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ORIGINAL ARTICLE

Diabetes as a prognostic factor in HER-2 positive breast cancer patients treated with targeted therapy

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Abstract

Purpose Recent studies revealed that metabolic stress influences the outcomes of breast cancer treatment. We sought to evaluate the prognostic effect of type 2 diabetes and find the molecular mechanism of relapses in postoperative HER-2+ breast cancer patients treated with HER-2 targeted therapy.

Materials and methods We evaluated 190 HER-2+ breast cancer patients (pT1-4N0-2M0) who were treated with surgical resection and trastuzumab (HER-2 targeted therapy) between 2006 and 2015. Survival outcomes and failure patterns were compared between such patients with ($n=12$) and without ($n=178$) type 2 diabetes.

Results The median follow-up period was 42.4 months (range 12.0–124.7 months). Twenty-one patients (11.1%) showed relapse (including nine patients with locoregional failure), and three patients (1.6%) died as a result of cancer relapse. One-third of the patients with diabetes experienced relapse (4/12, 33.3%). The 3-year disease-free survival (DFS) and overall survival (OS) rates were 90.7% and 98.6%, respectively. Diabetic patients showed shorter DFS compared with non-diabetic patients ($p=0.006$, 74.1% vs. 91.9%). OS was also shorter in diabetic patients compared with non-diabetic patients ($p=0.017$, 91.7% vs. 99.1%). Of our interest, the levels of HER-3 and its ligand neuregulin-1 were significantly increased in the tumor specimen in HER-2+ breast cancer patients suffering with type 2 diabetes than that in the euglycemic control group.

Conclusions Type 2 diabetes was associated with detrimental effects on survival in postoperative HER-2+ breast cancer patients who were treated with trastuzumab. The poor prognostic effect of diabetes in HER-2+ breast cancer patients could be associated with the high levels of HER-3 and neuregulin 1, thus it should be considered and evaluated more.

Keywords HER-2 positive breast cancer · Trastuzumab · Diabetes · Survival · Prognostic factor

Introduction

Recent studies showed that diabetes is correlated with cancer incidence [1, 2], and diabetic patients often have a poor prognosis after cancer treatment. Cancer growth requires a

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metabolic program change [3, 4]. Cancer cell proliferation is able to alter the cellular metabolic status, including glucose levels. Recent studies [3, 5] have revealed that metabolic stress influences the treatment outcomes of specific cancers. Clinically, metabolic syndrome, including diabetes and obesity, can influence tumor progression. Obesity was shown to increase the incidence of breast, colon, and endometrial cancers [2]; investigation into the prognostic relationship between diabetes and cancer followed thereafter [6]. Increased levels of insulin-like growth factor are considered a risk factor for carcinogenesis. Conversely, metformin, an old anti-diabetic drug, is known to have anti-cancer effects [7], even in breast cancer patients after resection [8].

Human epidermal growth factor receptor 2 (HER-2) gene amplification or protein overexpression is observed in 20% of invasive breast cancers. The treatment outcome of HER-2+ breast cancer has significantly improved following the introduction of HER-2 receptor targeting agents. The use of trastuzumab for the systemic treatment of HER-2+ breast cancer increases the disease-free survival (DFS) and overall survival (OS) by approximately 50%.

The mechanism of action of HER-2 is correlated with several known metabolic pathways, including the phosphoinositide 3-kinase (PI3K)-AKT signal transduction pathway [9]. To overcome the treatment resistance to trastuzumab, usage of everolimus, a new mTOR inhibitor, was tried. The epidermal growth factor receptor (EGFR)-related pathways also can influence tumor cell progression, proliferation, and invasion, while also altering the glucose metabolism pathway involved in diabetes [6]. Neuregulin 1 (Nrg1) is also receiving attention as one of the treatments resistant to trastuzumab-related molecules.

Overall, breast cancer prognosis is affected by the metabolic disease [10–12]. Diabetes weakly increases the incidence of breast cancer [10] and is associated with breast cancer prognosis [13, 14]. A large-scale cohort study by Bonifazi et al. [15] revealed that diabetes is a prognostic factor for Italian women with early breast cancer treated with trastuzumab. If high risk of recurrence in patients can be detected beforehand, it will be helpful to find the appropriate treatment for those patients. Therefore, the prognostic effect of diabetes on HER-2+ breast cancer in the era of treatment with trastuzumab should be evaluated more.

