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Original Article

Gene Expression Profiling between Patient Groups with High and Low Ki67 Levels after Short-term Preoperative Aromatase Inhibitor Treatment for Breast Cancer

Yukiko Kajiwara[a], Takayuki Iwamoto[b]*, Yidan Zhu[a], Mariko Kochi[b], Tadahiko Shien[b], Naruto Taira[b], Hiroyoshi Doihara[b], and Shinichi Toyooka[a]

[a]*Department of General Thoracic Surgery and Breast and Endocrinological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences,*
[b]*Departments of Breast and Endocrine Surgery, Okayama University Hospital, Okayama 700-8558, Japan*

According to a recent report, a low Ki67 level after short-term preoperative hormone therapy (post-Ki67) might suggest a more favorable prognosis compared with a high post-Ki67 level in patients with hormone receptor-positive/human epidermal growth factor 2-negative (HR+/HER2−) breast cancer with high levels of Ki67. This study aimed to evaluate the pre-treatment genetic differences between these two patient groups. Forty-five luminal B-like patients were stratified into two groups, namely, a group with high (H→H) and one with low (H→L) Ki67 levels after short-term preoperative aromatase inhibitor (AI) treatment. We compared pre-treatment gene expression profiles between the two groups. In gene level analysis, there was no significant difference between the two groups by the class comparison test. In pathway analysis, five metabolism-related gene sets were significantly upregulated in the H→L group ($p \leq 0.05$). In the search for novel targets, five genes (*PARP*, *BRCA2*, *FLT4*, *CDK6*, and *PDCD1LG2*) showed significantly higher expression in the H→H group ($p \leq 0.05$). Several metabolism-related pathways were associated with sensitivity to AI. In the future, it will be necessary to seek out new therapeutic strategies for the poor prognostic group with high post-Ki67.

Key words: breast cancer, short-term hormone therapy, gene expression profiling, Ki-67, targeted therapy

Hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2−) breast cancer with high Ki67 levels is a subtype of a luminal B-like breast cancer, which is associated with a poor prognosis [1]. Historically, clinical pathological markers, especially immunohistochemistry (IHC) Ki67 levels and histologic grade, have been widely used by clinicians to identify which patients with early-stage HR+/HER2− breast cancer should receive adjuvant chemotherapy [2]. Recently, genomic signatures that are highly associated with cell proliferation have been used to estimate prognoses and response to chemotherapy and are being used to guide decisions on adjuvant systemic chemotherapy because they may have better predictive power than classical pathological biomarkers (*e.g.*, histological grade and IHC-Ki67) [3,4]. Several genomic prognostic markers for early-stage HR+/HER2− breast cancers in the adjuvant setting have been tested [5]. The National Comprehensive Cancer Network (Version 8.2021) recommends using the 21-gene recurrence score with NCCN category "1" as both a prognostic and predictive marker for HR+/HER2− breast cancers. Reducing unnecessary chemotherapy brings

Received November 26, 2021; accepted February 16, 2022.
*Corresponding author. Phone: +81-86-235-6502; Fax: +81-86-235-6502
E-mail: tiwamoto@cc.okayama-u.ac.jp (T. Iwamoto)

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numerous benefits to patients as well as to the medical community. However, the high cost of genomic signature testing limits clinical access to this diagnostic technology in many countries outside of the US and some regions in Europe. Furthermore, most patients with HR+/HER2− breast cancers are prescribed hormone therapy (HT) as an adjuvant therapy, regardless of their response to HT; so far, there is no clinically available genomic signature to predict response to HT [6].

Recently, it was reported that IHC-Ki67 levels after two weeks of preoperative HT showed better prognostic power than baseline IHC-Ki67, and patients with high (≥10%) baseline IHC-Ki67 were sub-divided into poor and good prognostic groups based on low or high IHC-Ki67 after short-term HT, respectively [7]. Several gene expression analyses have been performed between before and after short-term HT. Miller *et al.* assessed gene expression patterns before and two weeks after letrozole (2.5 mg/day) treatment and showed that oestrogen receptor (ER) and proliferation-associated genes in tumors were downregulated after treatment [8]. Similarly, Dunbier *et al.* reported that proliferation and oestrogen-associated genes were strongly downregulated after HT, whereas collagens and chemokines were upregulated [9]. Interestingly, higher expressions of immune response-related genes such as *SLAMF8* and *TNF*, as well as lymphocytic infiltration, were associated with a worse response to HT [9]. We also compared the predictive power of IHC-Ki67 after two weeks of HT using 30 paired cases (60 samples) and showed that IHC-Ki67 after HT predicted more low-risk cases (83.3%, 25/30) than did the genomic signature (66.7%: 20/30) [10]. We assumed that luminal B-like breast cancers could be potentially classified by response to HT. However, the differences in gene expression profiles in luminal B-like breast cancers between groups with high and low IHC-Ki67 after short-term HT are unclear.

