Pathol. Oncol. Res. (2019) 25:71–79
DOI 10.1007/s12253-017-0326-z

ORIGINAL ARTICLE

NRG-1 Stimulates Serum DJ-1 Increase in Breast Cancers

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Received: 30 June 2017 / Accepted: 21 September 2017 / Published online: 30 September 2017

Abstract To explore the relationship between the expression of DJ-1/HER3 and tumor grade in breast cancer, and investigate the effect of HER3 on NRG-1-mediated serum DJ-1 level in vivo. We analyze the expression level of DJ-1 and HER3 in 68 patients with different grades of breast cancer by immunostaining the tissue microarray. Besides, we investigated the serum DJ-1 level by ELISA. We found that the detectable DJ-1 protein expression is decreased, and the HER3 expression is increased in tumor tissue with the progression of breast cancer. There is a significant rise of DJ-1 in serum in vivo with the stimulation of NRG-1. Meanwhile, we found that HER3 knockdown abolishes NRG-1-induced serum DJ-1 increase and HER3 overexpress improves NRG-1-induced serum DJ-1 increase. This study provides a serum biomarker for breast cancer. The results showed that DJ-1 was associated with clinical stage of breast cancer, and NRG-1 increased the dissociation of HER3 and DJ-1, with promoting the level of DJ-1 in peripheral blood. It is suggested that the level of DJ-1 in peripheral blood may be conducive to assess the prognosis of patients with breast cancer and serum DJ-1 levels can serve as an indicator of therapeutic effectiveness for the development of HER3 targeting breast cancer antibody therapies.

Keywords Serum DJ-1 · HER3 · NRG-1 · Breast cancers · MCF-7 cells

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Introduction

Breast cancer is the second leading cause of cancer death for female, including different types of phenotypes and morphology for the reason of highly heterogeneous [5, 8]. 20% of breast cancer is HER2 positive, tending with the characteristics of rapid growth, high invasive, prompting worse prognosis [3, 21]. The application of targeted therapy of HER2 antibodies has radically changed the prognosis of HER2 positive breast cancer in the past few years, however, resistance of the therapies still leads to treatment failure and tumor progression [22]. Recently, HER3 has been identified as a pivotal role in carcinogenic signaling pathways without therapeutic tumor progression and posttreatment response, especially, in the activation of the HER2/HER3 heterodimerization [13, 17]. Besides, when anti-HER2 antibodies were used to inhibit the PI3K/AKT signaling pathway, the feedback mechanism led to the activation of HER3, causing an important resistance to therapy. Therefore, HER3 targeted therapy for auxiliary of the comprehensive therapy in breast cancer caught the attention [6]. However, the lack of convenient biomarkers for HER3-driven cancer poses a big challenge for the clinical development of HER3 targeting antibody therapies [25].

Protein 7 (DJ-1/PARK 7) was previously considered a novel interaction partner of HER3 [2]. Overexpression of DJ-1 has been reported in many cancer types, which indicates DJ-1

is an oncogene [14]. DJ-1 mRNA expression was increased in most breast cancer [24], but approximately half of breast cancer cases exhibited low expression of DJ-1 protein despite upregulation of mRNA levels. It is demonstrated that some cancer cells secrete DJ-1 [15], including uveal malignant melanoma, NIH3T3, human SHSY5Y and breast cancer cells [9, 16, 23]. However, the physiological and pathological significance of DJ-1 secretion is not clearly understood. In fact, a high level of DJ-1 protein has been detected in peripheral blood of patients with breast cancers [11], which could be a biomarker candidate for breast cancer.

In the previous reports, we have found that NRG-I promotes the decoupling of DJ-I with HER3 and activates the heterodimerization of HER2/HER3 [26]. In this study, we analyzed the relationship between the expression of DJ-1 and the grades of breast cancer in the tumor tissue chip. Furthermore, to explore the effect of NRG-1 on tumor growth and DJ-1 level in vivo, we investigated the effect of HER3 knockdown and overexpression on NRG-1-induced DJ-1 in transplanted tumor tissue and peripheral blood of nude mice by ELISA.

Materials and Methods

Patients and Specimens

Tissue samples from 68 breast invasive ductal carcinoma patients, including clinical stage 1, 2, 3 and pathological gradingI,II,III grade (The sixth edition), were selected from Shanghai Outdo biotech Co., Ltd. The chip batch number: HBre-Duc068Bch-01. Pathological cancer stages were categorized by using the TNM classification affiliated with the International Union against Cancer [19]. All of the 68 patients are ER and HER2 positive. Portions of breast cancer tissue 4 mm thick were trimmed routinely by pathologists immediately after mastectomy in each case. In order to exclude the influence of variations in fixation time, the tissue was then fixed for approximately 5 h in 10% formalin and processed into paraffin blocks.

Tissue Microarray (TMA)

Two to three portions of cancer and non-cancerous tissue were identified and marked in specific blocks for immunostaining by matching with haematoxylin and eosin (H&E)-stained reference slides. TMA blocks were prepared using a custom-made puncher / arrayer (Beecher Instruments, Silver Spring, Maryland, USA). Each marked block of non-cancerous or cancerous tissue was sampled two to three times with a 0.6-mm diametercorer arrayed in a rectangular pattern with a distance of 1 mm between the center of the cores, creating a quadrupl-icate TMA layout. H&E staining and immunostaining of ER, PR and HER2 were performed, the presence of carcinoma and⁄or-non cancerous tissue was confirmed and histological grade, hormone receptor status, and HER2 expression were also confirmed to correspond to the immunostained slides used for pathological diagnosis.

