

Circulating Tumor DNA in a Breast Cancer Patient's Plasma Represents Driver Alterations in the Tumor Tissue

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Tumor tissues from biopsies or surgery are major sources for the next generation sequencing (NGS) study, but these procedures are invasive and have limitation to overcome intratumor heterogeneity. Recent studies have shown that driver alterations in tumor tissues can be detected by liquid biopsy which is a less invasive technique capable of both capturing the tumor heterogeneity and overcoming the difficulty in tissue sampling. However, it is still unclear whether the driver alterations in liquid biopsy can be detected by targeted NGS and how those related to the tissue biopsy. In this study, we performed whole-exome sequencing for a breast cancer tissue and identified *PTEN* p.H259fs*7 frameshift mutation. In the plasma DNA (liquid biopsy) analysis by targeted NGS, the same variant initially identified in the tumor tissue was also detected with low variant allele frequency. This mutation was subsequently validated by digital polymerase chain reaction in liquid biopsy. Our result confirm that driver alterations identified in the tumor tissue were detected in liquid biopsy by targeted NGS as well, and suggest that a higher depth of sequencing coverage is needed for detection of genomic alterations in a liquid biopsy.

Keywords: breast neoplasms, CDK4 amplification, circulating tumor DNA, liquid biopsy, next generation sequencing, PTEN mutation

Next generation sequencing (NGS) technology has not only revolutionized cancer research but also is currently being used to guide clinicians' decision-making for cancer treatments. Although tumor tissues from biopsies or surgery are major sources for the NGS study of primary, metastatic and resistant tumors, these serial tumor biopsies are often invasive procedures limited to certain locations and not easily acceptable in the clinic. More importantly, tissue biopsy has a severe limitation in view of the pronounced genomic and phenotypic heterogeneity of the tumor tissues [1]. To overcome the limitations of tissue biopsies or

surgery, a less invasive technique capable of both capturing the tumor heterogeneity and overcoming the difficulty in tissue sampling during the course of therapy is needed. Circulating tumor DNA (ctDNA) is comprised of small fragments of DNA released from cells undergoing apoptosis or necrosis in tumor tissues [2]. Recent studies have shown that driver alterations in tumor tissues can be detected by liquid biopsy for ctDNA [3]. However, it is still uncertain whether the driver alterations in ctDNA can be detected by targeted NGS and how those related to the tissue biopsy.

A 56-year old woman was referred to the oncology

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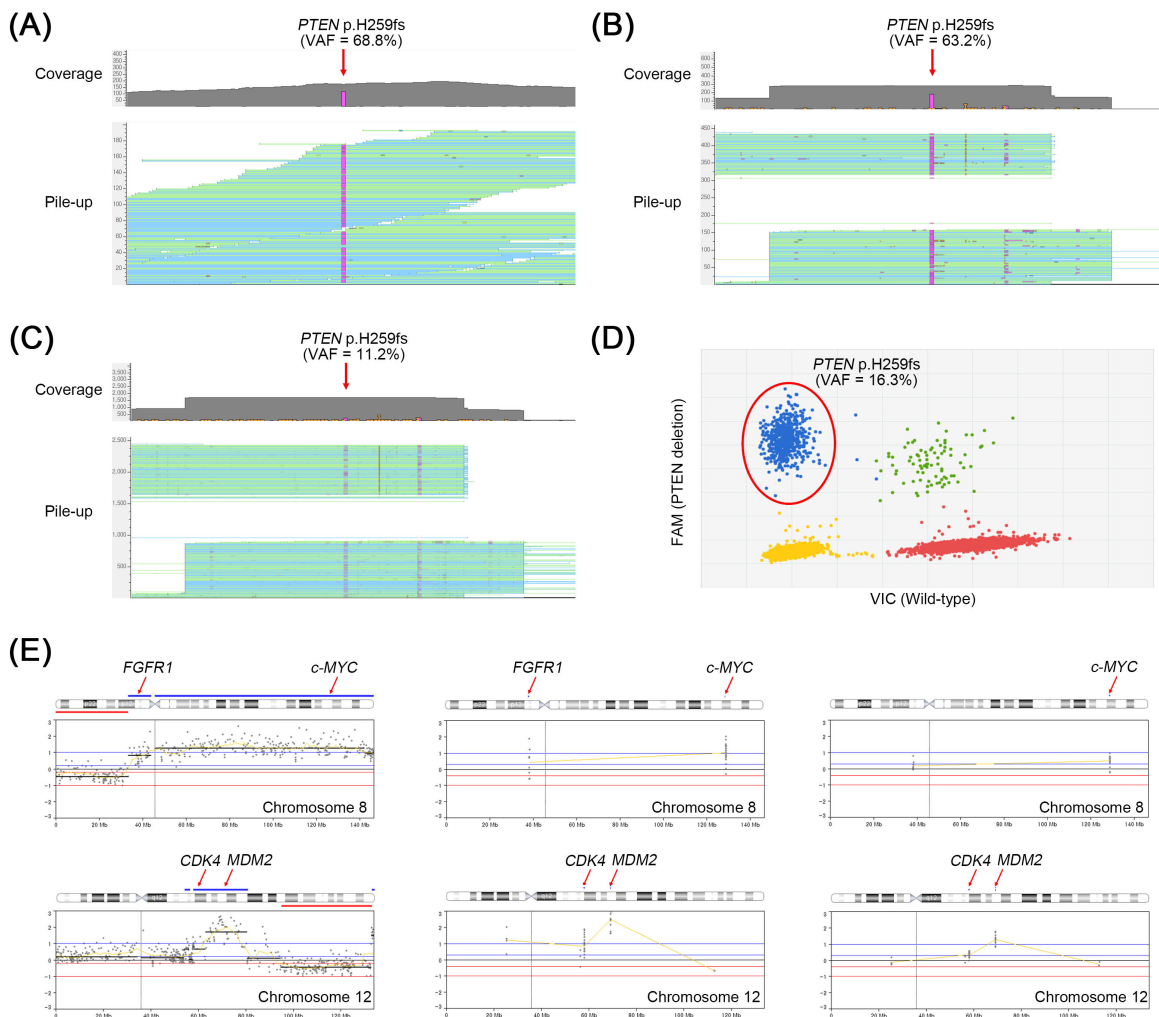