To address this gap in knowledge, this study aimed: 1) to evaluate pre-treatment gene expression differences between luminal B-like breast cancer patients with good and poor prognosis; and 2) to explore novel therapeutic targets for the poor prognostic group.

Materials and Methods

Patients and cohort. Publicly available cDNA microarray data of 77 primary HR+/HER2− breast cancer patients who received letrozole (2.5 mg/day) for two weeks before surgery were retrieved from the NCBI Gene Expression Omnibus (GEO https://www.ncbi.nlm.nih.gov/geo/: accessed date: Jan., 2019) repositories GSE80077 (19 cases) [10] and GSE20181 (58 cases) [8]. These data were from paired samples of patients before and after receiving short-term aromatase inhibitor (AI) treatment. All patients were postmenopausal women without evidence of distant metastatic disease. Hormone and HER2 statuses were determined in the diagnostic core needle biopsy samples before any therapies. ER and progesterone (PgR) status were assessed using IHC. Cases with ≥1% positive nuclear staining for ER and/or PgR were considered HR+. Cases with either 0 or 1 positive IHC staining for HER2 or with an *HER2* gene copy number <2.0 as detected by fluorescent *in situ* hybridization analysis were considered HER2-negative. We also assigned low or high proliferation status to each sample based on *MKI67* mRNA expression (probe set "212022_s_at"). The thresholds for defining low or high proliferation status based on *MKI67* expression values were determined in an independent prognostic data set by Wang *et al.* (GSE2034) [11], as previously described [3]. Briefly, we used the Youden index to identify optimal cut-off values on the receiver operating characteristic curves for 10-year distant event-free survival in the Wang data set. Patients with *MKI67* mRNA expression levels of greater than 8.489 were considered to have high Ki67 and the others to have low Ki67; these thresholds were applied to our analyses without further modification. All paired samples were stratified into three groups based on Ki67 levels before and after short-term AI: the L→L, H→L, and H→H groups.

This research study was conducted retrospectively from data obtained from public databases. Ethical approval was waived in view of the retrospective nature of the study. Informed consent was not required for this retrospective analysis.

Gene expression analysis. Data were annotated using the Affymetrix Human Genome Array system (Affymetrix Inc., Santa Clara, CA, USA). All gene expression data were generated using Affymetrix gene chips and normalized using the MAS5 algorithm (http://www.bioconductor.org: accessed Jan., 2019), with the mean expression centered to 600 and log 2 transformation. Probe sets with the lowest 15% mean expression value were removed from all higher-level

analyses to reduce noise from low-expression probe sets. When one gene had two or more probe sets, we retained only one probe set with the highest average gene expression. After applying these criteria, 11,192 probe sets were left for further analysis. All statistical analyses were performed using BRB Array Tools software (version 4.6.0; https://brb.nci.nih.gov/BRB-ArrayTools/: accessed Jan., 2019) and R software (version 3.5.1; https://www.r-project.org/: accessed Jan., 2019).

First, we compared the gene expression levels for three well-established clinical breast cancer markers (*ESR1*, *PGR*, and *ERBB2*) in pre-treatment samples between the H→L and H→H groups using the Wilcoxon rank sum test. We also performed a similar class comparison test for three immune-related gene sets (TILs-GS [12], B cell/plasma cell metagene [13] and dendritic cell metagene [13] and for one immune- and one inflammatory-related gene (*SLAMF8* and *TNF*) in two groups to assess the association between immune microenvironment and response to HT. High expression of immune-related genes such as *SLAMF8* and *TNF* were previously reported to be associated with a poor response to anastrozole [9].

