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Original Article

Impact of next-generation sequencing (NGS) for primary endocrine resistance in breast cancer patients

Ruoyang Li[1*], Tiantian Tang[1*], Tianli Hui[1], Zhenchuan Song[1], Fugen Li[2], Jingyu Li[2], Jiajia Xu[2]

[1]Breast Center, Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [2]Institute of Precision Medicine, 3D Medicines Inc., Shanghai, China. [*]Equal contributors.

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Abstract: Multiple mechanisms have been detected to account for the acquired resistance to endocrine therapies in breast cancer. In this study we retrospectively studied the mechanism of primary endocrine resistance in estrogen receptor positive (ER^+) breast cancer patients by next-generation sequencing (NGS). Tumor specimens and matched blood samples were obtained from 24 ER^+ breast cancer patients. Fifteen of them displayed endocrine resistance, including recurrence and/or metastases within 24 months from the beginning of endocrine therapy, and 9 patients remained sensitive to endocrine therapy for more than 5 years. Genomic DNA of tumor tissue was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks. Genomic DNA of normal tissue was extracted from peripheral blood mononuclear cells (PBMC). Sequencing libraries for each sample were prepared, followed by target capturing for 372 genes that are frequently rearranged in cancers. Massive parallel sequencing was then performed using Illumina NextSeq 500, and samples with a mean sequencing depth of 500× were analyzed. The analysis revealed that 8 (55%) of 15 patients showed phosphatidylinositol 3-kinase CA (PIK3CA) mutations, including 3 pathogenic variants in kinase domain, 3 pathogenic variants in helical domain, and 2 variants of unknown significance, in the endocrine-resistant group, while 3 (33%) of 9 patients displayed PIK3CA mutations, including 2 pathogenic variants in kinase domain and 1 pathogenic variant in helical domain, in the endocrine-sensitive group. In the endocrine-sensitive group, copy number gain of C11orf30 (EMSY) gene, copy number loss of CDH1 (E-cadherin) gene, and a missense mutation of splicing factor 3b (SF3B1) gene were also detected, which would probably decrease the expression of ESR1 and contribute to endocrine sensitivity. Collectively, the PIK3CA mutation rate in the resistance group is relatively higher than that in the sensitive group and thus PIK3CA mutations may contribute the primary endocrine resistance of breast cancer.

Keywords: Breast cancer, NGS, PIK3CA mutation, endocrine resistance

Introduction

Breast cancer is the most frequent non-skin malignant tumor in women and about 75% of patients with breast cancer are estrogen receptor-positive (ER^+) [1]. The majority of patients presenting with ER^+ disease can be successfully treated with endocrine therapies. Recommended therapy approaches depend on the patient's menopausal status and may include treatment with aromatase inhibitors (AI), selective estrogen receptor modulators (SERM) and selective estrogen receptor degraders (SERD) and appropriate combinations therapy. Unfortunately, resistance limits their therapeutic efficiency. However, the mechanism leading to endocrine resistance is still not fully understood. Several mechanisms of resistance to endocrine therapy, including loss of ER expression, alteration of several signaling pathways, damaged metabolism of tamoxifen (TAM), and converted expression of microRNAs, have been hypothesized [2]. Primary resistance in breast cancer can be classified into those categories: the loss of ER (the ERα isoform) expression; ER gene mutations, such as deletion, and frameshift; Patients with dormant alleles of cytochrome P4502D6 (CYP2D6) deficiency cannot transform TAM to its active metabolite, endoxifen, and therefore are resistant to TAM [3]. By comparison, we detect multiple mechanisms responsible for the acquired resistance to endocrine therapies. Furthermore, both genomic and non-genomic crosstalk and the complex interrelations between the ER subtypes and growth factors also contribute to endocrine resistance. There have been lines of evidence supporting that mutations of important genes,

