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Review Article

Mutation Detection of Phosphatidylinositol-4,5-bisphosphate 3-Kinase Catalytic Subunit Alpha for Treatment Guidance in Breast Cancer

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Abstract

Molecular analysis of breast cancer tissue has revealed that breast cancer is not a uniform disease. Each breast cancer patient has several molecular signatures that differ from those of others. Therefore, breast cancer therapy should be personalized, depending on its molecular signatures. Breast cancer with hormonal receptors can be treated with a selective estrogen receptor modulator or selective estrogen receptor degrader therapy, while breast cancer with overexpression of human epidermal growth factor receptor 2 *(HER2)-neu* gene responds excellently to anti-HER2-neu therapy. For patients with advanced breast cancer that already has distant metastasis and a poor prognosis, a new agent has been discovered. The phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) inhibitor has been proven effective in treating advanced breast cancer with a *PIK3CA* gene mutation. This therapy can be administered to HER2-negative breast cancer patients and in combination with selective estrogen receptor degrader therapy for post-menopausal patients with positive hormonal receptors. Although this treatment is effective, it cannot be given to every advanced breast cancer patient. Before administering the treatment, a *PIK3CA* mutation test is compulsory. *PIK3CA* mutation detection in breast cancer can predict the cancer's response to the PIK3CA inhibitor, providing information on which patients will benefit from the treatment.

Introduction

In women in the United States and worldwide, breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related death.[1,2] Approximately 287,850 women would be diagnosed with invasive breast cancer in the United States in 2022. In the same year, 40,920 deaths were recorded due to breast cancer. During that time, 92,700 women were projected to die from breast cancer in Europe.[3] Although breast cancer in men is rare,[4,5] accounting for less than 1% of breast cancer diagnoses, the same treatment is recommended for both sexes. Depending on mutations in breast cancer patients, the treatment can be adjusted if a mutation is detected.[3,4] Recently, one type of mutation affecting the therapy management of advanced breast cancer patients is a mutation in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene. Due to mutated *PIK3CA* genes, hyperactivation of the p110α subunit of phosphoinositide 3-kinase (PI3K) is induced.[6] Thus, targeted treatment can be guided by *PI3KCA* mutation testing. Hence, the importance and utility of *PIK3CA* mutation testing in advanced breast cancer patients increases to guide treatment decisions.[7] PIK3CA inhibitor therapy, such as Alpelisib, is primarily effective for somatic *PIK3CA* mutations in the context of breast cancer treatment, especially in human epidermal growth factor receptor 2 (HER2)-negative type and post-menopausal patients with positive hormonal receptors. Therefore, it is vitally relevant to perform *PIK3CA* mutation tests to detect genetic alterations in the *PIK3CA* gene, which plays an important role in the PI3K signaling pathway.[8–10]

Keywords: Breast cancer; Personalized medicine; *PIK3CA* mutation; PIK3CA inhibitor; Targeted therapy; Molecular classification.

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Review contents

Personalized therapy for breast cancer

Breast cancer therapy has evolved from conventional therapy to

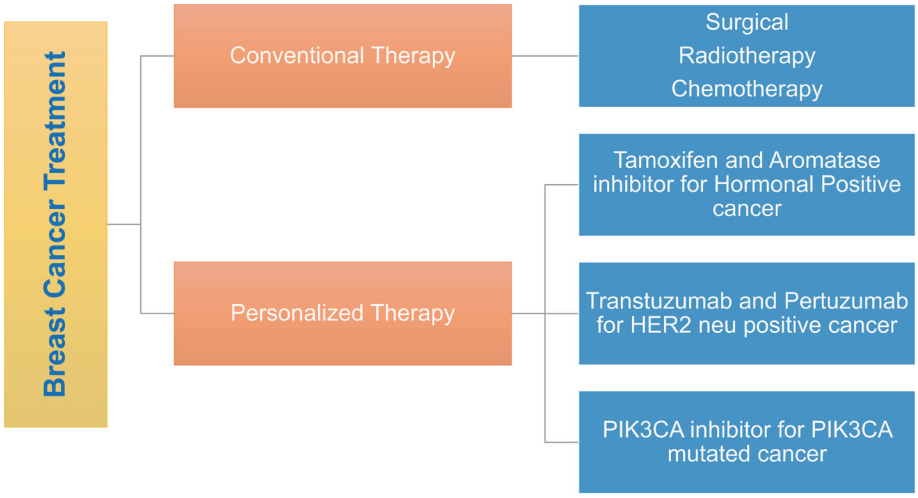

Fig. 1. Conventional therapy and personalized therapy in Breast cancer treatment. HER2, human epidermal growth factor receptor 2; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