Next, we performed a class comparison test for all 11,192 genes to identify broad molecular differences between the two groups. To adjust for the multiple comparisons, we calculated false discovery rates (FDR) and also assessed Global Significance using BRB Array tools. The FDR was calculated with the significance analysis of microarrays tool as the median number of false-positive genes from permutation testing divided by the number of nominally significant genes defined from the unperturbed data [14].

We also examined differential expression of *a priori* defined gene sets using gene set analysis (GSA) in pre-treatment samples. The goal of GSA is to determine whether members in a set of genes that correspond to a particular biological pathway tend to occur toward the top or the bottom of a rank-ordered gene list (rank ordered by differential expression between the H→H and H→L groups). The total number of gene sets included in this analysis was 178 from the KEGG PATHWAY Database (https://www.genome.jp/kegg/pathway.html: accessed Jan., 2019). Gene sets with a minimum number of 10 and maximum of 100 genes were selected for inclusion in this analysis. We used the Efron and Tibshirani GSA method to test whether gene sets were differentially expressed between the H→H and H→L groups, with statistical significance being determined by a permutation test [15]. Significance was estimated with the permutation test (n = 1,000). The null hypothesis was that the average degree of differential expression of members of a given gene set between the H→H and H→L groups would be the same as expected from a random set of genes of a similar size. Pre-treatment samples were used for the preceding analysis in order to confirm the essential difference between the two groups of patients with luminal B-like breast cancer with different responses to hormone therapy.

Finally, we selected 41 genes that either had been targeted by US FDA-approved drugs or had been assessed through clinical trials as molecular target agents for various solid cancer types including breast cancer. For these genes, we explored the possibility of novel adjuvant therapies added to the standard adjuvant AI for the H→H group with poor prognosis after the AI by the Wilcoxon rank sum test in post-treatment samples. These analyses were performed by post-treatment samples because gene expression differences caused by short-term AI may be associated with target therapy. Information on anticancer therapy drugs was obtained by reference to previous studies [16, 17] from the National Cancer Institute (https://www.cancer.gov/about-cancer/treatment/drugs: accessed Jan., 2019), Drug@FDA (https://www.accessdata.fda.gov/scripts/cder/daf/: accessed Jan., 2019), and ClinicalTrials. gov (https://clinicaltrials.gov/: accessed Jan., 2019). The 41 selected genes belonged to the following pathways or functions (Table 1): associated with breast cancer (*AR*, *ERBB3*, and *p53*); DNA damage repair pathways and BRCA functions (*BRCA1*, *BRCA2*, *PARP1*, and *PARP2*); the cyclin-dependent kinase (CDK) pathway (*CDK2*, *CDK4*, *CDK6*, *CCND1*, *CDKN2A*, and *RB1*); vascular endothelial growth factor (VEGF) and VEGF receptor pathways (*VEGF-A*, *VEGF-B*, *VEGF-C*, *EGFR*, *KDR*, *PGF*, and *FLT4*); modulation of DNA methylation and histone acetylation (*HDAC1*, *HDAC2*, *HDAC3*, *DNMT1*, *DNMT3A*, and *DNMT3B*); immune responses (*PDCD1LG2*) and the mTOR pathway (*PIK3CA*); and targeted by US FDA-approved drugs or under investigation (*AKT1*, *ALK*, *RAF1*, *CTNNB1*, *MET*, *STK11*, *PTEN*, *NF1*, *ROS1*, *NOTCH1*, *ATM*, *KITL*, and *KRAS*).

Table 1 Patient Characteristics (GSE80077)

Age	Median (min.-max) 66 (51–88)
	Number of cases
cTumor size	
T1	11
T2	7
T3	1
cN	
positive	0
negative	19
ER	
positive	19
negative	0
PgR	
positive	14
negative	5
HER2	
positive	0
negative	19
Historogical grade	
1	10
2	7
3	2

ER, estrogen receptor; PgR, progesteron receptor; HER2, human epidermar growth factor 2.

Results

A total of 77 paired samples were analyzed in this study. Patient characteristics for the 19 cases from GSE80077 are shown in Table 2. The median age was 66 years (range: 51-88). All cases were lymph node-negative and ER-positive. Five (26.3%) of 19 cases were PgR-negative. Almost half of them (52.6%: 10/19) were histological grade 1. Unfortunately, patient characteristics were not available for the remaining 58 cases from GSE20181, although the characteristics for 15 of the 58 who were clinically resistant to AI were previously reported [7].