Treatment response of trastuzumab may be affected by the Nrg1 expression status [16, 17]. According to a recent research by Park et al. [18], Nrg1 is involved in the phenomenon of hyperglycemic memory in breast cancer, showing an aggressive growth of cancer cells exposed to hyperglycemia compared to euglycemia. Nrg1 is a potent growth factor activating HER-3 signaling pathways as a ligand; often it activates HER-2 pathways when HER-2 form dimerize with HER-3 in the development and cancer progression [19, 20]. In the cancer perspective, various studies strongly suggest that amplification of HER-3 status in cancer cells is an index of poor prognosis, resulting in a higher rate of chemo-resistance and relapses [20–22]. Thus, it would be valuable to evaluate the levels of Nrg1 and HER3 in HER-2+ breast cancer patients who experienced relapses after targeted chemotherapy particularly in diabetic conditions. For these reasons, we sought to evaluate the prognostic effect of diabetes in HER-2+ breast cancer patients who were treated with surgery and trastuzumab.

Materials and methods

Patient selection

We included and evaluated 190 breast cancer patients who were treated in Busan and Haeundae Paik Hospital. All patients were pathologically diagnosed with a subtype of hormone receptor negative (non-luminal) HER-2+ breast cancer or hormone receptor positive (luminal B) HER-2+ breast cancer, according to the St. Gallen criteria [23]. Patients diagnosed with carcinoma in situ or bilateral breast cancers were excluded from the evaluation. Patients with distant metastases or a history of cardiac disease at the time of diagnosis were also excluded from this evaluation. All patients included in this study underwent breast cancer surgery. After surgical resection, all patients received trastuzumab for at least 6 months. All patients in this study were followed for at least 1 year after surgical resection.

Patients' medical records were retrospectively reviewed to evaluate their past medical history and treatment results. Diabetes was diagnosed in patients with fasting plasma glucose levels ≥ 126 mg/dL, hemoglobin A1c levels $> 6.5\%$, or random glucose levels > 200 ng/dL with clinical symptoms of the disease. Evaluation of patients for diabetes was performed at the time of surgery.

Ethical approval

This study was approved by the Institutional Review Board of Busan Paik Hospital and Haeundae Paik Hospital. Further biological research with tissue samples from the patients who had agreed to the use of their breast tumor tissue for cancer research (with written consent) was approved by both IRB and Inje University-Paik Hospital Biobank.

Perioperative treatment methods

Chemotherapy (CT) consisting of an anthracycline, taxane, and platinum-based regimen, was administered to the patients with a high risk of relapse. Trastuzumab was generally administered intravenously for 1 year as a targeted agent for HER-2+ breast cancer. Conventional

radiotherapy (RT) was administered to the patients who underwent breast-conserving surgery or who had a high risk of local relapse after undergoing a modified radical mastectomy based on pathological reports. Anti-estrogen hormone therapy was administered to patients with hormone receptor positive (Luminal B) HER-2+ breast cancer.

Immunohistochemical staining, Western blotting and RT-PCR

HER-2+ breast cancer was diagnosed based on pathologically observed overexpression (3+ according to the American Society of Clinical Oncology guidelines [24]) of ERBB2/HER-2 or HER-2 gene positivity in a fluorescence in situ hybridization assay, and negative expression for both the estrogen receptor (ER) and progesterone receptor (PR). For ER and PR, activity < 10% was considered negative. The primary antibodies used for pathologic evaluations were as follows: ER (1:90; Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), PR (1:170; Novocastra Laboratories Ltd.), and HER-2 (Ventana Medical Systems Inc., Tucson, USA).

Tumor tissue of five breast cancer patients was available for further molecular evaluation. Patient tissue samples were obtained under the approval of InJe biobank. For the additional experiments to find the mechanism of cancer treatment resistance-related diabetes, immunohistochemical staining of Nrg1, Western blotting, and quantitative real-time polymerase chain reaction (qRT-PCR) were performed to evaluate the tissue samples. Paraffin block and frozen section were used for tissue analyses. For immunochemical staining, 6-μm-sections of formalin-fixed and paraffin-embedded tumor tissue were used. Optical microscope was used for examining Nrg1 expression after immunochemical staining. Nrg1 monoclonal antibody (from R&D Systems™) was used for immunochemical (IHC) staining and Western blotting. Western blot analyses were performed to identify the cross-reactivity between patients' tissue and Nrg1 antibodies in protein level. Anti-beta lactamase antibody was used for loading of negative control. Additionally, qRT-PCR was performed to find the Nrg1 expression in messenger RNA level according to the presence of diabetes.

Outcome

The primary end-point of this study was DFS. OS and the pattern of failure were also evaluated as secondary end-points. The OS and DFS rates were calculated from the time of surgery. The mortality event in this study included all types of deaths.