Table 1. Clinical characteristics of breast cancer patients

Patients and disease features	Endocrine resistance group n = 15 (%)	Endocrine sensitive group n = 9 (%)	Total n = 24 (%)
Age (y)			
< 30	2 (13.3)	0 (0)	2 (8.3)
30-49	6 (40.0)	6 (66.7)	12 (50)
50-59	2 (13.3)	2 (22.2)	4 (16.7)
60+	5 (33.4)	1 (11.1)	6 (25)
Tumor size (cm)			
< 2	4 (26.7)	4 (44.4)	8 (33.3)
2-5	10 (66.7)	5 (55.6)	15 (62.5)
> 5	1 (6.7)	0 (1)	1 (4.2)
Clinical staging			
I	4 (26.7)	3 (33.3)	7 (29.2)
II	8 (53.3)	6 (66.7)	14 (58.3)
III+	3 (20.0)	0 (0)	3 (12.5)
Lymph nodes (+)			
0	5 (33.3)	6 (66.7)	11 (45.8)
1-4	6 (40.0)	3 (33.3)	9 (54.2)
> 4	4 (26.7)	0	4 (16.7)
Endocrine therapy			
TAM/Exemestane	12 (80.0)	7 (77.8)	19 (79.2)
Letrozole	3 (20.0)	2 (22.2)	5 (20.8)

such as PIK3CA and P53, may contribute to endocrine resistance [4]. New treatments targeting such oncogenic signaling pathway which blocks the crosstalk among those pathways have been proved useful in preclinical models [5, 6]. However, some clinical trial data have shown that whether PIK3CA is mutated or not does not significantly affect the therapeutic effect of TAM [7]. This study aimed to determine which mutation status is more causatively associated with endocrine resistance. Firstly, we retrospectively studied two groups of patients of early breast cancer with different prognosis to endocrine therapy and then explored the mechanism of those gene mutations that contribute to endocrine resistance.

Materials and methods

Patients

This study included a total of 24 patients diagnosed with breast cancer from the Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei, China. This study was approved by the Independent Ethics Committee of the Fourth Hospital of Hebei Medical University. All the patients had been provided with written informed consent for genomic testing used for this study. The patients were divided into two groups: 15 patients were primarily resistant to endocrine therapy and displayed endocrine resistance within 24 months and taken as the endocrine-resistant group while the other 9 patients kept sensitive to endocrine therapy for more than 5 years and were taken as the endocrine-sensitive group. The tumor specimens of all of the patients and the matched blood samples were obtained and analyzed. ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses were collected. Receptors statuses were assessed by immunohistochemistry with a positivity cutoff of more than 1% for ER and PR. HER2 was considered positive if overexpression was scored at 3+ in immunohistochemistry or if amplification ratio was > 2 in fluorescent *in situ* hybridization. Specimens were evaluated by board-certified pathologists to identify tumor-bearing areas for DNA extraction.

Collection for samples

Tumor tissues were obtained from biopsy or surgery upon the initial diagnosis of cancer at the primary site. Tumor genomic DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tumor tissue blocks. Only tissue blocks/slices with more than 20% tumor cells were further analyzed. Genomic DNA from normal tissue was extracted from peripheral blood mononuclear cells (PBMCs). Sequencing libraries for each sample were prepared, followed by targets capturing for 372 genes that are frequently rearranged in cancers. Massive parallel sequencing was then performed using Illumina NextSeq 500, and samples with a mean sequencing depth of 500× were analyzed.

Data processing and analysis

Sequencing data were mapped to the human genome (hg19) using BWA aligner v0.7.12. PCR

Table 2. ER, PR and HER2 status of the breast cancer patients

Sample	Endocrine therapy response	IHC			FISH	NGS
		ER	PR	HER2	HER2	HER2
R6-2	Resistant	+	+	NA	NA	-
R6-21	Resistant	+	+	1+	NA	-
R6-22	Resistant	+	+	-	-	-
R6-23	Resistant	+	+	NA	NA	-
R6-24	Resistant	+	+	1+	NA	-
R6-26	Resistant	+	+	2+	-	-
R6-27	Resistant	+	-	3+	NA	Copy number gain
R6-29	Resistant	+	+	2+	-	-
R6-30	Resistant	+	-	3+	NA	Copy number gain
R6-31	Resistant	+	+	2+	NA	-
R6-32	Resistant	+	+	NA	NA	-
R6-33	Resistant	+	+	1+	NA	-
R6-34	Resistant	+	+	3+	NA	Copy number gain
R6-35	Resistant	+	+	2+	-	-
R6-36	Resistant	+	+	1+	NA	-
R6-13	Sensitive	+	+	1+	NA	-
R6-14	Sensitive	+	+	1+	NA	-
R6-16	Sensitive	+	+	3+	NA	Copy number gain
R6-17	Sensitive	+	+	3+	NA	Copy number gain
R6-39	Sensitive	+	+	3+	NA	Copy number gain
R6-40	Sensitive	+	+	1+	NA	-
R6-41	Sensitive	+	+	2+	NA	-
R6-43	Sensitive	+	+	2+	NA	-
R6-44	Sensitive	+	+	2+	NA	-