Among the total 77 samples, 30 paired samples that had low Ki67 levels both before (pre-Ki67) and after (post-Ki67) short-term preoperative AI treatment were assigned to the L→L group. Two cases had low pre-Ki67 and high post-Ki67 levels (L→H). The remaining 45 paired samples were used in subsequent analyses to assess the differences between the H→H (N = 26) and H→L (N = 19) gene expression groups.

Class comparison test between the H→H and H→L groups. To explore the associations between short-term AI response and potential expression levels of breast cancer clinical markers, we compared the mRNA gene expression levels of *ESR1*, *PGR*, and *ERBB2* in pre-treatment samples between the H→H and H→L groups. There were no significant differences in the expressions of any of the three genes between the two groups (Fig. 1). A similar class comparison test was done for three immune response-related signature genes, one immune-related gene and one inflammatory-related gene, in the pre-treatment samples. A significant difference was found only for *TNF* mRNA: it was significantly under-expressed in the H→H group (Fig. 2). Next, we performed a class comparison test for all 11,192 genes by FDR in pre-treatment samples. There was no gene that showed a significant difference in expression level between the two groups (FDR < 0.1), indicating that the molecular differences between the two groups were subtle.

Gene sets analyses between the H→H and H→L groups. Individual gene level tests suggested relatively small differences in gene expressions between the two groups. Next, we investigated whether we could detect coordinated but relatively small-scale differences in the expression of gene sets belonging to functional pathways. We tested 178 gene sets from the KEGG PATHWAY Database (https://www.genome.jp/kegg/pathway.html: accessed Jan., 2019) in pre-treatment samples. Eighteen gene sets showed significantly higher expression in the H→L group compared with the H→H group ($p < 0.05$) (Table 3). Of these, 11 gene sets were associated with various metabolic pathways (tryptophan metabolism, propionate metabolism, beta-alanine metabolism, valine, leucine and isoleucine degradation, arginine and proline metabolism, pentose and glucuronate interconversions, valine, leucine and isoleucine biosynthesis, alanine, aspartate and glutamate metabolism, glyoxylate and dicarboxylate metabolism, and fatty acid degradation). In contrast, 9 gene sets had significantly higher expressions in the H→H group than in the H→L group ($p < 0.05$). Interestingly, 3 of these 9 genes sets were also associated with metabolism.

Molecular target makers according to short-term AI response. Next, we analyzed 41 molecular target markers to explore the efficacy of AI as a novel adjuvant therapy for patients with poor prognosis in the H→H group and performed a class comparison test between

Table 2 The list of selected 41 genes that are targeted by FDA-approved drugs or have been investigated with clinical trials as molecular target agents

Symbol	Name	Probe Set
AR	androgen receptor	211110_s_at
ERBB3	erb-b2 receptor tyrosine kinase 3	202454_s_at
TP53	tumor protein p53	201746_at
BRCA1	breast cancer 1, early onset	211851_x_at
BRCA2	breast cancer 2, early onset	214727_at
PARP1	poly (ADP-ribose) polymerase 1	208644_at
PARP2	poly (ADP-ribose) polymerase 2	204752_x_at
PDCD1LG2	programmed cell death 1 ligand 2	220049_s_at
CDK2	cyclin-dependent kinase 2	204252_at
CDK4	cyclin-dependent kinase 4	202246_s_at
CDK6	cyclin-dependent kinase 6	214160_at
CCND1	cyclin D1	208712_at
CDKN2A	cyclin-dependent kinase inhibitor 2A	209644_x_at
*RB*1	retinoblastoma 1	203132_at
VEGF-A	vascular endothelial growth factor A	210512_s_at
VEGF-B	vascular endothelial growth factor B	203683_s_at
VEGF-C	vascular endothelial growth factor C	209946_at
EGFR	epidermal growth factor receptor	201984_s_at
PGF	placental growth factor	209652_s_at
KDR	kinase insert domain receptor	203934_at
FLT4	fms-related tyrosine kinase 4	210316_at
HDAC1	histone deacetylase 1	201209_at
HDAC2	histone deacetylase 2	201833_at
HDAC3	histone deacetylase 3	206846_s_at
DNMT1	DNA (cytosine-5-)-methyltransferase 1	201697_s_at
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	218457_s_at
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	220668_s_at
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	204369_at
AKT1	v-akt murine thymoma viral oncogene homolog 1	207163_s_at
ALK	anaplastic lymphoma receptor tyrosine kinase	208211_s_at
RAF1	Raf-1 proto-oncogene, serine/threonine kinase	201244_s_at
CTNNB1	catenin (cadherin-associated protein), beta 1, 88kDa	201533_at
MET	MET proto-oncogene, receptor tyrosine kinase	211599_x_at
STK11	serine/threonine kinase 11	41657_at
PTEN	phosphatase and tensin homolog	204053_x_at
NF1	neurofibromin 1	212676_at
ROS1	ROS proto-oncogene 1, receptor tyrosine kinase	207569_at
NOTCHI	notch 1	218902_at
ATM	ATM serine/threonine kinase	210858_x_at
KITL	KIT ligand	207029_at
KRAS	Kirsten rat sarcoma viral oncogene homolog	214352_s_at