Immunostaining

Three-1 mm-thick sections were reacted with an identical mouse monoclonal antibody against human DJ-1 or HER3 that was used for immunoblot analysis. Briefly, after blocking of any endogenous peroxidase activity, the antigen was retrieved in 0.01 M sodium citrate buffer (pH 6.0) by autoclaving for 10 min. The sections were then allowed to cool to room temperature and incubated overnight at room temperature with the anti-DJ-1 or anti-HER3 antibody diluted 1:10,000. The sections were subsequently stained using Simple Stain MAX-PO (Nichirei Bioscience, Tokyo, Japan) and visualized using stable diaminobenzidene (DAB solution) (Invitrogen, San Diego, CA, USA), followed by counterstaining with haematoxylin.

Overexpression and Stable Knockdown of HER3 in Breast Cancer Cells

For the construction of stable HER3 overexpression cell line, the pcDNA3/FRT vector (GenScript, China) was used to transfect breast cancer cells MCF-7, which containing the gene of human HER-3, then they were treated by the addition of G418 (20 μg/ml) to co-culture for 3–4 weeks to select successful transfected cells. To generate stable HER-3 knockdown cells, Plasmid DNA of shRNA targeting HER-3 were amplified in *E. coli* (Clontech) and lenti-viral particles were produced after 24 h of co-transfection with the shRNA constructs, using lipofectamine (Invitrogen). MCF-7 cells was transfected with the lentivirus particles and cells were selected in RMPI (1640) media containing puromycin (4 μg/ml) for 3 weeks as described previously.

Mouse Xenograft Study

Mouse tumor xenograft studies were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication no. 85–23, revised 1985) and approved by IACUC (Institutional Animal Care and Use Committee of Nanjing Medical University). HER-3 shRNA knockdown, scramble shRNA control, HER-3 overexpression and pcDNA control MCF-7 cells were subcutaneously (s.c.) implanted in nu/nu mice as previously reported [12]. Tumor growth was measured using a digital caliper and recorded twice a week. Xenograft mouse tumor tissues and the serum were collected at the end of in vivo study and stored at −80 °C for ex vivo analysis.

NRG-1 Administration

Mice were administered either PBS or NRG1 (R&D Systems; dissolved in phosphate-buffered saline (PBS), administered at a constant rate of 10 μ g/d; n = 8/group) for 24 h through subcutaneously-implanted osmotic mini-pumps (Alzet), as performed and described previously by Mahar [10]. Post-operatively, mice were placed on a heating pad to recover, monitored for complications, and received an anti-inflammatory Carprofen tablet placed in the cage.

Enzyme-Linked Immunosorbent Assay (ELISA)

The DJ-1 concentration in mouse serum samples were determined by the sandwich ELISA method. ELISA was carried out using the DJ-1 /PARK7 ELISA kit, obtained from R&D Systems, Inc. (Minneapolis, MN, USA), according to the manufacturer's protocol. Briefly, 96-well immuno-module microplates (Corning Inc., Coring, NY, USA) were pre-coated with DJ-1 capture antibody by incubation overnight at room temperature followed by blocking for 1 h at room temperature. Then 100 μL sera or standards were added to the pre-treated plates. After incubation at room temperature for 2 h the plates were washed five times with 300 μL PBS containing 0.05% (v/v) Tween-20 and then immuno-reacted with another biotinylated goat anti-human DJ-1 antibody at room temperature for 30 min. After removing any unbound antibody with five washes, 100 μL horseradish peroxidase (HRP)-labeled streptavidin was added to each well followed by incubation at room temperature for 30 min. After another five washes, 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (R&D Systems, Inc.) was added to the wells and allowed to react for 10 min. The reaction was then immediately stopped with 2 mol/L H_2SO_4, and optical density (OD) was determined using a photometer at a wavelength of 450 nm and a reference wavelength of 570 nm. Each sample was diluted to 1:20, and was examined in triplicate.

Statistical Analysis

Mean and standard deviation (SD) of each sample's data collected from data in vivo. Statistical differences between two or