Table 1 Patient characteristics ($n = 190$)

Characteristics	No. (%)
Age (year)	
< 50	64 (33.7)
≥ 50	126 (66.3)
Diabetes	
Positive	12 (6.3)
Negative	178 (93.7)
Pathologic subtype	
Luminal B HER-2	79 (41.6)
HER-2	111 (58.4)
p53	
Positive	118 (62.1)
Negative	72 (37.9)
Resection margin	
Positive	7 (3.7)
Negative	183 (96.3)
T stage	
T1	107 (56.3)
T2	77 (40.5)
T3	5 (2.6)
T4	1 (0.5)
N stage	
N0	116 (61.1)
N1	54 (28.4)
N2	10 (5.3)
N3	10 (5.3)
Tumor location	
Right	96 (50.5)
Left	94 (49.5)
Types of surgery	
BCS	117 (61.6)
MRM	73 (38.4)
Radiotherapy	
Yes	120 (63.2)
No	70 (36.8)
Chemotherapy	
Yes	138 (72.6)
No	52 (27.4)
Hormone therapy	
AI	48 (25.3)
TMX	26 (13.7)
None	116 (61.1)

HER-2 human epidermal growth factor receptor 2, *BCS* breast-conserving surgery, *MRM* modified radical mastectomy, *TMX* Tamoxifen, *AI* aromatase inhibitor

Statistical analysis

As for the statistical methods, the Fisher's exact test and Chi square test were used for comparisons to evaluate the clinical factors related to treatment failure and diabetes.

Table 2 Comparison of clinical characteristics between diabetic and non-diabetic patients

Characteristics	Diabetic patients ($n=12$)	Non-diabetic patients ($n=178$)	p value
	No.	No.	
Age (year)			0.344
< 50	2	62	
≥ 50	10	116	
p53			0.768
Positive	7	111	
Negative	5	67	
Pathologic subtype			0.365
Luminal B HER-2	3	76	
HER-2	9	102	
Resection margin			1
Positive	0	7	
Negative	12	171	
T stage			0.233
T1	9	97	
T2–4	3	81	
N stage			1
N0	7	109	
N1–3	5	69	
Tumor location			0.565
Right	5	91	
Left	7	87	
Types of surgery			0.541
BCS	6	111	
MRM	6	67	
Radiotherapy			0.129
Yes	5	115	
No	7	63	
Chemotherapy			0.739
Yes	8	130	
No	4	48	
Hormone therapy			0.373
Yes	3	71	
No	9	107	

HER-2 human epidermal growth factor receptor 2, *BCS* breast-conserving surgery, *MRM* modified radical mastectomy

The Kaplan–Meier method and log-rank test were used for univariate survival analysis. The Cox proportional hazard model was used for multivariate survival analysis. Student's *t* test was used for analyzing the results of qRT-PCR. For all of the statistical analyses, SPSS version 22.0 software (IBM, Armonk, NY, USA) was used. Statistical significance was defined as $p<0.05$ in cross-tabulation and univariate analyses, whereas $p<0.10$ was considered to indicate borderline significance. In multivariate analysis, $p<0.0125$ (0.05/4) denoted significance according to the Bonferroni correction.

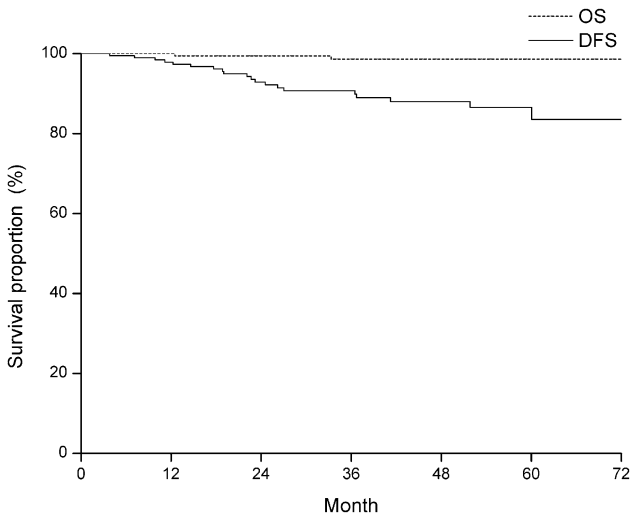

Fig. 1 Disease-free survival and overall survival

The Bonferroni correction was used for p value adjustment to reduce the false positive results from type I error. Thus, the p value was divided by the number of comparisons being made.

Results

Patient characteristics

The median follow-up period was 42.4 months (range 12.0–124.7 months). The patients' clinical features are summarized in Table 1. All of the patients were female. Their median age was 54 years (range 30–80 years). Diabetes was diagnosed in 12 patients (6.7%) prior to surgery. All of the diabetic patients had type 2 diabetes. More than half of the patients ($n=107$, 56.3%) had pathologic T1 stage breast cancer. Seventy-four patients (38.9%) had regional lymph node metastases, as determined based on the pathologic reports after surgery. As for the surgical treatment, breast-conserving surgery was performed in 117 patients (61.6%). The remaining 73 patients (38.4%) were treated with modified radical mastectomy. After surgical resection, postoperative RT was performed in 120 patients (63.2%). Overall, CT was administered to 138 patients (72.6%). Among them, 22 patients received neo-adjuvant CT. As for the hormone therapy, 48 patients (25.3%) received tamoxifen and 26 patients (13.7%) received aromatase inhibitor.