personalized therapy (Fig. 1). Targeted therapy for breast cancer involves drugs or treatments that specifically target certain molecules or pathways involved in the growth and survival of cancer cells. The choice of targeted therapy depends on the specific characteristics of the breast cancer, such as the presence of certain receptors on the cancer cells.[11,12] There are several common targeted therapies used in the treatment of breast carcinoma. (1) Tamoxifen and Aromatase Inhibitors: These are used for hormone receptor-positive breast cancers. Tamoxifen blocks the estrogen receptor, while aromatase inhibitors reduce the production of estrogen. (2) Trastuzumab: This monoclonal antibody targets the HER2 protein, which is overexpressed in some breast cancers. Trastuzumab can be used alone or in combination with chemotherapy. (3) Pertuzumab: Another HER2-targeted monoclonal antibody, often used in combination with trastuzumab. (4) Poly(ADP-ribose) polymerase (PARP) (c) Inhibitors: Drugs such as olaparib, talazoparib, and niraparib are used for breast cancers with *BRCA1* or *BRCA2* gene mutations. They inhibit the PARP enzyme, leading to DNA damage and cell death. (5) PI3K Inhibitors: Alpelisib is a drug that targets the PI3K pathway and is used in combination with fulvestrant for hormone receptor-positive and HER2-negative breast cancer with PIK3CA mutations.[13,14]

Hormone receptor status and endocrine-based therapy for breast cancer

Estrogen receptor (ER) and progesterone receptor (PR) assays help classify breast cancers into subtypes based on hormonal receptor status. This classification is crucial for tailoring treatment strategies.[15,16] Hormone receptor-positive breast cancers are often more responsive to hormonal therapies, while receptor-negative cancers may be treated with other approaches, such as chemotherapy. ER-positive (ER+)/PR-positive (PR+) breast cancers tend to have a relatively better prognosis compared to ER-negative (ER-)/PR-negative (PR-) cancers. ER- and PR- breast cancers may have a less favorable prognosis compared to hormone receptor-positive tumors. These receptors allow cancer cells to respond to hormones like estrogen and progesterone, promoting their growth.[17] ER+/PR+ breast cancer, constituting approximately 70% of breast cancers, is the most common subtype.[18] Since ER+/PR+ tumors overexpress multiple ER/PR, hormone therapies are often used for their treatment.[17,19] Endocrine-based therapies are standard for hormonal receptor (HR)-positive breast cancer. These therapies target hormone receptors (ER and PR) and hormone signaling pathways driving HR-positive (HR+) breast cancer cell growth. Endocrine therapies include aromatase inhibitors (e.g., letrozole, anastrozole), selective estrogen receptor modulators (SERMs, e.g., tamoxifen), and selective estrogen receptor degraders (SERDs, e.g., fulvestrant).[17,19,20]

Tamoxifen, a common SERM in breast cancer treatment, has a dual effect on estrogen receptors. It acts as an agonist (mimicking the action of estrogen) in certain tissues (e.g., bone), where estrogen is beneficial, and as an antagonist in breast tissue, blocking estrogen receptors to inhibit estrogen's growth-promoting effects on cancer cells. Tamoxifen is used in both premenopausal and postmenopausal women with ER+/PR+ breast cancer as adjuvant therapy to reduce recurrence risk post-surgery and in advanced stages of breast cancer.[21] Fulvestrant, a common SERD in breast cancer treatment, works by binding to estrogen receptors and promoting their degradation, reducing their numbers within the cell. Unlike SERMs, SERDs do not have agonistic effects and act purely as antagonists, effectively blocking the estrogen signaling pathway. Fulvestrant is typically used in postmenopausal women with advanced ER+/PR+ breast cancer that has progressed after other hormonal therapies. The key difference between SERMs and SERDs lies in their mechanisms of action. SERMs have dual effects, acting as both agonists and antagonists, while SERDs specifically promote estrogen receptor degradation without exhibiting agonistic effects.[22]