Table 1 The expression of DJ-1/HER3 in different groups of breast cancer

Factor		Total (n = 68)	DJ-1 level(average)	P-value	Factor		Total (n = 68)	DJ-1 level(average)	P-value
T	T1	15	1.37(20.5/15)	P < 0.05	age group	30–39	6	1.25 (7.5/6)	NS
	T2	41	1.26(51.5/41)			40–49	12	1.33(16/12)	
	T3	5	1.2(6/5)			50–59	12	1.13(13.5/12)	
	T4	2	1(2/2)			60–69	16	1.41(22.5/16)	
	unknown	5	1.1(5.5/5)			70–79	8	1.31(10.5/8)	
N	N0	37	1.3(48.5/37)	P < 0.05		80–89	4	1.5(6/4)	
	N1	10	1.3(13/10)			NR	10	1.1(11/10)	
	N2/N3	16	1.19(19/16)		Cinical stage	1	12	1.29(15.5/12)	NS
	unknown	5	1.2(6/5)			2A	23	1.33(30.5/23)	
M	M0	68	0			2B	11	1.27(14/11)	
Size, cm3	≤2 cm3	3	1.83(5.5/3)	P < 0. 05		3A	14	1.39(19.5/14)	
	2-5 cm3	11	1.39 15/11)			3B	1	1.5(1.5/1)	
	≥5 cm3	54	1.3(7054)			3C	1	1(1/1)	
Factor		Total(n = 68)	her3 level(average)	P-value	Factor		Total (n = 68)	her3 level(average)	P-value
T	T1	15	1.03(15.5/15)	P < 0.05	age group	30–39	6	1.33(8/6)	NS
	T2	41	1.17(48/41)			40–49	12	0.83(10/12)	
	T3	5	1.2(6/5)			50–59	12	1.17(14/12)	
	T4	2	1.25(2.5/2)			60–69	16	1.13(18/16)	
	unknown	5	0.8(4/5)			70–79	8	1.38(11/8)	
N	N0	37	1.03(38/37)	P < 0 05		80–89	4	1.5(6/4)	
	N1	10	1.1(11/10)			NR	10	0.6(6/10)	
	N2/N3	16	1.25 (20/I6)		Clinical stage	1	12	0.87(10.5/12)	P < 0.05
	unknown	5	1.2(6/5)			2A	23	1.10(25.5/23)	
M	M0	68	0			2B	11	1.11(10/9)	
Size, cm3	≤ 2 cm3	3	1(3/3)	P < 0 05		3A	14	1.28(18/14	
	2-5 cm3	11	1.14(17/11)			3B	1	2(2/1)	
	≥5 cm3	54	1.21(65.5/54)			3C	1	0	

TNM staging is in accordance with the UICC 6th version in 2002; NS, no statistical difference

more groups were tested using the unpaired Student's t-test or one-way ANOVA, respectively, from STATA 11.0 software (StataCorp, College Station, TX, USA) and Prism 5.0 software (GraphPad, La Jolla, CA, USA). The chi-square test was used to investigate the significance of the relationships between DJ-1 expression and other factors, such as tumor topography, tumor size, lymph node status metastasis and histological grade, difference at $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the StatView version 5.0 software package (SAS Institute, Cary, NC, USA).

Results

Correlations between the Expression of DJ-1 and Clinic-Pathological Parameters

We carried out the immunostaining of DJ-1 and HER3 with the tissue samples respectively, according to the color intensity and the color rate. We grouped the pathological parameters of conventional breast cancer, such as TNM, tumor size, age, clinical stage, etc. (UICC 6th version in 2002), and used effective statistics. The characteristics of the data are described in the following form. We found that the expression level of DJ-1 in tumor cytoplasm decreased gradually with the increase of T, N stage and tumor diameter of breast cancer tissue samples, showing significant statistically differences ($P < 0.05$). However, there was not significant difference in group of age and clinical stages (Table 1). Meanwhile, the level of HER3 expression in the cytoplasm gradually increased with that of the tissue, showing statistical differences ($P < 0.05$), except data of age groups (Table 1).

The Expression of DJ-1 in Tumor Cells Decreased with the Progress of Breast Cancer

We analyzed the data of breast cancer tissue microarray after specific immunostaining include DJ-1 and HER3 with the 68 patients. We found that the expression of DJ-1 protein in