Diabetic patients ($n=12$) had no statistically significant differences in their clinical risk factors compared with non-diabetic patients ($n=178$), such as T stage, N stage, and resection margin (Table 2).

Table 3 Survival analysis for disease-free survival by univariable and multivariable analyses

Characteristics	Univariable analysis			Multivariable analysis	
	No.	3-year DFS (%)	*p* value	*p* value	HR(95% CI)
Age (year)			0.976		
< 50	7/64	93.1			
≥ 50	14/126	89.5			
Diabetes			0.006	0.009	4.5 (1.454–14.045)
Positive	4/12	74.1			
Negative	17/178	91.9			
p53			0.992		
Positive	13/118	90.6			
Negative	8/72	90.8			
Pathologic subtype			0.053	0.937	0 (0–2.320×10^{90})
Luminal B HER-2	6/79	96.1			
HER-2	15/111	86.1			
Resection margin			0.367		
Positive	0/7	100			
Negative	21/183	90.4			
T stage			0.895		
T1	12/106	90			
T2–4	9/84	91.6			
N stage			0.098	0.019	1.7 (1.086–2.536)
N0	9/116	95.7			
N1–3	12/74	83.2			
Tumor location			0.305		
Right	8/96	91.6			
Left	13/94	89.8			
Types of surgery			0.199		
BCS	9/117	92.6			
MRM	12/73	87.8			
Radiotherapy			0.342		
Yes	11/120	90.9			
No	10/70	90.7			
Chemotherapy			0.664		
Yes	14/138	90.8			
No	7/52	90.8			
Hormone therapy			0.084	0.944	2475.7 (0–3.46×10^{97})
Yes	6/74	100			
No	15/131	86.7			

HER-2 human epidermal growth factor receptor 2, *BCS* breast-conserving surgery, *MRM* modified radical mastectomy

Pattern of treatment failure

Ipsilateral breast tumor recurrence occurred in 3 patients, 6 patients experienced regional lymph nodal recurrence, and distant metastasis occurred in 13 patients. The most common distant metastasis site was the lung ($n = 7$). Overall, 21 cases of treatment failure occurred (1 patient showed both regional and distant failure). The occurrence of treatment failure was significantly increased in diabetic patients ($p = 0.031$).

Survival analysis

The 3-year DFS and OS rates were 90.7% and 98.6%, respectively (Fig. 1). For the patients with the HER2+ subtype ($n = 111$), the 3-year DFS and OS rates were calculated as 86.3% and 97.5%, respectively. For the patients with the luminal B HER2+ subtype ($n = 79$), the 3-year DFS and OS rates were 96.7% and 100%, respectively. Table 3 shows the results of the univariate survival analysis for DFS. Diabetes was the only significant poor prognostic factor associated