Despite the initial effectiveness of endocrine-based therapies, resistance can develop over time in advanced cases of ER+/PR+ breast cancer, as well as in some early stages of this malignancy.[18] Tumor cells may adapt and find ways to bypass or resist the inhibitory effects of these therapies. In a recent study, HR+ and HER2- metastatic breast cancers were found to respond poorly to certain chemotherapy treatments and showed lower survival rates. In HR+ breast cancer, PIK3CA mutations can contribute to resistance to endocrine-based therapies and may drive cancer progression.[19,20] To improve progression-free survival rates, new treatments are needed. Since PIK3CA mutations are associated with HR+, HER2- breast cancer, a PIK3CA inhibitor was designed. It is expected to reduce hyperactivation of PIK in postmenopausal women and men

with advanced breast cancer whose tumors possess this combination of mutations. In addition to endocrine-based therapy, which hinders estrogen's impact on cancer cells, this oral inhibitor drug is administered.[7,10,18,23–27]

HER2/ c-erbB2 status in breast cancer

HER2 status is a critical factor in the diagnosis and treatment of breast cancer. HER2 is a protein that can promote the growth of cancer cells when it is overexpressed or amplified. Determining HER2 status is crucial because it helps classify breast cancer into different subtypes and guides treatment decisions. HER2 status assays in breast cancer patients have been used for (1) Classification of breast cancer subtypes: If the breast cancer cells overexpress or have amplified levels of the HER2 protein, the tumor is classified as HER2-positive (HER2+). Approximately 15–20% of breast cancers are HER2+. HER2-negative (HER2-): Tumors without overexpression or amplification of HER2 are classified as HER2-. (2) Prognostic Information: HER2+ breast cancers tend to be more aggressive and have a higher risk of recurrence compared to HER2- tumors. (3) Treatment Implications: HER2+ breast cancers are typically more responsive to targeted therapies directed against the HER2 protein, such as Trastuzumab.[28]

Some breast cancers overexpress the HER2 protein and are classified as HER2+. HER2 is a receptor tyrosine kinase that can lead to aggressive growth and behavior of cancer cells when overexpressed.[17] HER2+ breast cancer is typically treated with targeted therapies such as HER2 inhibitors, e.g., trastuzumab, pertuzumab, or ado-trastuzumab emtansine, which specifically target HER2. In some cases, breast cancers may have both HER2 overexpression and PIK3CA mutations. HER2- breast cancer lacks overexpression or amplification of the *HER2* gene and HER2 protein.[19]

PIK3CA mutation and PIK3CA inhibitor therapy for breast cancer

The PI3K signaling pathway is a crucial cellular pathway that regulates various processes such as cell growth, survival, proliferation, and metabolism. It plays a pivotal role in transmitting signals from cell surface receptors to intracellular effectors, influencing cellular responses to extracellular stimuli. The key functions of the PI3K pathway include (1) Cell growth and survival: The PI3K pathway promotes cell growth and survival by activating signaling cascades that stimulate protein synthesis and inhibit programmed cell death (apoptosis). Activation of Akt plays a central role in promoting cell survival by regulating the expression of anti-apoptotic proteins and inhibiting pro-apoptotic factors. (2) Cell proliferation: The pathway is involved in regulating the cell cycle and promoting cell proliferation. Activation of PI3K signaling can stimulate cell division by influencing the expression of genes involved in cell cycle progression.[29,30]

Negative regulation of the PI3K signaling pathway: To maintain cellular homeostasis, the PI3K pathway is tightly regulated by negative feedback mechanisms. For example, the tumor suppressor phosphatase and tensin homolog dephosphorylates PIP3, counteracting the action of PI3K.[31]

Germline mutations in the *PIK3CA* gene are hereditary mutations present in a patient's inherited DNA and are typically found in all cells of the body, including normal and cancerous cells. While germline PIK3CA mutations can cause various conditions, such as PIK3CA-related overgrowth syndromes, somatic PIK3CA mutations occur specifically within cancer cells.[32–34] These somatic mutations are genetic changes that develop during a person's lifetime and are acquired mutations not present in the patient's inherited DNA.[35] Somatic mutations in the *PIK3CA* gene are found in many types of cancer, including brain, breast, lung, ovary, stomach, and colorectal cancers. These mutations alter single amino acids in the p110α protein, leading to activation of the PI3K signaling pathway in cancer cells.[35–39]

Functions regulated by this pathway in cancer cells include angiogenesis, cell proliferation, cell migration, glucose metabolism, survival, and translational regulation of protein synthesis.[40] In tumor tissues, activating somatic missense mutations of the *PIK3CA* gene have been identified to increase the kinase activity of the PI3Kα protein, contributing to cellular transformation in different human cancers.[41] However, researchers suspect that *PIK3CA* gene mutations alone do not cause cancer but rather influence cancer risk, often in combination with mutations in other genes. The oral drug alpelisib has been approved by the U.S. Food and Drug Administration in combination with endocrine therapy fulvestrant to treat breast cancer with mutations in the *PIK3CA*.[36]

Detection of molecular signature in cancer cells

The molecular signature of cancer cells can be detected by immunohistochemistry techniques for protein expression, such as ER/PR and HER2 neu protein. On the other hand, molecular techniques such as polymerase chain reaction (PCR) are needed for the detection of *HER2* neu gene amplification and *PIK3CA* gene mutation (Fig. 2).