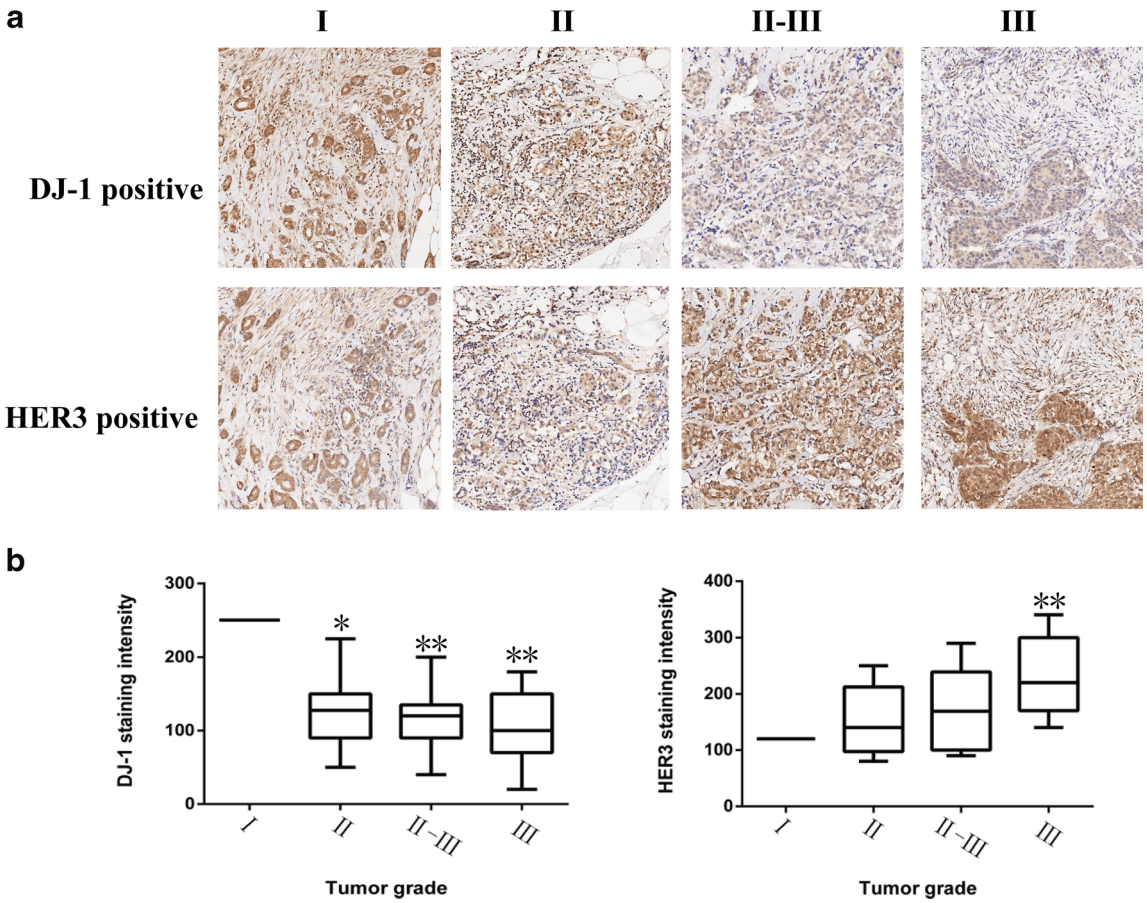

Fig. 1 Expression of DJ-1 and HER3 in breast cancer tissue microarray from different tumor grades. **a** The intracellular intensity of DJ-1 was decreased with the grade increased in breast cancer. The increase of intracellular HER3 expression with the grade increased in breast cancer. Immunohistochemical staining of DJ-1 and HER3 in breast cancer tissue microarray from different tumor grades (I, II, II-III, III). Scale bars, 500 μm. **b** Quantitative analysis of the statistical differences in the expression of DJ-1/HER3 between the various groups.* indicates $P < 0.05$, ** indicates $P < 0.01$

tumor tissue was decreased both in dyeing strength and positive staining rate with the breast cancer classification improved from I to III. On the other hand, HER3 showed a clear upward trend with the classification of breast cancer increased. We selected the representative pictures shown in Fig. 1a. In order to analyze the results of immunostaining more intuitively and quantitatively, we accumulated staining intensity and positive staining rate and according to different tumor grading group, used professional statistical software and found that with the increase of tumor grade in breast cancer, the expression of DJ-1 in tissue microarray was significantly decreased, both II-III and III groups showed significant statistical differences compared with group I. While the expression level of HER3 increased in group III showed significant differences (Fig. 1b) ($P < 0.01$).

NRG-1-Induced Serum DJ-1 Increase In Vivo

NRG-1 is an important ligand for HER3/HER2 activation which can promote the dissociation of DJ-1 and HER3 in breast cancer. In order to explore the effect of NRG-1 on the growth of breast cancer and DJ-1 level in tumor tissue and peripheral blood in vivo. We grouped and transplanted tumor with or without NRG-1 to nude mice, observed the process of tumor growth and detailed records of tumor volume changes from 10th to 42th day. The data revealed that the xenografts grew significantly faster from 109 mm^3 to 1616 mm^3 with the stimulant of NRG-1 compared to PBS group (from 56 mm^3 to 638 mm^3), showing a statistically significant difference (Fig. 2a) ($P < 0.01$).

To explore the interaction between NRG-1 and DJ-1, we detected the level changes of DJ-1 in serum and transplanted tumor tissues by quantitative method ELISA. The data demonstrated that the level of DJ-1 in serum is significantly increased from 11.95 ng/ml to 27.05 ng/ml with the stimulant of NRG-1 (Fig. 2b) ($P < 0.01$). At the same time, the expression of DJ-1 in tumor tissue is significantly decreased from 36.9 ng/ml to 21.89 ng/ml with the stimulant of NRG-1 (Fig. 2c), which may be related to NRG-1 improved the decoupling of DJ-1 and HER3. Previous studies have reported that DJ-1 and HER3 are in coupled state, and NRG-1 has a decoupling effect on DJ-1 and HER3 (18). In order to further explore whether the NRG-1-stimulated serum DJ-1 increase was affected by the expression level of HER3, we constructed HER3 knockdown and overexpression MCF-7 cells and compared tumor growth to the control with or without NRG-1 in vivo.