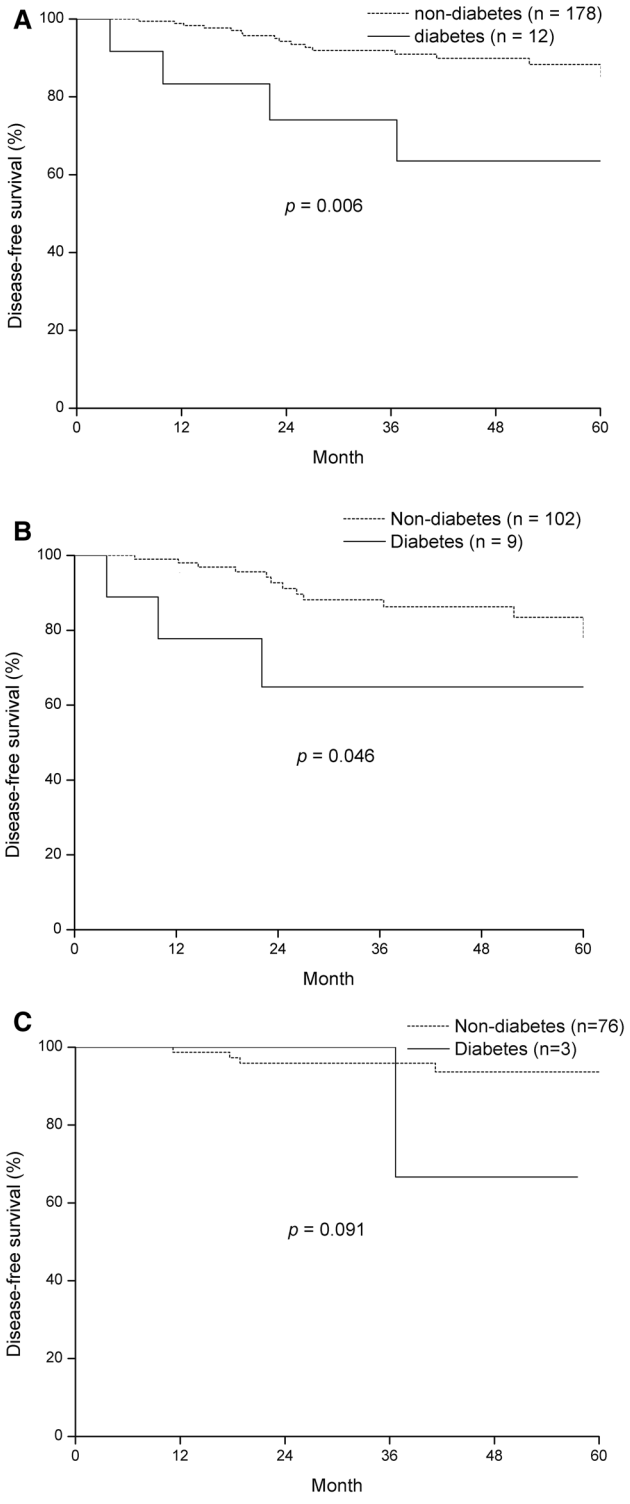

Fig. 2 **a** Disease-free survival according to diabetes. **b** Diabetes and disease-free survival in hormone receptor negative (non-luminal B) HER-2 positive breast cancer. **c** Diabetes and disease-free survival in hormone receptor positive (Luminal B) HER-2 positive breast cancer

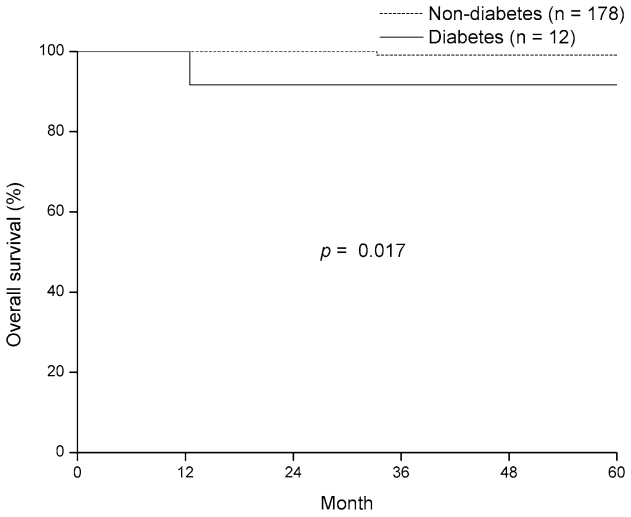

Fig. 3 Overall survival according to diabetes

with DFS ($p = 0.006$, Fig. 2a). Diabetes also reduced the OS ($p = 0.017$, Fig. 3). There were no other clinical factors that significantly influenced the DFS and OS. Pathologic node positivity ($p = 0.098$), HER-2+ subtype ($p = 0.053$), and hormone therapy ($p = 0.084$) tended to be related to DFS (Table 3). However, p53 positivity did not influence DFS ($p = 0.978$, Table 3). Neither CT ($p = 0.664$) nor RT ($p = 0.342$) significantly affected DFS (Table 3). Multivariate analysis showed that diabetes was a significant prognostic factor for DFS ($p = 0.009$, HR 4.5, 95% CI 1.454–14.045).

Pathologic subgroup analysis

In the subgroup analysis of patients with the HER2+ subtype, diabetes significantly shortened DFS ($p = 0.046$, Fig. 2b). Additionally, DFS tended to be shorter in patients with diabetes compared with those without diabetes according to the subgroup analysis of patients with the luminal B HER-2+ subtype ($p = 0.091$, Fig. 2c).

Molecular analysis of HER-2 positive breast cancer with diabetes

In subgroup analyses using patients' tissue samples, four patients out of five patients were diagnosed as type 2 diabetes, and 50% of these diabetic breast cancer patients experienced recurrence during the follow-up periods, whereas the patients in the non-diabetic control group did not experience recurrence after the targeted therapy. We directly analyzed the tumor specimens from diabetic- versus non-diabetic HER-2+ breast cancer patients by utilizing immunohistochemistry for both Nrg1 and HER-3. Notably, the levels of

Nrg1 and HER-3 in tumors were significantly elevated in diabetic groups compared to the levels in the non-diabetic control group (Fig. 4a). In the same line, the protein levels of Nrg1 and HER-3 were consistently upregulated in tumor tissues isolated from diabetic breast cancer patients compared to that in non-diabetic control group (Fig. 4b). We also confirmed the increased levels of Nrg1 and HER-3 in tumor tissues in diabetic breast cancer patient compared to that in control tumor tissues by utilizing qRT-PCR (Fig. 4c, d).