ER = Estrogen receptor, PR = Progesterone Receptor, HER2 = Human epidermal growth factor receptor 2.

duplicate read removal and sequence metric collection were performed using Picard 1.130 (https://github.com/broadinstitute/picard/releases/tag/1.130) and Samtools 0.1.19. Base substitution analysis, indel analysis, copy number variation (CNV) analysis, and rearrangement analysis were performed by using variants calling pipelines developed in 3D Medcines Inc. All variants called were verified by checking integrative genomics viewer (IGV) images visually.

Results

Patients and tumor characteristics

A total of 24 patients were included in this study and 24 specimens were obtained. Patient characteristics are presented in (**Table 1**). For the patients with HER2$^+$, they were also identified by NGS (**Table 2**).

Distribution of PIK3CA mutations in the two groups

Among the total 24 patients, 11 PIK3CA mutations were detected in 45.8% of these cases. The most common mutations in the PIK3CA gene were found in exon 20 (p.H1047R; ~20.8% of cases), followed bymutations in exon 9 (p.E542K; 12.5%) detected in all the patients. In the two groups, 8 of endocrine resistant group showed PIK3CA mutation, including 3 in exon 20 H1047R; 2 in exon 9 E542K and 3 of the endocrine sensitive group show PIK3CA mutation (2 in exon 20 H1047R; 1 in exon 9 E542K), respectively (**Figure 1A**; **Table 3**). Other PIK3CA mutations included exon 9 E545K (8.3%) and exon 9 N345K (4.1%), respectively.

Though no significant difference in PIK3CA mutation rate was seen between the two groups, we found that the PIK3CA mutation rate in the endocrine-resistant group was higher than that in the endocrine-sensitive group (53.3% vs 33.3%, $P = 0.423$).

Other mutations in the two groups

In our study, patients with PIK3CA mutation also had other mutations, including SNVs and CNVs (**Table 4**). Especially, 3 endocrine-sensitive patients displayed a PIK3CA mutation.

Discussion

Several types of gene mutations have been reported in breast cancer patients with endocrine resistance. The aim of the present study was to determine which gene mutations are more closely associated with endocrine resistance. We detected 11 phosphoinositide 3-