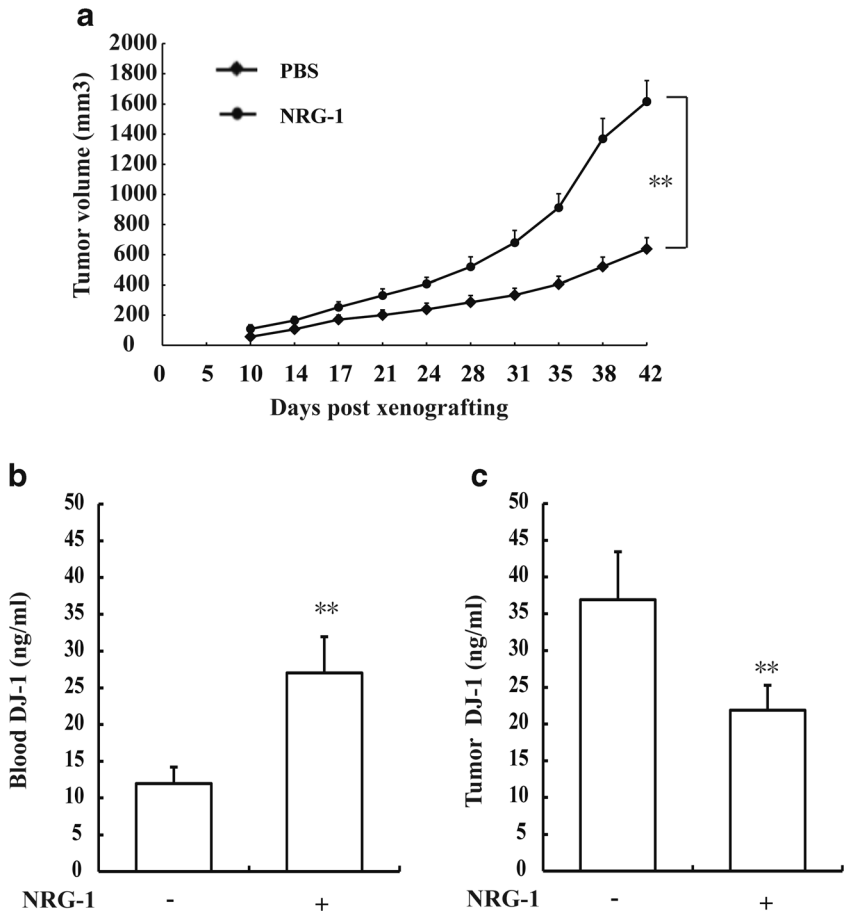

Fig. 2 Effect of NRG-1 on tumor growth and DJ-1 level in vivo. The proliferation of tumor was significantly faster with the stimulant of NRG-1than the PBS group. The graph shows mean tumor volume ± standard deviation (s.d.) (**a**). The level of DJ-1 was significantly increased in peripheral blood with NRG-1 (**b**). The DJ-1 level in tumor tissue was quantitatively analyzed, and showed significantly decreased with NRG-1 (**c**). ** indicates $P < 0.01$ compared to without NRG-1group

HER3 Knockdown Inhibits NRG-1 Induced Serum DJ-1 Increase

As shown in Fig. 3a, the tumor growth was decreased in HER3 knockdown group. The tumor grow ability from the 10th day to the 42th day was significantly inhibited from 36.3 mm^3 to 350.3 mm^3 in the HER3 knockdown group when compared to the scramble group (from 58.2 mm^3 to 608.2 mm^3). However, NRG-1 had no effect on the tumor proliferation in HER3 knockdown group. ELISA was used to detect the level of DJ-1 in tumor tissue and peripheral blood. We found that DJ-1 level increased from 11.8 ng/ml to 26.6 ng/ml in peripheral blood and decreased from 36.6 ng/ml to 23.8 ng/ml in tumor tissue with NRG-1 stimulation in scramble group ($P < 0.01$). However, there was no significant change in HER3 knockdown groups with NRG-1 stimulation (Fig. 3b, c). These results suggest that HER3 knockdown abolishes NRG-1-induced serum DJ-1 increase.

HER3 Overexpression Stimulates NRG-1 Induced Serum DJ-1 Increase

To further explore the effect of HER3 on NRG-1-induced serum DJ-1 level, we constructed HER3 overexpressing cell lines and transplanted in nude mice. We regularly recorded the growth of the tumor, and detected the level of DJ-1 in the peripheral blood and tumor tissue by ELISA.

As shown in Fig. 4a, the tumor growth from the 10th day to the 31th day was significantly increased from 88.3 mm^3 to 839.9 mm^3 in the HER3 overexpression group than the pcDNA control group (from 59.6 mm^3 to 362.7 mm^3). As expected, NRG-1 improved tumor growth both in HER3 overexpress group and pcDNA control group. Importantly, NRG-1 showed significantly more increase of tumor growth in HER3 overexpress group as compared to pcDNA control group. We also found that the serum DJ-1 level was higher in the HER3 overexpression group than the pcDNA control group in both presence (33.2 ng/ml vs 24.6 ng/ml) and absence (17.8 ng/ml vs 12.1 ng/ml) of NRG-1. And the DJ-1 level of tumor tissue was lower in the HER3 overexpression group than the pcDNA control group in both presence (18.6 ng/ml vs 24.2 ng/ml) and absence (31.7 ng/ml vs 37.1 ng/ml) of NRG-1. NRG-1 significantly improved the serum DJ-1 level and inhibited the DJ-1 level of tumor tissue in both the pcDNA control and HER3 overexpress groups (Fig. 4b, c). These results suggest that HER3 overexpress improves NRG-1-induced serum DJ-1 increase.