the H→H and H→L groups using the post-treatment samples. We found 5 target genes (*PARP2*: $p=0.004$; *CDK6*: $p=0.005$; *FLT4*: $p=0.023$; *PDCD1LG2*: $p=0.021$; and *MET*: $p=0.046$) with significantly higher expressions in the H→H group than in the H→L group (Fig. 3).

Discussion

To our knowledge, this is the first study to assess gene expression data in luminal B-like breast cancers in patients with high versus low Ki67 levels after short-term preoperative AI administration (post-Ki67). Moreover, we also explored candidate target genes for patients who are resistant to AI. Luminal B breast can-

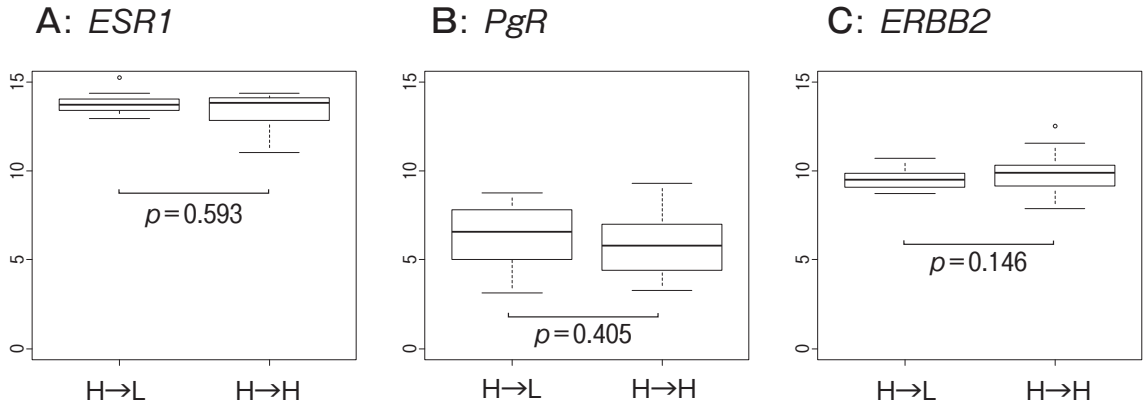

Fig. 1 Comparison of mRNA expression levels for 3 genes related to breast cancer treatment between patients with high and low Ki67 levels. Boxplots showed the distribution of mRNA gene expression based on a five number summary ("minimum", first quartile, median, third quartile, and "maximum") for *ESR1* (**A**), *PgR* (**B**) and *ERBB2* (**C**). *P* values were calculated by the Wilcoxon test comparing differences between the high- and low-Ki67 subgroups. HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