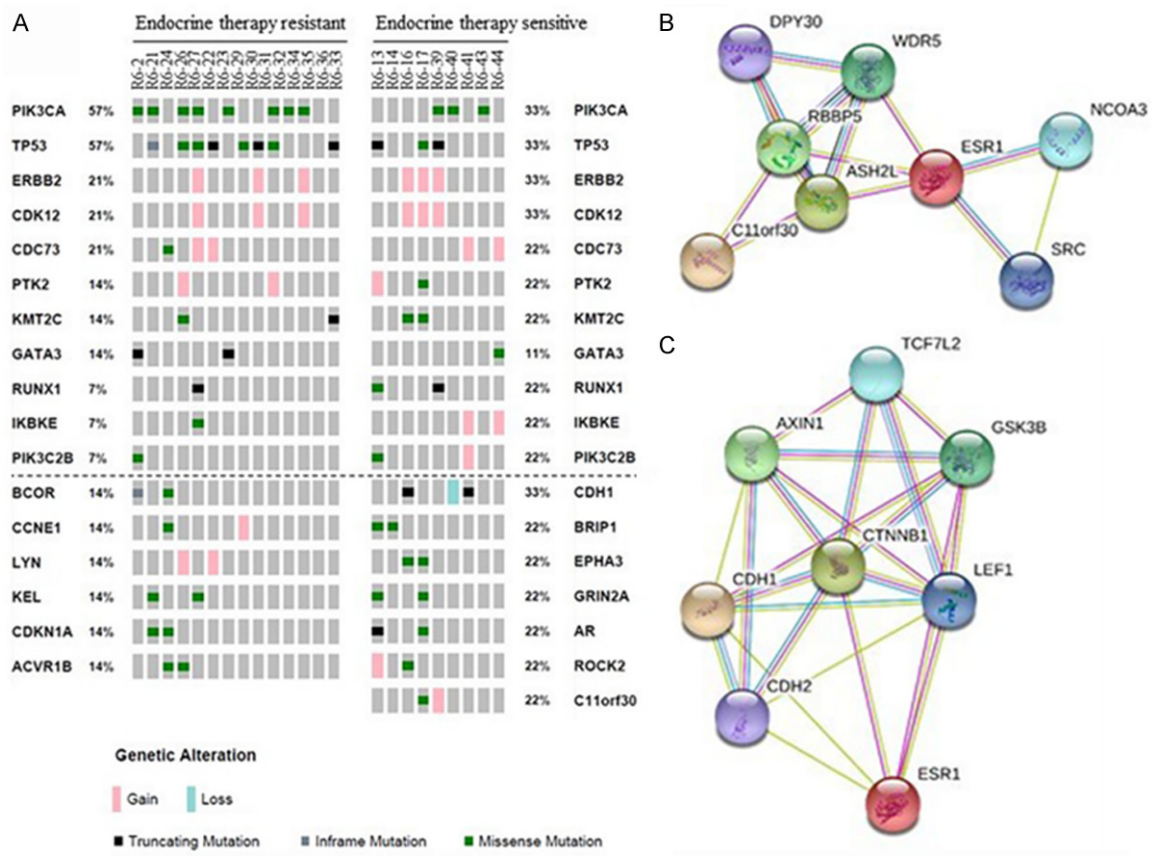

Figure 1. Distribution of PIK3CA mutations in the two groups and the predicted protein-protein interaction by STRING analysis. A. The recurrent genes and their mutation frequencies in endocrine therapy resistant and endocrine therapy sensitive groups. B, C: (http://string-db.org/version 10.0). B. Interaction between C11orf30 and ESR1. C. Interaction between CDH1 and ESR1.

kinase (PIK3CA) mutations in 45.8% of cases of breast cancer patients with the mutations H1047R in exon 20 being the most frequent one and the E542K in the exon 9 being the second most common one. We also detected other mutations, including SNVs and CNVs in 3 patients who were endocrine-sensitive and carried a PIK3CA mutation.

The PI3K signaling pathway has been shown to be of great importance in breast cancer. Under usual circumstances, it is a pathway to induce cell proliferation, development, and differentiation [8]. Of late, activation of the PI3K pathway is known to be involved in the acquired resistance to anticancer therapy. Consistent with our finding in Chinese breast cancer patients, mutation in PIK3CA is the most common genetic abnormality identified in ER^+ breast cancer (30%) [9]. Of note, PIK3CA mutations incline to be massively focused on the helicase (exon 9, residues E542 and E545) and kinase (exon 20, residue H1047) domains. E542 and E545 are frequently mutated to lysine and H1047 is frequently altered to arginine [10].

PI3Ks fall into three categories: classes I, II, and III. Class I is further subdivided into class IA and class IB, which are composed of a catalytic (p110) and regulatory (p85 for class IA) components. Class IA is activated by various types of cell surface receptors and has 3 isoforms, namely p110α, p110β, and p110δ. PIK3CA (PI3K alpha catalytic subunit) is the gene that encodes for the catalytic component p110α. When PIK3CA mutation occurs, p85 and p11 integrate together to stabilize p110, thus inhibiting their kinase activities. Then the complex binds phosphotyrosine in activated receptors or adaptors. The binding of p85 and phosphotyrosine activates the kinase activity of p110 by releasing the p85-mediated inhibition of p110 [11]. PIP2 is phosphorylated by the activated catalytic subunit of PI3K (p110), inducing PIP3,

Table 3. Genes involved in druggable targets (80 genes), druggable targets-related signal pathways (~200 genes), DNA damage repair (~50 genes), epigenetics (~25 genes) and other frequently mutated genes