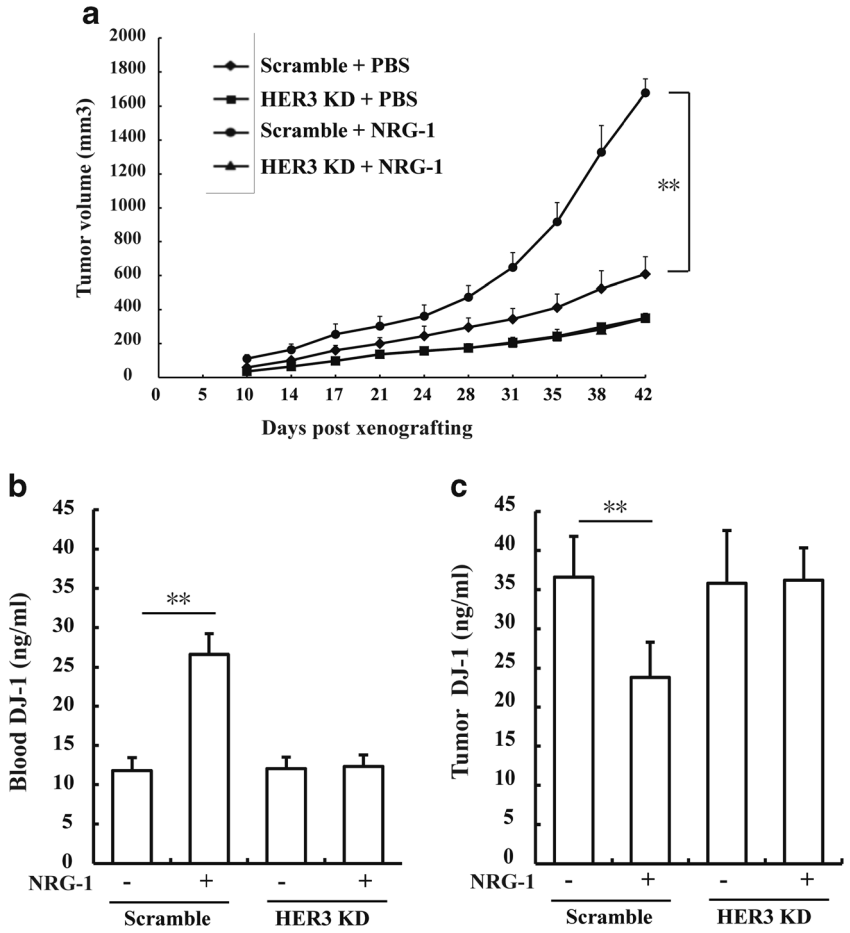

Fig. 3 Effect of HER3 knockdown on tumor growth and DJ-1 level with NRG-1 in vivo. The tumor growth was decreased in HER3 knockdown group when compared to the scramble group. NRG-1 had no effect on the tumor growth in HER3 knockdown group The graph shows mean tumor volume ± standard deviation (s.d.) (**a**). HER3 knockdown abolishes NRG-1-induced serum DJ-1 increase (**b**) and tumor tissue DJ-1 decrease (**c**). ** indicates $P < 0.01$ compared to without NRG-1group

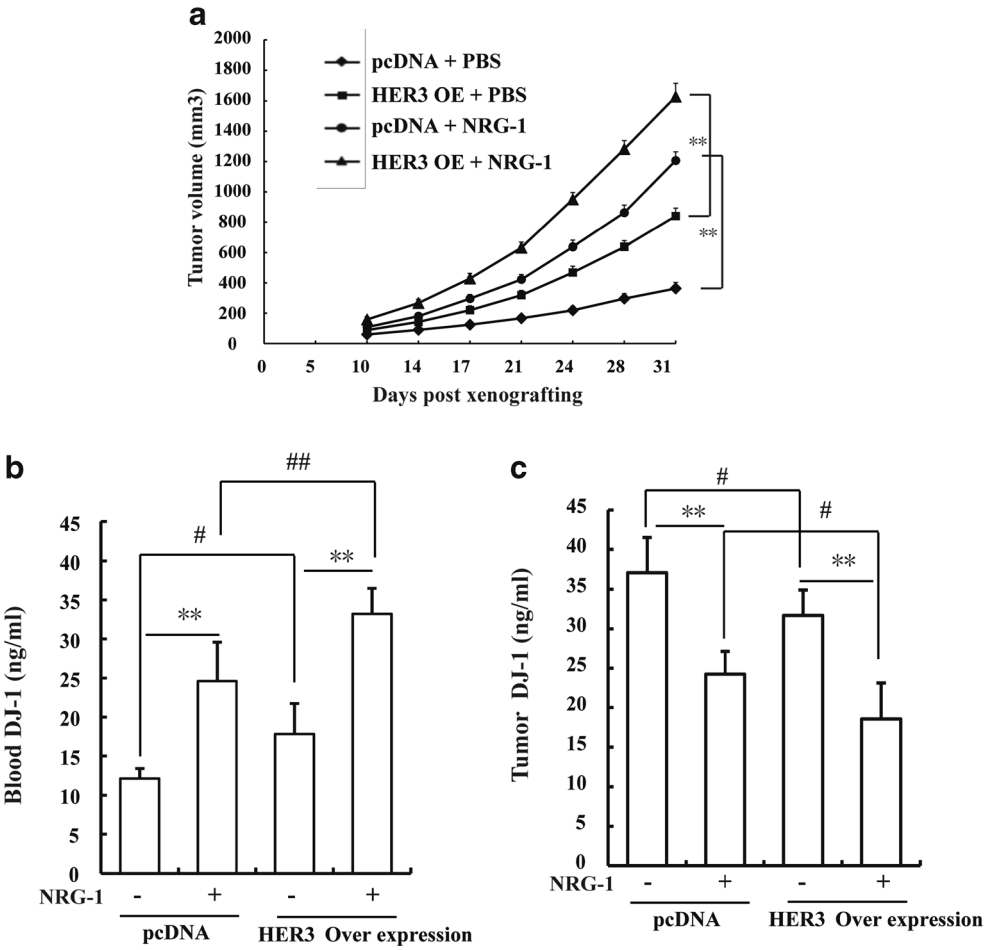

Fig. 4 Effect of HER3 overexpression on tumor growth and DJ-1 level with NRG-1 in vivo. The tumor growth was significantly improved in the HER3 overexpression group with NRG-1. The graph shows mean tumor volume ± standard deviation (s.d.) (**a**). HER3 overexpress improves NRG-1-induced serum DJ-1 increase (**b**) and tumor tissue DJ-1 decrease (**c**)..* indicates $P < 0.05$, ** indicates $P < 0.01$ compared to without NRG-1group. # indicates $P < 0.05$, ## indicates $P < 0.01$ compared to pcDNA control group