Discussion

Metabolic stress can influence breast cancer outcomes. In this study, we showed that diabetes is a poor prognostic factor for survival in patients with the HER-2+ positive breast cancer who were treated with trastuzumab. Consistent with previous report [18], increased levels of the Nrg1-HER-3 pathway in diabetic breast cancer patients seem to be correlated with poor prognosis in our cohort study, nevertheless the case number is limited.

Breast cancer consists of several different subtypes, each with different characteristics. In particular, the cellular response to metabolic stress seems to be diverse among the subtypes [25]. The proliferation of HER-2+ breast cancer cells could be related to the cellular glucose level. Moreover, the downstream expression levels of members of the EGFR and insulin-like growth factor receptor pathways are quite similar, in that they both signal through the PI3K and AKT pathway.

Alteration of glucose metabolism is related to diabetes and cancer progression, and increased levels of insulin-like

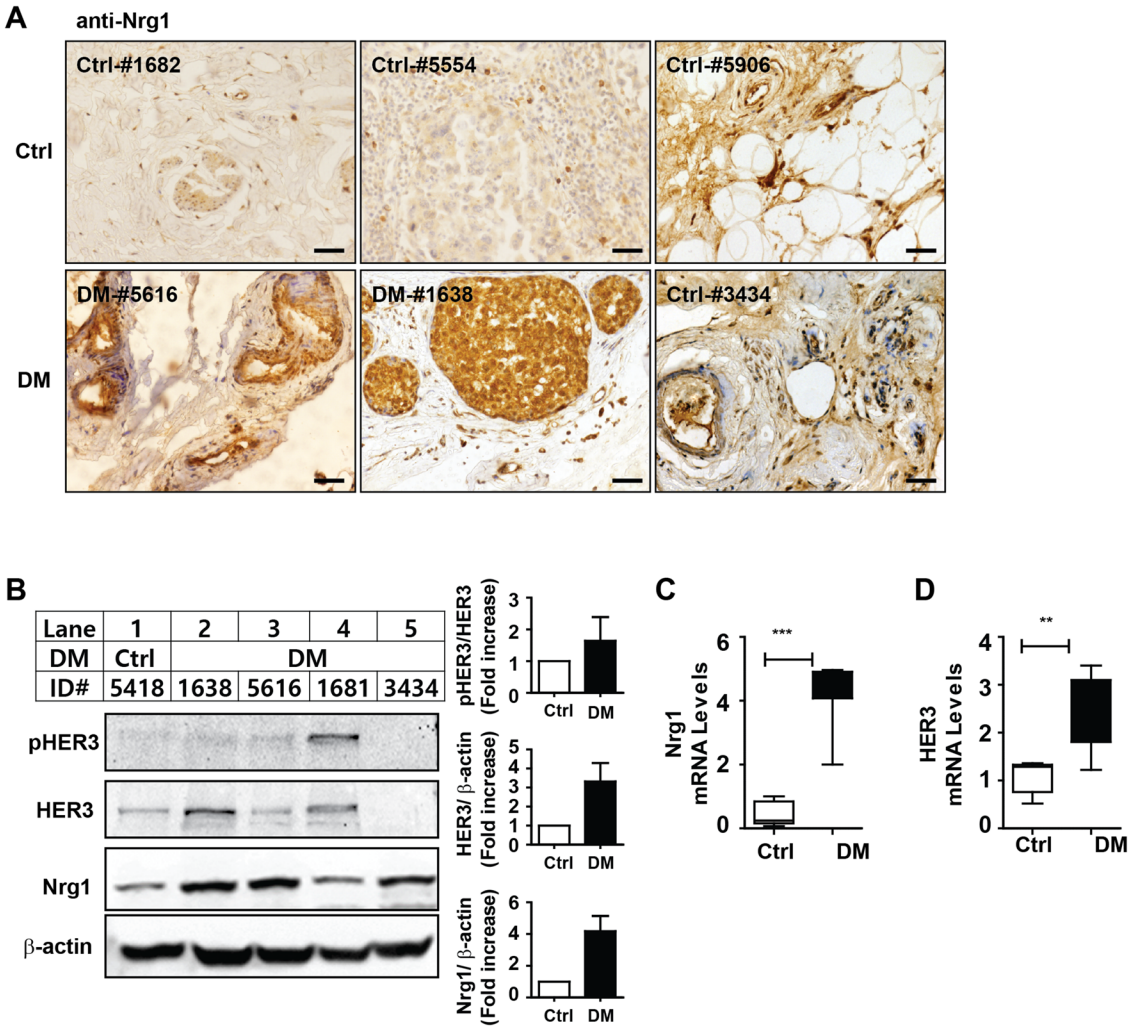

Fig. 4 **a** Immunostaining for Nrg1 in tumor specimens from HER-2+ breast cancer patients in either control or DM condition. Scales: 100 µm. **b** Protein levels of Nrg1, its receptor HER-3 and phospho-HER-3 (pHER-3) were determined by Western blotting. Quantitation of protein levels is represented as a bar graph (left). (Ctrl = 1, DM = 4). **c** mRNA levels for Nrg1 and **d** HER-3 in breast tumor tissues from control ($n = 3$) and DM ($n = 5$) were determined by qRT-PCR. Data are presented as the mean ± SEM. Statistical significance was evaluated by unpaired two-tailed Student's t test. $^{**}p < 0.01$; $^{***}p < 0.001$