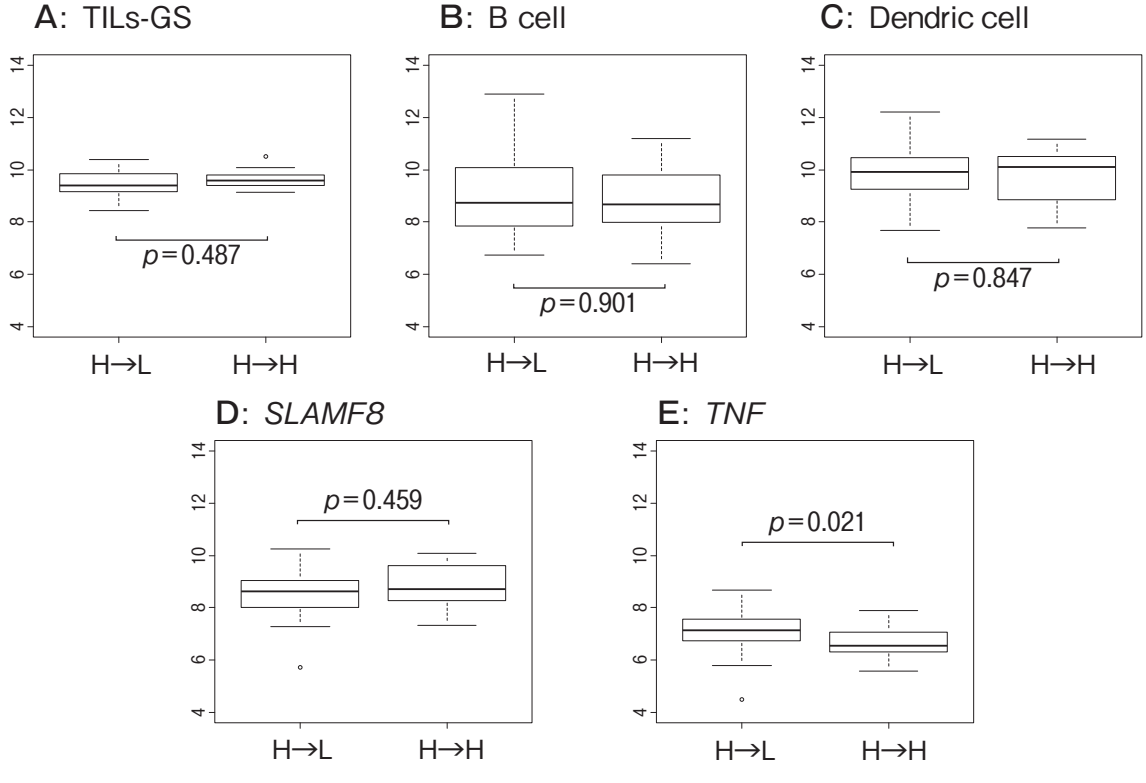

Fig. 2 Comparison of mRNA expression levels for immune-related gene sets and immune- and inflammatory-related genes between patients with high and low Ki67 levels. Boxplots show the distribution of mRNA gene expression based on a five-number summary ("minimum", first quartile, median, third quartile, and "maximum") for TILs-GS (**A**), B cells (**B**), Dendritic cells (**C**), *SLAMF8* (**D**) and *TNF* (**E**). *P* values were calculated by Wilcoxon test comparing differences between the high- and low-Ki67 subgroups.

Table 3 Pathways identified as differentially expressed between H→H and H→L by gene set comparison analysis*

	Pathway description	Number of genes	*P*-value
H→H > H→L			
1	Maturity onset diabetes of the young	11	< 0.005
2	Linoleic acid metabolism	19	0.010
3	Pancreatic secretion	79	0.015
4	alpha-Linolenic acid metabolism	11	0.015
5	African trypanosomiasis	29	0.020
6	Cytosolic DNA-sensing pathway	40	0.020
7	Olfactory transduction	50	0.025
8	Long-term depression	61	0.030
9	Glycosphingolipid biosynthesis - lacto and neolacto series	21	0.040
H→L > H→H			
1	Tryptophan metabolism	32	< 0.005
2	Propanoate metabolism	28	< 0.005
3	beta-Alanine metabolism	20	< 0.005
4	SNARE interactions in vesicular transport	31	0.005
5	Nucleotide excision repair	42	0.005
6	Butanoate metabolism	26	0.015
7	Valine, leucine and isoleucine degradation	42	0.015
8	Arginine and proline metabolism	42	0.020
9	Pentose and glucuronate interconversions	13	0.020
10	Parkinson's disease	97	0.025
11	Valine, leucine and isoleucine biosynthesis	10	0.025
12	Alanine, aspartate and glutamate metabolism	27	0.025
13	Glyoxylate and dicarboxylate metabolism	15	0.030
14	Protein export	19	0.030
15	RNA polymerase	22	0.035
16	Homologous recombination	26	0.040
17	Fatty acid degradation	39	0.040
18	Peroxisome	69	0.045

*Gray-highlighted rows: metabolism-associated pathways.

cer is a heterogeneous disease characterized by a higher proliferation rate [18]. In spite of combination therapies, such as adjuvant AI and chemotherapy, luminal B has a poorer prognosis than luminal A, because of its high malignant potential and resistance to these therapies [1]. So far, no clinically available genomic marker has been identified which can help with decision-making regarding the adjuvant AI regimen [6]. Therefore, the majority of patients with HR+ breast cancer are prescribed an AI regardless of their response to it. The use of a short-term AI to predict the response to AIs is more affordable than genomic testing and has few critical side effects.

