Droplet digital polymerase chain reaction assay for screening of *ESR1* mutations in 325 breast cancer specimens

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Droplet digital polymerase chain reaction (ddPCR), which could perform thousands of PCRs on a nanoliter scale simultaneously, would be an attractive method to massive parallel sequencing for identifying and studying the significance of low-frequency rare mutations. Recent evidence has shown that the key potential mechanisms of the failure of aromatase inhibitors–based therapy involve identifying activating mutations affecting the ligand-binding domain of the *ESR1* gene. Therefore, the detection of *ESR1* mutations may be useful as a biomarker predicting an effect of the treatment. We aimed to develop a ddPCR-based method for the sensitive detection of *ESR1* mutations in 325 breast cancer specimens, in which 270 primary and 55 estrogen receptor–positive (ER+) metastatic breast cancer (MBC) specimens. Our ddPCR assay could detect the *ESR1* mutant molecules with low concentration of 0.25 copies/μL. According to the selected cutoff, *ESR1* mutations occurred in 7 (2.5%) of 270 primary breast cancer specimens and in 11 (20%) of 55 ER+ MBC specimens. Among the 11 MBC specimens, 5 specimens (45.5%) had the most common *ESR1* mutation, Y537S, 4 specimens (36.3%) each had D538G, Y537N, and Y537C. Interestingly, 2 patients had 2 *ESR1* mutations, Y537N/D538G and Y537S/Y537C, and 2 patients had 3 *ESR1* mutations, Y537S/Y537N/D538G. Biopsy was performed in heterochrony in 8 women twice. In 8 women, 4 women had primary breast cancer and MBC specimens and 4 women had 2 specimens when treatment was failure. Four of these 8 women acquired *ESR1* mutation, whereas no *ESR1* mutation could be identified at first biopsy. ddPCR technique could be a promising tool for the next-generation sequencing-free precise detection of *ESR1* mutations in endocrine therapy resistant cases and may assist in determining the treatment strategy. (Translational Research 2015;166:540–553)

Abbreviations: LBD = ligand-binding domain; ERα = estrogen receptor α; NGS = next generation sequencing; ER+ = ER-positive; AIs = aromatase inhibitors; MBC = metastatic breast cancer; ddPCR = droplet digital polymerase chain reaction; FFPE = formalin-fixed paraffin

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Submitted for publication July 15, 2015; revision submitted September 3, 2015; accepted for publication September 5, 2015.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2015.09.003

embedded; PR = partial response; SD = stable disease; PD = progressive disease; gDNA = genomic DNA; LMD = laser microdissection; MGB = minor groove binding; PgR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; AR = androgen receptor; IBTR = ipsilateral breast tumor recurrence; HR = hormone receptor; cfDNA = cell-free DNA; DCIS = ductal carcinoma in situ

AT A GLANCE COMMENTARY

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Background

Droplet digital polymerase chain reaction (ddPCR), which could perform thousands of PCRs on a nanoliter scale simultaneously, would be an attractive method for identifying the significance of low-frequency rare mutations. The detection of rare activating mutations in the ligand binding domain of *ESR1* gene has a possibility to predict response to endocrine therapies in metastatic breast cancer (MBC) patients. We have developed a ddPCR-based method for the sensitive detection and quantification of 4 representative *ESR1* mutations, Y537S, Y537N, Y537C, and D538G, in 325 breast cancer specimens, in which 270 primary breast cancer and 55 MBC specimens.

Translational Significance

ddPCR analysis could detect *ESR1* mutations in 7 (2.5%) of 270 primary breast cancer specimens and in 11 (20%) of 55 MBC specimens. Among the 11 MBC specimens, 5 specimens (45.5%) had the most common *ESR1* mutation, Y537S, 4 specimens (36.3%) each had D538G, Y537N, and Y537C. ddPCR technique could be a promising tool for the next-generation sequencing-free precise detection of *ESR1* mutations in endocrine therapy resistant cases and may assist in determining the treatment strategy.

INTRODUCTION

In the case of hormone-responsive tumors without life-threatening visceral metastases, endocrine therapy is the first-line treatment of choice and can be administered repeatedly and consistently although it continues to be effective because it has fewer adverse events than chemotherapy and has been shown to improve the survival rate.[1] However, eventually, all initial responders develop resistance over time[2] and finally progress with antiestrogen resistant, hormone-independent disease. Recent evidence, from comprehensive sequencing programs for breast cancer patients, has shown that the key potential mechanisms of the failure of endocrine therapy involve identifying activating mutations affecting the ligand-binding domain (LBD) of the *ESR1* gene which encodes estrogen receptor α (ERα; reviewed in Jordan et al[3]). Fuqua et al[4] pioneered studies on *ESR1* mutations in metastases when her group identified a single-point mutation in LBD of the *ESR1* gene, Y537N, that was predicted to cause a conformational change in the ER analogous to hormone binding. Weis et al[5] reported that functional mutations in the *ESR1* regions encoding the LBD of ERα, mainly at amino acids 537–538 in helix 12. Those mutations cause ligand-independent ER transcriptional activity that does not respond to endocrine manipulation. More recently, Li et al[6] reported the significance of *ESR1* 537–538 mutations in xenografts derived from primary or metastatic breast cancers that were resistant to endocrine therapy. Furthermore, 3 independent studies describing the next-generation sequencing (NGS) showed that *ESR1* 537–538 mutations appeared to be acquired in advanced estrogen receptor–positive (ER+) hormone-resistant breast cancer at metastatic lesions in the context of estrogen deprivation therapy, in particular, aromatase inhibitors (AIs)–based therapy.[7-9] The estimated frequency from these 3 studies was 20%–50% for *ESR1* mutations in metastatic breast cancer (MBC), but that was little present in primary breast cancer. In vitro and preclinical data suggest that *ESR1* mutations lead to complete AI resistance and to partial resistance to ER agonists and antagonists.[6,9] These features indicate that the detection of *ESR1* mutations may be useful as a biomarker predicting an effect of the treatment. Given its valuable predictive potential, molecular diagnostic tests, designed for higher precision determination of activating *ESR1* mutations, are gaining increasing significance. This is because biopsy specimens taken from metastatic lesions generally provide only a small amount of tissue, and *ESR1* mutations are rare point mutations in a background of wild-type sequences.

Traditional direct sequencing method is the gold standard in DNA variation analysis, but this method compromises its feasibility and accuracy in the detection of rare mutations. NGS technologies could identify these mutations, but it may be still difficult to use it