growth factor in diabetes can lead to cancer cell growth [2]. Ferroni et al. [26] suggested that the mechanism of breast cancer relapse in diabetic patients is a result of both pro-inflammatory conditions and oxidative stress, which together result in cellular metabolic change. Coller et al. [3] claimed that glutamine could be an anaplerotic source for cancer cells. And, a recent study shows both breast cancer and metabolic syndrome involved in the insulin-leptin-adiponectin axis [27].

Immunologic changes in diabetes can also influence cancer treatment outcomes. Generally, T cells, natural killer cells, and macrophages are involved in the response to adjuvant treatment, such as RT [28]. Diabetic patients showed decreased innate cellular immunity related to the function of their leucocytes and macrophages. Therefore, the effect of immunologic changes can influence the breast cancer treatment outcomes.

New drugs targeting specific metabolic pathways can improve the outcome of HER-2+ breast cancer. AKT inhibitors or PI3K inhibitors seem to be promising. Also, lapatinib, a receptor tyrosine kinase inhibitor, can be useful in treating HER-2 positive breast cancer with diabetes. In addition, amplification of Nrg1 and HER-3 per se as well as the activation of HER-3 downstream signaling pathways potentially by its ligand Nrg1 could be promising targets for new treatment of breast cancer patients with diabetes [29]. The development of dual blocking agents, affecting both glucose metabolism and cancer progression, may be feasible to prevent breast cancer recurrence. In addition, it may be useful to identify the gene status associated with diabetes in selecting more effective treatment methods for relapsed HER-2 positive breast cancer patients [30, 31].

As for the limitations of this study, it employed a retrospective design, therefore, interpreting the results requires attention. As this study was initially designed as a retrospective study, only a few patients were able to agree to further PCR, Western blotting, and IHC staining. It would be better if we could analyze more patients. Nevertheless, this study may have a meaning as a pilot study to show the possibility of future research. On the other hand, treatment-related toxicity, such as cardiotoxicity [32], may influence breast cancer survival outcomes indirectly. Additionally, the anticancer effect of metformin for HER-2+ breast cancer should be evaluated more [33].

Conclusions

We demonstrated that diabetes has detrimental effects in postoperative HER-2+ breast cancer patients treated with trastuzumab. Positive NRG 1 status seems to attribute to HER axis activation. The prognostic importance of diabetes in HER-2+ breast cancer patients should not be ignored. To overcome treatment resistance related to diabetes, more effective treatment strategies for breast cancer patients with diabetes need to be developed.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

References

1. Ohkuma T, Peters SAE, Woodward M. Sex differences in the association between diabetes and cancer: a systematic review and meta-analysis of 121 cohorts including 20 million individuals and one million events. Diabetologia. 2018;61:2140–54.
2. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. Diabetes Care. 2010;33:1674–85.
3. Coller HA. Is cancer a metabolic disease? Am J Pathol. 2014;184:4–17.
4. Seyfried TN, Flores RE, Poff AM, D'Agostino DP. Cancer as a metabolic disease: implications for novel therapeutics. Carcinogenesis. 2014;35:515–27.
5. Gristina V, Cupri MG, Torchio M, Mezzogori C, Cacciabue L, Danova M. Diabetes and cancer: a critical appraisal of the pathogenetic and therapeutic links. Biomed Rep. 2015;3:131–6.
6. Chowdhury TA. Diabetes and cancer. Qjm. 2010;103:905–15.
7. Kasznicki J, Sliwinska A, Drzewoski J. Metformin in cancer prevention and therapy. Ann Transl Med. 2014;2:57.
8. Calip GS, Yu O, Hoskins KF, Boudreau DM. Associations between diabetes medication use and risk of second breast cancer events and mortality. Cancer Causes Control. 2015;26:1065–77.
9. Yang SX, Polley E, Lipkowitz S. New insights on PI3K/AKT pathway alterations and clinical outcomes in breast cancer. Cancer Treat Rev. 2016;45:87–96.
10. Redaniel MT, Jeffreys M, May MT, Ben-Shlomo Y, Martin RM. Associations of type 2 diabetes and diabetes treatment with breast cancer risk and mortality: a population-based cohort study among British women. Cancer Causes Control. 2012;23:1785–95.
11. Choi Y, Park SK, Ahn KJ, Cho H, Kim TH, Yoon HK, et al. Being overweight or obese increases the risk of progression in triple-negative breast cancer after surgical resection. J Korean Med Sci. 2016;31:886–91.
12. Hauner D, Hauner H. Metabolic syndrome and breast cancer: is there a link? Breast Care (Basel). 2014;9:277–81.
13. Mu L, Zhu N, Zhang J, Xing F, Li D, Wang X. Type 2 diabetes, insulin treatment and prognosis of breast cancer. Diabetes Metab Res Rev. 2016;33:e2823.