First, we evaluated gene expression levels in luminal B-like patients, comparing those with H→H and H→L expressions. In a global comparison of the H→H versus H→L groups, we found no significant intergroup differences in gene expression at the level of individual genes after adjusting for multiple comparisons. Indeed, this is consistent with the current understanding that there are no clinically available genomic markers that can detect resistance to HT, except for IHC ER and PgR status. Several gene expression signatures to predict prognosis and response to treatments have been developed for breast cancer patients, and various other genomic signatures have been shown to predict specific

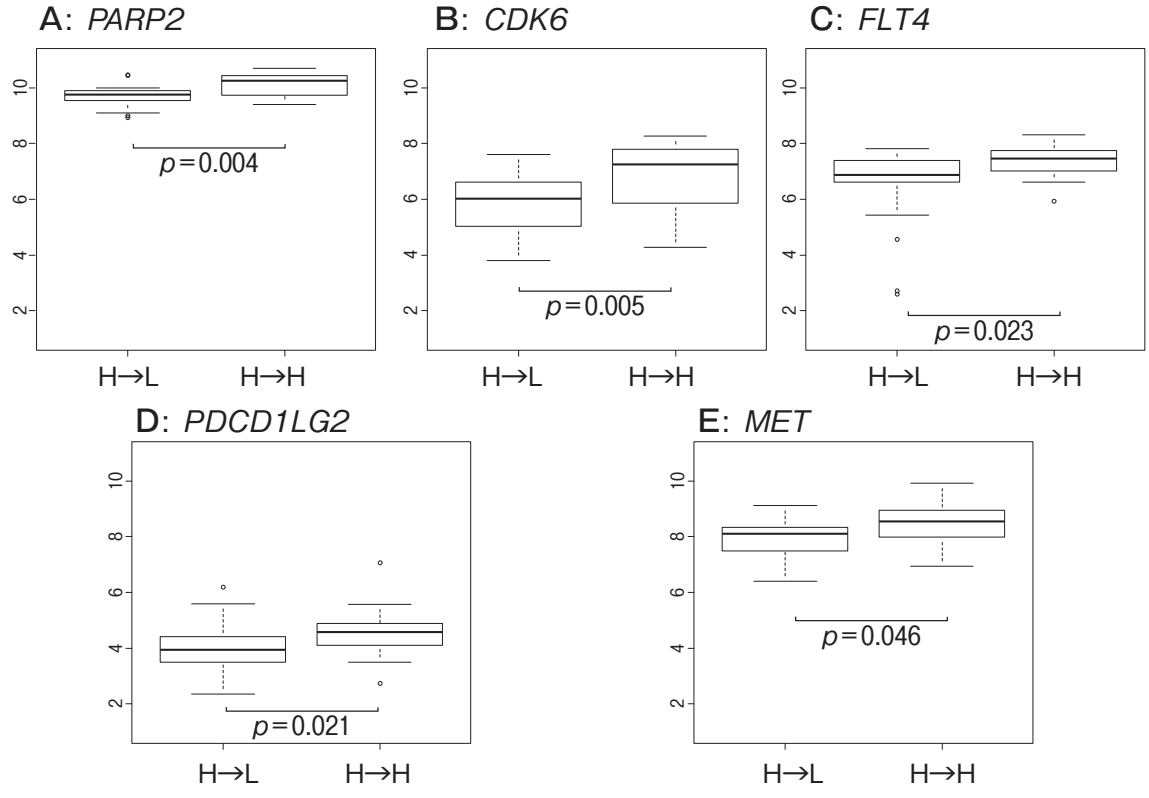

Fig. 3 Comparison of mRNA expression levels for 5 potentially targeted genes between patients with high and low Ki67 levels. Boxplots show the distribution of mRNA gene expression based on a five-number summary ("minimum", first quartile, median, third quartile, and "maximum") for *PARP2* (**A**), *CDK6* (**B**), *FLT4* (**C**), *PDCD1LG2* (**D**) and *MET* (**E**). *P* values were calculated by Wilcoxon test comparing differences between the high- and low-Ki67 subgroups.