Discussion

It is reported that some of the breast cancer cells, in which the DJ-1 protein is weakly stained, retain the function of secretion of DJ-1 and poorly differentiation. In contrast, other cell lines that cannot secrete DJ-1 show strong staining of DJ-1 protein, and the growth is relatively slow [24]. In addition, DJ-1 has a chaperone activity and may transport some molecules into extracellular space [27], that DJ-1 has the effect of promoting invasion and metastasis [7]. The potential level of DJ-1 assessment is necessary for early diagnosis of breast cancer. In this study, we have found that the level of the DJ-1 with negative correlation with the tumor size, grade, T stage, and lymphatic metastasis in tumor tissues microarray of the breast cancer. It is suggested that the level of DJ-1 in breast cancer cells is related to the clinical stage and prognosis of breast cancer. However, we cannot confirm that the relation of the DJ-1 in serum with the tumor grade from above information, so we carried out nude mouse tumor xenograft studies to identify the speculation.

It is reported that NRG-1 promotes the heterodimerization of HER3 with other ligands, especially HER2, further activates the PI3K/AKT cell signaling pathways [20], which leading to tumor cell proliferation and differentiation, inhibiting the role of tumor suppressor genes such as PTEN [4]. NRG-1 has been proposed as a biomarker for clinical development of HER3 antibody cancer therapies [1, 12, 18]. Our team found that NRG-1 plays a key role in regulating the interaction of HER3 and DJ-1. In the absence of NRG-1, DJ-1 was associated with HER3 in a state of low phosphorylation, and the effect of DJ-1 also prevent HER3 from degradation via the ubiquitin-proteasomal pathway. In the presence of NRG-1, HER3 transform to a state of high phosphorylation and dissociates from DJ-1 [26]. In this study, we compared the tumorigenesis of HER3 knockdown group and overexpression group to the control with or without NRG-1, and the changes of DJ-1 expression in serum and tumor tissues in vivo. We found that NRG-1-induced serum DJ-1 increased and decreased DJ-1 in tumor tissues. Furthermore, HER3 knockdown abolishes NRG-1-induced serum DJ-1 increase, and HER3 overexpress improves NRG-1-induced serum DJ-1 increase in vivo. So we confirmed that NRG-1-stimulated serum DJ-1 increase in breast cancer was affected by the expression level of HER3 in the tumor tissue.

However, there are some defects of this experiment. We only have the information on tissue microarrays and lack of

the information of patients' serum. The lack of an effective long term follow up and identification, only reflect the relationship between HER3 and serum DJ-1 expression in specific stage of breast cancer in vivo, which does not adequately account for long-term dynamic results, as well as the prognosis and overall survival of patients.

Conclusion

The current study provides a serum biomarker for breast cancer. The information from tissue microarray showed that DJ-1 was associated with clinical stage of breast cancer, and NRG-1 increased the dissociation of HER3 and DJ-1, with promoting the level of DJ-1 in peripheral blood. Taken together, our results suggest that the level of DJ-1 in peripheral blood may be conducive to assess the prognosis of patients with breast cancer and serum DJ-1 levels can serve as an indicator of therapeutic effectiveness for the development of HER3 targeting breast cancer antibody therapies.

