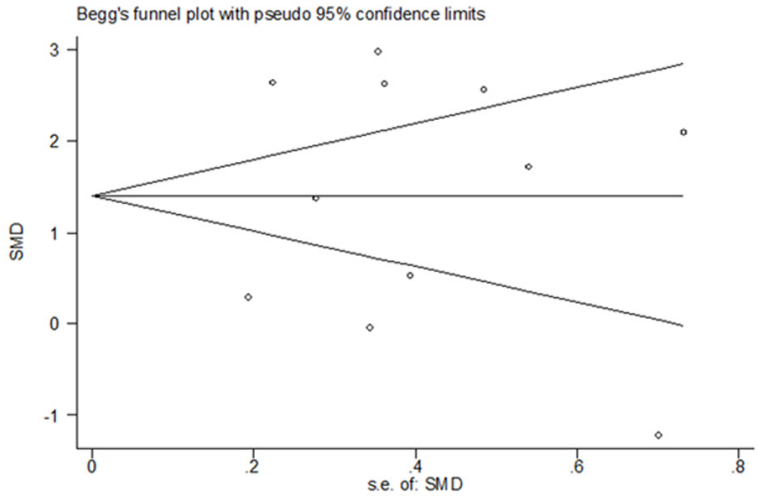

Figure 6. The publication bias. The symmetrical funnel plot indicated that no publication bias was detected.

(**Figure 5**). Additionally, no publication bias was detected from the symmetrical funnel plot produced by Begg's test (P>0.05) (**Figure 6**).

Diagnostic ability of MAD2L1

According to the SROC plotted with TP, FP, FN and TN of all the 11 GSE datasets, MAD2L1 exhibited significantly preferable diagnostic ability for BRCA with an area under curve (AUC) value of 0.9642 (**Figure 7**).

Discussion

The mitotic cycle of normal cells is under the rigorous regulation of multiple check points in cells. If the checkpoints are destroyed, the loss or wrongly distribution of chromosomes during uncontrolled mitosis would bring about aneuploidy cells. In general, solid tumor cells are aneuploid cells [32]. Thus, many researchers hypothesized that the development of aneuploidy might lead to the initiation of tumor [33, 34].

MAD2L1, an important component of spindle assembly checkpoint, is located on chromosome 14 and consists of 205 amino acid residues, with a molecular weight of approximately 25 KD. MAD2 is the protein encoded by MAD2L1 gene [20, 35]. Wei YC et al. found that low levels of MAD2 caused chromosomal instability and overexpression of MAD2 correlated with mitotic arrest and chromosomal in mouse embryos [35]. Sotillo et al. also reported that both low and MAD2 expression would produce aneuploidy and ultimately result in tumorigenesis in a study of adult mice [36]. These findings revealed the pivotal role of MAD2L1 in supervising mitosis of cells. Recently, a growing number of evidence suggested that MAD2L1 is majorly overexpressed in tumors and overexpression of MAD2L1 promoted the formation of tumors from multiple organs [37, 38].

In this study, we investigated MAD2L1 expression in BRCA through IHC, GEPIA and GEO meta-analysis. Consistently, results from IHC, GEPIA and GEO meta-analysis proved that MAD2L1 was remarkably overexpressed in BRCA tissues. Though several studies have discovered the overexpression of MAD2L1 in BRCA

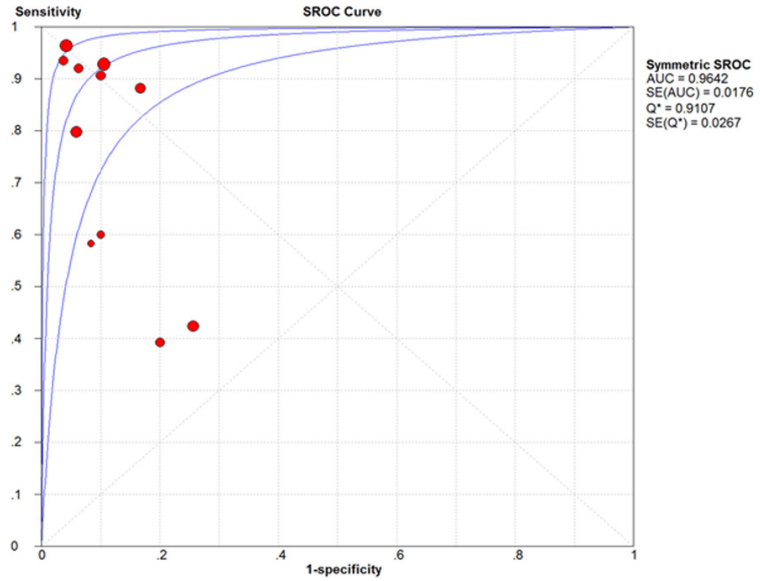

Figure 7. The SROC curve. An AUC value of 0.9642 from SROC curve revealed that MAD2L1 was of significant diagnostic value for BRCA.