Mutation genes												
ABL1	BCL2L2	CDK4	DDR2	FAS	GATA4	INPP4B	MAGI2	NF2	PIK3CG	RBM10	SOX9	TSC2
ABL2	BCL6	CDK6	DICER1	FAT1	GATA6	IRF2	MAP2K1	NFE2L2	PIK3R1	RET	SPEN	TSHR
ACVR1B	BCOR	CDK8	DNMT3A	FBXW7	GID4	IRF4	MAP2K2	NFKBIA	PIK3R2	RICTOR	SPOP	TYK2
ACVR2A	BCORL1	CDKN1A	DOT1L	FGF10	GLI1	IRS2	MAP2K4	NKX2-1	PKD2	RNF43	SPTA1	U2AF1
ADAM29	BIRC5	CDKN1B	EGF	FGF14	GLI2	ITK	MAP3K1	NOTCH1	PLA2G1B	ROCK1	SRC	VEGFA
AKT1	BLK	CDKN2A	EGFR	FGF19	GLI3	JAK1	MAP4K5	NOTCH2	PLCG2	ROCK2	SRMS	VHL
AKT2	BLM	CDKN2B	EP300	FGF23	GNA11	JAK2	MCL1	NOTCH3	PMS2	ROS1	STAG2	WEE1
AKT3	BMX	CDKN2C	EPHA2	FGF3	GNA13	JAK3	MDM2	NPM1	POLD1	RPTOR	STAT3	WEE2
ALK	BRAF	CEBPA	EPHA3	FGF4	GNAQ	JUN	MDM4	NRAS	POLE	RUNX1	STAT4	WISP3
AMER1	BRCA1	CHD2	EPHA5	FGF6	GNAS	KAT6A	MED12	NRG1	PPP2R1A	RUNX1T1	STK11	WT1
APC	BRCA2	CHD4	EPHA7	FGFR1	GPR124	KDM5A	MEF2B	NRG3	PRDM1	RXRA	STK24	XIAP
AR	BRD4	CHEK1	EPHB1	FGFR2	GRIN2A	KDM5C	MEN1	NSD1	PREX2	SDHA	SUFU	XPO1
ARAF	BRIP1	CHEK2	ERBB2	FGFR3	GRM3	KDM6A	MET	NTRK1	PRKAR1A	SDHB	SYK	YES1
ARFRP1	BTG1	CIC	ERBB3	FGFR4	GSK3B	KDR	MITF	NTRK2	PRKCI	SDHC	TAF1	ZBTB2
ARID1A	BTK	CRBN	ERBB4	FGR	H3F3A	KEAP1	MLH1	NTRK3	PRKDC	SDHD	TBX3	ZNF217
Mutation genes												
ARID1B	C11orf30	CREBBP	ERCC1	FH	HCK	KEL	MPL	NUP93	PRSS8	SETD2	TCF7L2	ZNF703
ARID2	CARD11	CRKL	ERG	FLCN	HGF	KIT	MRE11A	PAK3	PTCH1	SF3B1	TEK	ZNF750
ASXL1	CBFB	CRLF2	ERRFI1	FLT1	HNF1A	KLHL6	MS4A1	PALB2	PTEN	SIK1	TET2	
ATM	CBL	CSF1R	ESR1	FLT3	HRAS	KMT2A	MSH2	PARK2	PTK2	SLIT2	TGFBR1	
ATR	CCND1	CSK	EZH2	FLT4	HSD3B1	KMT2C	MSH6	PAX5	PTK6	SMAD2	TGFBR2	
ATRX	CCND2	CSNK1A1	FAM135B	FOXL2	HSP90AA1	KMT2D	MST1R	PBRM1	PTPN11	SMAD3	TIE1	
AURKA	CCND3	CTCF	FAM46C	FOXP1	IDH1	KRAS	MTOR	PDCD1LG2	QKI	SMAD4	TNFAIP3	
AURKB	CCNE1	CTNNA1	FANCA	FRS2	IDH2	LCK	MUTYH	PDGFRA	RAC1	SMARCA4	TNFRSF14	
AXIN1	CD274	CTNNB1	FANCC	FUBP1	IGF1R	LIMK1	MYC	PDGFRB	RAD50	SMARCB1	TNFSF11	
AXL	CD79A	CUL3	FANCD2	FYN	IGF2	LMO1	MYCL	PDK1	RAD51	SMO	TNK2	
BAP1	CD79B	CXCR4	FANCE	GABRA6	IKBKE	LRP1	MYCN	PIK3C2B	RAF1	SNCAIP	TOP1	
BARD1	CDC73	CYLD	FANCF	GATA1	IKZF1	LRP1B	MYD88	PIK3CA	RANBP2	SOCS1	TOP2A	
BCL2	CDH1	DAXX	FANCG	GATA2	IL7R	LYN	NEK11	PIK3CB	RARA	SOX10	TP53	
BCL2L1	CDK12	DDR1	FANCL	GATA3	INHBA	LZTR1	NF1	PIK3CD	RB1	SOX2	TSC1	
Fusion genes												
ALK	BCR	BRAF	BRCA1	BRCA2	BRD4	ETV1	ETV4	ETV5	ETV6	FGFR1	FGFR2	FGFR3
MYB	NOTCH2	NTRK1	NTRK2	PDGFRA	RAF1	RARA	RET	ROS1	TMPRSS2	MET	DDR2	