clinical outcomes. However, the majority of genomic signatures yielded low accuracy and reproducibility in the independent cohort [19]. A previous report showed that immune- and inflammatory-related genes were associated with resistance to the HT [9]. Another paper reported that neither change nor increase in the expression level of genes responsible for proliferation were observed in the cases with resistance to HT [8]. Importantly, these previously published papers assessed gene expression data comparing pre- and post-treatment conditions in a number of different cancers. In the current study, we compared H→H and H→L only among luminal B patients.

Next, we performed gene expression analyses at the level of gene sets to search for subtle differences between the two groups with the aim of identifying related biological pathways. We found that various metabolism-related pathways were associated with the efficacy of AI. Indeed, 7 of the top 10 gene sets with higher expression in the H→L patients were associated with metabolism, and the most highly expressed set, the tryptophan metabolic pathway, has already been assessed in the study of balance between Treg and Th17, the two associated populations of CD4+ T cells with opposing functions during immune response [20]. The levels of expression of *IDO1*, *IDO2*, and *TDO2* in the early segments of the kynurenine pathway of tryptophan metabolism were correlated with high *AHR* expression and shown to play an important role in breast tumorigenesis [21]. Moreover, ER status in breast cancer likely influenced the AHR activity involved in tryptophan metabolism [21]. The gene set with the second-highest expression, propionate metabolism, also plays important roles in the regulation of the hormone environment during carcinogenesis. Propionate reduces BaF3 cell growth through a cAMP-dependent pathway [22]. Furthermore, the activation of free fatty acid receptor 2, a Gi/Gq-protein-coupled receptor that

binds propionate, reduced the proliferation of BaF3 and human cancer cell lines [22]. Interestingly, Budczies *et al.* reported associations between several metabolic pathways and breast cancer subtypes using GC–TOFMS-based metabolomics, and the pathway with the 3rd-highest expression, associated with beta-alanine metabolism, showed the greatest difference between ER-positive and -negative breast cancers [23]. Further, beta-alanine treatment reduced extracellular acidity, a constituent of the invasive microenvironment that promotes breast cancer progression; it was also shown to reduce both breast cancer cell migration and proliferation without causing cytotoxicity [24]. As far as we know, these previously published papers might not show direct associations between hormone resistance and breast cancer progression. These findings should be validated in other independent data sets in a future analysis.

Finally, we performed gene expression analysis of 41 molecular target markers to explore novel therapeutic targets for the poor prognostic group. Our results suggested that 5 genes had a potential for target therapy in addition to standard adjuvant therapy. Our findings suggested that the efficacy of therapeutic targets in this subtype of breast cancer based on gene expression analyses is still poorly understood. Further clinical studies to validate the efficacy of these targeted candidate therapies are needed.

This study had several limitations. First, the sample sizes were relatively small in each group; therefore, some valid but weaker variables might not have been detected as significant in our study. Future validation studies are needed in a different external dataset with the larger sample size. Second, some of the cases showing resistance to an AI might be sensitive to the chemotherapy and therefore might not require additional targeted therapies. The HR+/HER2− subgroup with high recurrence risk might be treated by chemotherapy and AI as the adjuvant standard therapy, although so far, we have no clinically available biomarkers to predict a response to co-administration of chemotherapy and AI. Third, we assessed mRNA data for gene expression to seek the novel target genes; however, these might not be true targetable driver genes. These genes should be validated in future trials. Unfortunately, as far as we know, there is no other gene expression dataset containing information for pre- and post-HT. Finally, we defined high and low Ki67 by mRNA gene expression level and not by the IHC assay that was originally reported from POETIC trial [7]. These assays may be similar, but they do not show the same level of expression [25]. Such subtle differences might have had an effect on our results.

In conclusion, several metabolism-related pathways were associated with sensitivity to AI in two groups stratified by post-Ki67 levels. Several candidate targets for cases with resistance to AI were suggested as novel add-on treatments. Our observations support the need for further investigation of the mechanisms of resistance to AIs and validations in future clinical trials.

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