14. Jiralerspong S, Kim ES, Dong W, Feng L, Hortobagyi GN, Giordano SH. Obesity, diabetes, and survival outcomes in a large cohort of early-stage breast cancer patients. Ann Oncol. 2013;24:2506–14.
15. Bonifazi M, Franchi M, Rossi M, Zambelli A, Moja L, Zambon A, et al. Long term survival of HER2-positive early breast cancer treated with trastuzumab-based adjuvant regimen: a large cohort study from clinical practice. Breast. 2014;23:573–8.
16. de Alava E, Ocana A, Abad M, Montero JC, Esparis-Ogando A, Rodriguez CA, et al. Neuregulin expression modulates clinical response to trastuzumab in patients with metastatic breast cancer. J Clin Oncol. 2007;25:2656–63.
17. Yang L, Li Y, Shen E, Cao F, Li L, Li X, et al. NRG1-dependent activation of HER3 induces primary resistance to trastuzumab in HER2-overexpressing breast cancer cells. Int J Oncol. 2017;51:1553–62.
18. Park J, Sarode VR, Euhus D, Kittler R, Scherer PE. Neuregulin 1-HER axis as a key mediator of hyperglycemic memory effects in breast cancer. Proc Natl Acad Sci USA. 2012;109:21058–63.
19. Britsch S. The neuregulin-I/ErbB signaling system in development and disease. Adv Anat Embryol Cell Biol. 2007;190:1–65.
20. Ocana A, Vera-Badillo F, Seruga B, Templeton A, Pandiella A, Amir E. HER3 overexpression and survival in solid tumors: a meta-analysis. J Natl Cancer Inst. 2013;105:266–73.
21. Chiu CG, Masoudi H, Leung S, Voduc DK, Gilks B, Huntsman DG, et al. HER-3 overexpression is prognostic of reduced breast cancer survival: a study of 4046 patients. Ann Surg. 2010;251:1107–16.
22. Lee Y, Ma J, Lyu H, Huang J, Kim A, Liu B. Role of erbB3 receptors in cancer therapeutic resistance. Acta Biochim Biophys Sin (Shanghai). 2014;46:190–8.
23. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22:1736–47.
24. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol. 2013;31:3997–4013.
25. Garcia Vicente AM, Soriano Castrejon A, Leon Martin A, Chacon Lopez-Muniz I, Munoz Madero V, Munoz Sanchez Mdel M, et al. Molecular subtypes of breast cancer: metabolic correlation with (1)(8)F-FDG PET/CT. Eur J Nucl Med Mol Imaging. 2013;40:1304–11.
26. Ferroni P, Riondino S, Buonomo O, Palmirotta R, Guadagni F, Roselli M. Type 2 diabetes and breast cancer: the interplay between impaired glucose metabolism and oxidant stress. Oxid Med Cell Longev. 2015;2015:183928.
27. Davis AA, Kaklamani VG. Metabolic syndrome and triple-negative breast cancer: a new paradigm. Int J Breast Cancer. 2012;2012:809291.
28. Park B, Yee C, Lee KM. The effect of radiation on the immune response to cancers. Int J Mol Sci. 2014;15:927–43.
29. Ma J, Lyu H, Huang J, Liu B. Targeting of erbB3 receptor to overcome resistance in cancer treatment. Mol Cancer. 2014;13:105.
30. Davis NM, Sokolosky M, Stadelman K, Abrams SL, Libra M, Candido S, et al. Deregulation of the EGFR/PI3K/PTEN/Akt/mTORC1 pathway in breast cancer: possibilities for therapeutic intervention. Oncotarget. 2014;5:4603–50.
31. Leung WY, Roxanis I, Sheldon H, Buffa FM, Li JL, Harris AL, et al. Combining lapatinib and pertuzumab to overcome lapatinib resistance due to NRG1-mediated signalling in HER2-amplified breast cancer. Oncotarget. 2015;6:5678–94.
32. Guenancia C, Lefebvre A, Cardinale D, Yu AF, Ladoire S, Ghiringhelli F, et al. Obesity as a risk factor for anthracyclines and trastuzumab cardiotoxicity in breast cancer: a systematic review and meta-analysis. J Clin Oncol. 2016;34:3157–65.
33. Kim HJ, Kwon H, Lee JW, Kim HJ, Lee SB, Park HS, et al. Metformin increases survival in hormone receptor-positive, HER2-positive breast cancer patients with diabetes. Breast Cancer Res. 2015;17:64.