Table 4. SNVs and CNVs of breast cancer patients with PIK3CA mutation

Sample	Endocrine therapy response	PIK3CA mutation	PIK3CA mutation annotation	Other SNV	Copy number gain	Copy number loss
R6-2	Resistant	PIK3CA, p.H1047R	MUT	MET, PIK3C2B, NOTCH2, ZBTB2, GRM3, WEE2, XIAP, FANCD2, BCOR, GLI2, KDM6A, CBFB, GATA3	-	-
R6-21	Resistant	PIK3CA, p.E542K	MUT	CDKN1A, KEL, TP53, RAD50, CDKN2A, NOTCH3, FBXW7	-	-
R6-26	Resistant	PIK3CA, p.E545K/ PIK3CA, p.E726K	MUT/VOUS	KDR, GATA2, ACVR1B, POLD1, GLI1, BRCA1, IL7R, FOXL2, TP53, ROS1, PRDM1, TBX3, CEBPA, EZH2, KMT2C	LYN/PRKDC/PREX2/ MYC/PTK2/FAM135B	-
R6-27	Resistant	PIK3CA, p.R108C	VOUS	IKBKE, ERG, RAF1, SPEN, KEL, TNFAIP3, TSC1, GNAS, TP53, RUNX1	ERBB2/CDK12/SPOP/ RNF43, AKT3/CDC73, PIK3R1	AMER1
R6-23	Resistant	PIK3CA, p.H1047R	MUT	GATA3	PRKAR1A	-
R6-31	Resistant	PIK3CA, p.E542K	MUT	TP53	PTK2	-
R6-32	Resistant	PIK3CA, p.H1047R	MUT	FGFR4	ZNF703	-
R6-34	Resistant	PIK3CA, p.N345K	MUT	-	ERBB2/CDK12	-
R6-39	Sensitive	PIK3CA, p.H1047R	MUT	ERBB3, TP53, ATR, RUNX1	ERBB2/CDK12, C11orf30	-
R6-40	Sensitive	PIK3CA, p.E545K	MUT	-	-	CDH1, PLCG2, FANCA
R6-43	Sensitive	PIK3CA, p.H1047R	MUT	SF3B1, SDHA	-	LZTR1

MUT: pathogenic or likely pathogenic variants; VOUS: variants of unknown significance.

which then attaches to AKT (protein kinase B), a serine/threonine protein kinase, then initiating the protocol to exposure the two amino acid residues required for phosphorylation. PIP3 also attaches to phosphoinositide-dependent protein kinase-1 (PDK1), by which those two residues will be phosphorylated, activating the function of AKT. AKT, one of the downstream targets of PI3K, promotes cell proliferation and anti-apoptotic responses [12]. Knockdown of the suppressor phosphatase and tensin homolog (PTEN, a negative regulator of AKT), and increased PI3K and AKT phosphorylation in ER^+ breast cancer cell lines, result in hormone-independent growth and resistance to tamoxifen and fulvestrant [13, 14]. Endocrine-resistant tumor cells display the up-regulation of expression levels of IGF-1R, HER2, and EGFR, as well as increased PI3K/AKT/mTOR activation.

In some *in vitro* experiments, the addition of PI3K pathway inhibitors increases the pro-apoptotic effects of tamoxifen, primarily in the cell line with the highest endogenous levels of AKT activity, supporting the notion that either high expression of AKT or altered activity of the PI3K/AKT pathway can be associated with endocrine resistance [15, 16]. AKT activate another serine threonine protein kinase, mTOR, which is quite pivotal in cell proliferation by monitoring cellular requisites, such as water, oxygen and nutrients. mTOR leads to compose two distinctive complexes with other proteins, both of which are associated to tumorigenesis [17].

Nonetheless, PI3K/AKT/mTOR pathway activation is associated with lower levels of ERα expression. Besides estrogen, the ER signaling pathway can also be regulated by membrane receptor tyrosine kinases, including epidermal growth factor receptor (EGFR), HER2, and insulin-like growth factor receptor (IGF1-R) [18-22]. Activation of the PI3K/AKT and the p42/44 mitogen-activated protein kinases (MAPK) pathways by these receptors, in turn, down-regulates the expression of ER and PR [23, 24]. While these receptor tyrosine kinases can activate the transcriptional function of ER, they can also reduce estrogen dependence by down-regulating the expression of ER, perhaps contributing to the relative resistance to endocrine therapies in tumors amplified for HER2.