[18, 39], our study was the first one to comprehensively explore the aberrant expression of MAD2L1 in BRCA through a combination of IHC, GEPIA and GEO meta-analysis. We originally attempted to validate MAD2L1 expression in BRCA by literature analysis. Unfortunately, only a small number of studies have concentrated on identifying MAD2L1 expression in BRCA and none of the studies provided available data of MAD2L1 expression in BRCA and normal tissues for extraction. Therefore, we only conduct meta-analysis using microarray chips containing MAD2L1 expression in BRCA and normal tissues from GEO database. Although the pooled SMD with 95% CI confirmed MAD2L1 overexpression in BRCA, we failed to explain the obvious heterogeneity between studies. We assumed that various platforms of GEO datasets and different number of BRCA and normal samples between GEO datasets might contribute to the heterogeneity between studies. It should be noted that only five cases of BRCA and normal tissues were included in GSE25407, which was contrasted sharply with the large sample size of other GSE datasets.

Apart from comparing MAD2L1 expression in BRCA and normal tissues, we also investigated the relationship between clinicopathological features of MAD2L1. From the significant correlation between MAD2L1 expression and the advanced histological grade and clinical stage of BRCA, we can deduce that MAD2L1 might promote the malignant potential of BRCA. Previous studies have reported that MAD2 overexpression played an essential role in tumor invasion and metastasis of diverse human cancers including non-small cell lung cancer, oral squamous cell carcinoma and osteosarcoma [40-42], which indicated that MAD2L1 served as an oncogene in most cancers. As for the contribution of MAD2L1 to tumor development, the underlying mechanism was far from elucidated. Schvartzman et al. demonstrated through a cell culture experiment that MAD2 overexpression was required for chromosome instability of p53 and retinoblastoma (Rb) mutant tumor model, which subsequently lead to the inactivation of p53 and Rb tumor suppressor pathways. Thus, inhibition of p53 and Rb are closely associated with MAD2 overexpression [43]. We hypothesized that down-regulation of p53 and Rb might interpret the carcinogenic effect of MAD2L1 in breast cancer. Additionally, we explored the association between MAD2L1 expression and common biomarkers for the detection of BRCA such as ER, P53, HER-2 and Ki-67. The significant relationship between MAD2L1 and ER, P53, HER-2 and Ki-67 implied that MAD2L1 might interact with these biomarkers to function in the pathogenesis of BRCA. Ki-67 played a crucial role in cell proliferation and served as a diagnostic target for cancer [44]. In large B-cell lymphoma and oral squamous cell carcinoma, MAD2 was observed to accelerate tumor proliferation and was significantly correlated with Ki-67 index [41, 45], we speculated that MAD2L1 and Ki-67 exerted synergistic effect on tumor growth. With regard to p53, the significant statistical result enhanced the reliability of our hypothesis that the inhibition of p53 might contribute to overexpression of MAD2L1. MAD2 expression has also been reported to associate with HER-2 expression in invasive ductal BRCA [46]. Up to now, there is insufficient evidence to support the potential interaction between MAD2L1 and

these biomarkers, further studies with in vivo or in vitro experiments are warranted to validate this assumption.

Since we have confirmed overexpression of MAD2L1 and its relationship between clinicopathological features of BRCA, we furthermore examined the diagnostic value of MAD2L1 in BRCA. The significant diagnostic ability of MAD2L1 in BRCA from SROC revealed that MAD2L1 had a broad application prospect as a novel diagnostic target for BRCA.

Conclusion

In conclusion, MAD2L1 overexpression played critical role in the development of BRCA and we anticipated that MAD2L1 could function as an effective therapeutic and diagnostic marker for BRCA. Since we only verified overexpression and clinical significance of MAD2L1 in BRCA in this study, future studies were needed to investigate the molecular basis of the oncogenic impact of MAD2L1 on BRCA.

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Disclosure of conflict of interest

None.

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