Acknowledgements This project was sponsored by the National Natural Science Foundation of China (No. 81373398); a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Allard L, Burkhard PR, Lescuyer P, Burgess JA, Walter N, Hochstrasser DF, Sanchez JC (2005) PARK7 and nucleoside diphosphate kinase A as plasma markers for the early diagnosis of stroke. Clin Chem 51:2043–2051. https://doi.org/10.1373/clinchem.2005.053942
2. Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M, van Dongen JW, Vanacore N, van Swieten JC, Brice A, Meco G, van Duijn CM, Oostra BA, Heutink P (2003) Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299:256–259. https://doi.org/10.1126/science.1077209
3. Brenton JD, Carey LA, Ahmed AA, Caldas C (2005) Molecular classification and molecular forecasting of breast cancer: ready for clinical application? J Clin Oncol 23: 7350–7360. https://doi.org/10.1200/JCO.2005.03.3845
4. Choi BK, Cai X, Yuan B, Huang Z, Fan X, Deng H, Zhang N, An Z (2012) HER3 intracellular domains play a crucial role in HER3/HER2 dimerization and activation of downstream signaling pathways. Protein Cell 3:781–789. https://doi.org/10.1007/s13238-012-2065-y
5. Chung C, Lee S, Hwang S, Park E (2013) Systematic review of exercise effects on health outcomes in women with breast cancer. Asian Nurs Res (Korean Soc Nurs Sci) 7:149–159. https://doi.org/10.1016/j.anr.2013.07.005
6. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA (2007) MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 316:1039–1043. https://doi.org/10.1126/science.1141478
7. Honbou K, Suzuki NN, Horiuchi M, Niki T, Taira T, Ariga H, Inagaki F (2003) The crystal structure of DJ-1, a protein related to male fertility and Parkinson's disease. J Biol Chem 278:31380–31384. https://doi.org/10.1074/jbc.M305878200
8. Karagianni M, Kaitelidou D, Kalokairinou A, Mantas J (2014) Breast cancer in social media: a literature review. Stud Health Technol Inform 202:321
9. Le Naour F, Misek DE, Krause MC, Deneux L, Giordano TJ, Scholl S, Hanash SM (2001) Proteomics-based identification of RS/DJ-1 as a novel circulating tumor antigen in breast cancer. Clin Cancer Res 7:3328–3335
10. Mahar I, Tan S, Davoli MA, Dominguez-Lopez S, Qiang C, Rachalski A, Turecki G, Mechawar N (2011) Subchronic peripheral neuregulin-1 increases ventral hippocampal neurogenesis and induces antidepressant-like effects. PLoS One 6:e26610. https://doi.org/10.1371/journal.pone.0026610
11. Maita C, Tsuji S, Yabe I, Hamada S, Ogata A, Maita H, Iguchi-Ariga SM, Sasaki H, Ariga H (2008) Secretion of DJ-1 into the serum of patients with Parkinson's disease. Neurosci Lett 431: 86–89. https://doi.org/10.1016/j.neulet.2007.11.027
12. Meetze K, Vincent S, Tyler S, Mazsa EK, Delpero AR, Bottega S, McIntosh D, Nicoletti R, Winston WM, Weiler S, Feng B, Gyuris J, Weng Z (2015) Neuregulin 1 expression is a predictive biomarker for response to AV-203, an ERBB3 inhibitory antibody, in human tumor models. Clin Cancer Res 21:1106–1114. https://doi.org/10.1158/1078-0432.CCR-14-2407
13. Mujoo K, Choi BK, Huang Z, Zhang N, An Z (2014) Regulation of ERBB3/HER3 signaling in cancer. Oncotarget 5:10222–10236. https://doi.org/10.18632/oncotarget.2655
14. Nagakubo D, Taira T, Kitaura H, Ikeda M, Tamai K, Iguchi-Ariga SM, Ariga H (1997) DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras. Biochem Biophys Res Commun 231:509–513. https://doi.org/10.1006/bbrc.1997.6132
15. Oda M, Makita M, Iwaya K, Akiyama F, Kohno N, Tsuchiya B, Iwase T, Matsubara O (2012) High levels of DJ-1 protein in nipple fluid of patients with breast cancer. Cancer Sci 103:1172–1176. https://doi.org/10.1111/j.1349-7006.2012.02267.x
16. Pardo M, Garcia A, Thomas B, Pineiro A, Akoulitchev A, Dwek RA, Zitzmann N (2006) The characterization of the invasion phenotype of uveal melanoma tumour cells shows the presence of MUC18 and HMG-1 metastasis markers and leads to the identification of DJ-1 as a potential serum biomarker. Int J Cancer 119: 1014–1022. https://doi.org/10.1002/ijc.21942
17. Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM, Moasser MM (2007) Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature 445:437–441. https://doi.org/10.1038/nature05474
18. Shames DS, Carbon J, Walter K, Jubb AM, Kozlowski C, Januario T, Do A, Fu L, Xiao Y, Raja R, Jiang B, Malekafzali A, Stern H, Settleman J, Wilson TR, Hampton GM, Yauch RL, Pirzkall A, Amler LC (2013) High heregulin expression is associated with activated HER3 and may define an actionable biomarker in patients with squamous cell carcinomas of the head and neck. PLoS One 8: e56765. https://doi.org/10.1371/journal.pone.0056765
19. Sobin LH, Compton CC (2010) TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. Cancer-Am Cancer Soc 116: 5336–5339. https://doi.org/10.1002/cncr.25537

20. Soltoff SP, Carraway KR, Prigent SA, Gullick WG, Cantley LC (1994) ErbB3 is involved in activation of phosphatidylinositol 3-kinase by epidermal growth factor. Mol Cell Biol 14:3550–3558
21. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 100:8418–8423. https://doi.org/10.1073/pnas.0932692100
22. Sotiriou C, Pusztai L (2009) Gene-expression signatures in breast cancer. N Engl J Med 360:790–800. https://doi.org/10.1056/NEJMra0801289
23. Tsuboi Y, Munemoto H, Ishikawa S, Matsumoto K, Iguchi-Ariga SM, Ariga H (2008) DJ-1, a causative gene product of a familial form of Parkinson's disease, is secreted through microdomains. FEBS Lett 582:2643–2649. https://doi.org/10.1016/j.febslet.2008.06.043
24. Tsuchiya B, Iwaya K, Kohno N, Kawate T, Akahoshi T, Matsubara O, Mukai K (2012) Clinical significance of DJ-1 as a secretory molecule: retrospective study of DJ-1 expression at mRNA and protein levels in ductal carcinoma of the breast. Histopathology 61:69–77. https://doi.org/10.1111/j.1365-2559.2012.04202.x
25. Zhang N, Chang Y, Rios A, An Z (2016) HER3/ErbB3, an emerging cancer therapeutic target. Acta Biochim Biophys Sin Shanghai 48:39–48. https://doi.org/10.1093/abbs/gmv103
26. Zhang S, Mukherjee S, Fan X, Salameh A, Mujoo K, Huang Z, Li L, To'A SG, Zhang N, An Z (2016) Novel association of DJ-1 with HER3 potentiates HER3 activation and signaling in cancer. Oncotarget 7:65758–65769. https://doi.org/10.18632/oncotarget.11613
27. Zhou W, Zhu M, Wilson MA, Petsko GA, Fink AL (2006) The oxidation state of DJ-1 regulates its chaperone activity toward alpha-synuclein. J Mol Biol 356:1036–1048. https://doi.org/10.1016/j.jmb.2005.12.030