In this study, we made an important finding that patients with PIK3CA mutation are sensitive to endocrine therapy. We found that 3 patients (R6-39, R6-40, and R6-43) who carried PIK3CA mutation were sensitive to endocrine therapy for more than 5 years. Using their blood samples, these 3 patients were selected and analyzed to predict its possible molecular mechanism on a case-by-case basis.

For patient R6-39, we detect the increased copy number of gene C11orf30 (EMSY), which may decrease ASH2L (a component of MLL complex that confers H3K4 trimethylation). MLL complex is co-purified with ERα and required for ERα-regulated transcription. ASH-2L is a component of the Set1/Ash2 histone methyltransferase (HMT) complex, which specifically methylates Lys-4 of histone H3, but does not when the neighboring Lys-9 residue is already methylated. As part of the MLL1/MLL complex, it is involved in methylation and demethylation at Lys-4 of histone H3. Thus, it may function as a transcriptional regulator. Both EMSY and Ash2L are components of NIF-1 complex containing a H3 methyl-transferase activity. As part of a histone H3-specific methyltransferase complex, it may mediate ligand-dependent transcriptional activation by nuclear hormone receptors. Thus, depletion of ASH2L suppressed ERα expression in breast cancer cells. C11orf30 gene is a regulator capable of repressing transcription, possibly via its interaction with a multi-protein chromatin remodeling complex that modifies the chromatin. The interaction between C11orf30 and ESR1 are shown in **Figure 1B**. Its interaction with BRCA2 suggests that it may play a central role in the DNA repair function of BRCA2 [25, 27]. The increased copy number of gene C11orf30 detected in this patient indicates presence of ER activity and BRCA2 activity, conferring the sensitivity to endocrine therapy.

For patient R6-40, decreased copy number of CDH1 (E-cadherin) was found. This gene encodes a classical cadherin of the cadherin superfamily. We also detected a decreased copy number of CTNNB1 (Beta catenin), which encodes an important component of the Wnt signaling pathway, which binds to E-cadherin at the cell membrane, where the complex of these two proteins functions in the stabilization of cell adhesion. The protein encoded by this

gene is a part of a complex of proteins constituting adherence junctions (AJs). AJs are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. The encoded protein also anchors the actin cytoskeleton and may be responsible for transmitting the contact inhibition signal that causes cells to stop dividing once the epithelial sheet is complete. ER-α and β-catenin were co-precipitated within the same immunocomplexes, reciprocally enhanced the transactivation of cognate reporter genes, and were reciprocally recruited to cognate response elements in the promoters of endogenous target genes. The interaction between CDH1 and ESR1 are shown in **Figure 1C**. With the reduced expression levels of both CDH1 and CTNNB1, we predicted a down regulation of ESR1 expression [28, 29].

For patient R6-43, we detected a point mutation, E890K, in splicing factor 3B1 (SF3B1). This mutation may be harmful. The splicing factor SF3B belongs to the minor U12-dependent spliceosome, which is involved in the splicing of rare class of nuclear pre-mRNA intron. SF3B1 is identified by being associated with ligand-activated ERα in nuclei of human breast cancer cells by tandem affinity purification and nano LC-MS/MS. It may decline the expression of the ESR1 [30].

In our study, we found that the mutation rate of PIK3CA in the endocrine-resistant group (55%) was relatively higher than that in the endocrine-sensitive group (33%). Though the difference between the two groups was not statistically significant, we incline to conclude that PIK3CA mutation status may contribute to endocrine resistance. When future studies with a larger spectrum of patients are performed and analyzed, more conclusive results may be obtained.

Acknowledgements

This study was approved by the independent ethics committee of the Fourth Hospital of Hebei Medical University. All the patients had been provided with written informed consent for genomic testing used for this study.

Disclosure of conflict of interest

None.

Address correspondence to: Zhenchuan Song, Breast Center, Fourth Hospital of Hebei Medical University, No. 169, Tianshan Street, Yuhua District, Shijiazhuang, Hebei, China. Tel: +86 13739745699; Fax: (+86 311) 66696310; E-mail: songzhch@hotmail.com

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