Fig. 1. *PTEN* frameshift mutation identified in a breast tumor tissue and circulating tumor DNA (ctDNA). H259fs mutation is detected by the whole-exome sequencing (WES) (A) and OncoChase targeted sequencing (B) in the breast tumor. (C) The same mutation is also detected by the OncoChase targeted sequencing in the ctDNA. (D) Validation of the *PTEN* mutation by a digital polymerase chain reaction in ctDNA. In all experiments, the *PTEN* mutation was not detected in the matched normal DNA. (E) Copy number alterations identified in breast tumor or ctDNA by next generation sequencing. Amplification of *FGFR1*, *c-MYC*, *CDK4*, and *MDM2* are detected in the tumor tissue by WES (left panel). Among them, *c-MYC*, *CDK4*, and *MDM2* amplifications are consistently detected in the tumor tissue (middle panel) or ctDNA (right panel) by OncoChase targeted panel sequencing. The x-axis represents genomic position and the y-axis represents the relative depth ratio (tumor/matched normal) in log₂ scale. VAF, variant allele frequency.

department for evaluation of her right shoulder pain. She was previously diagnosed as a breast cancer 6 years ago. The patient was treated with quadrantectomy along with axillary node dissection (invasive ductal carcinoma, pT2N0M0, estrogen receptor [ER] positive, progesterone receptor positive and human epidermal growth factor receptor 2 [HER2] negative), adjuvant radiation of the left breast, and adjuvant toremifen (an oral selective ER modulator) for 5 years. Seven months after the termination of adjuvant toremifen, the patient a pathologic fracture of right distal clavicle, multiple bone, lung and liver metastases (Supplementary Fig. 1). During 3 years of the systemic treatment,

the patient had progression of chest, liver and bone metastases (Supplementary Fig. 2).

To identify the genetic alterations in the tumor tissue, we performed whole-exome sequencing (WES) for the cancer tissue metastasized to femur along with her matched normal DNA from peripheral blood. Generation and processing of the sequencing data were performed as previously described [4]. In the WES, a total of 53 nonsilent somatic mutations and 23 copy number alterations (CNAs) were detected (Supplementary Tables 1 and 2). Among them, *PTEN* p.H259fs*7 frameshift mutation (Fig. 1A) as well as *FGFR1*, *c-MYC*, *CDK4*, and *MDM2* amplifications (Fig. 1E) were identified as

driver alterations in this tumor.

To validate these, the metastatic tumor and matched normal samples of the patient were re-analyzed by a targeted NGS. Targeted NGS was performed using the OncoChase Cancer Panel v0.9 (ConnectaGen, Seoul, Korea) consisting of 78 well-characterized cancer genes (Supplementary Table 3) with Ion PGM Dx system (Thermo Fisher Scientific, Waltham, MA, USA). Coverage of depth was 416× for the metastatic tumor sample and 754× for the normal sample. In the OncoChase analysis, all five driver alterations previously identified in the WES were consistently detected (Fig. 1B and 1E). Of note, variant allele frequency (VAF) of *PTEN* frameshift mutation detected by WES (68.8%) were almost similar to that detected by OncoChase (63.2%), suggesting the OncoChase Cancer Panel may be reliable for the detection of driver alterations.

Next, we analyzed the plasma DNA (liquid biopsy) of the patient using the same cancer panel (OncoChase Cancer Panel v0.9). Coverage of depth was 1,213× for the liquid biopsy sample. The *PTEN* frameshift mutation initially identified in the tumor tissue was also detected in her liquid biopsy with a VAF of 11.2% (Fig. 1C). This mutation was subsequently validated by digital polymerase chain reaction (Fig. 1D). The ctDNA also harbored *c-MYC*, *CDK4* and *MDM2* amplification but signal intensities of the CNAs were relatively lower than those of the tissue biopsy (Fig. 1E). One CNA (*FGFR* amplification) identified in the tumor tissue was not detected in the ctDNA.

Comprehensive review of the hormone status (ER positive) and genetic alteration (*CDK4* amplification) status strongly suggested the use of *CDK4/6* inhibitors such as palbociclib [5] combined with aromatase inhibitor [6]. However, due to her dismal hepatic function, palbociclib was not administered, and she passed away after 52 months of cancer recurrence. In this study, we showed an example that driver alterations identified in the tumor tissue were

detected in liquid biopsy by targeted NGS as well. The low VAF of mutation and attenuated CNA signals in the liquid biopsy compared to the tissue biopsy suggest that a higher depth of sequencing coverage is required for detection of genomic alterations in a liquid biopsy than in a tissue biopsy.

Supplementary materials

Supplementary data including three tables and two figures can be found with this article online at <http://www.genominfo.org/src/sm/gni-15-48-s001.pdf>.

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