Different methods for screening PIK3CA mutations have been described in the past. With the approval of the novel drug alpelisib, detection of mutations in the *PIK3CA* gene has become increasingly important. Immunohistochemistry assays can detect mutated proteins expressed from the *PIK3CA* gene, although they are not intended to predict response to alpelisib therapy. While not directly detecting PIK3CA mutations, immunohistochemistry for protein phosphatase and tensin homolog, a key negative regulator of the PI3K pathway, may provide indirect information about pathway dysregulation. Hu *et al*.[42] detected PIK3CA mutations using immunohistochemistry assays in triple-negative breast cancer. Fluorescent in situ hybridization (FISH) assays can also be used to detect PIK-3CA mutations, but their results are not intended to predict therapy response with alpelisib.[43] FISH, although less commonly used for PIK3CA specifically, can detect gene amplifications or structural alterations by using fluorescent probes that bind to specific DNA sequences, visualized under a microscope. In cervical carcinoma, both FISH and PCR can assess *PIK3CA* gene amplification and mutation.[44] PCR selectively amplifies the region of interest in the *PIK3CA* gene, followed by sequencing to identify mutations. Techniques such as allele-specific PCR or quantitative PCR can detect specific mutations. Cossu-Rocca *et al*.[45] utilized a real-time PCR procedure with the cobas® DNA Sample Preparation Kit (Roche Mannheim, Germany) to detect mutations in the *PIK3CA* gene in isolated genomic DNA from triple-negative breast cancer patients. For analyzing tumor tissue specimens and/or plasma specimens with isolated circulating tumor DNA to select patients with *PIK3CA* gene mutations, the U.S. Food and Drug Administration approved the *therascreen*® PIK3CA RGQ PCR Kit in May 2019.[8]

The importance of PIK3CA mutation detection in breast cancer and detection method

Personalized therapy for breast cancer depends on its molecular signature. In hormone receptor-positive breast cancer (HR+), patients can be treated with tamoxifen or aromatase inhibitors. However, when the cancer progresses to an advanced stage, there is new hope with PIK3CA inhibitor treatment. Pathology examination

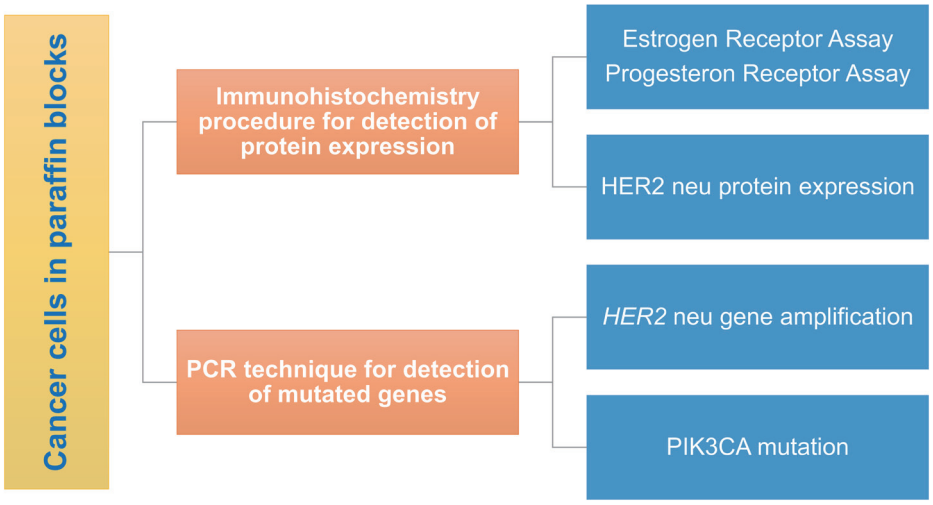

Fig. 2. Detection techniques of molecular signature in cancer cells. HER2, human epidermal growth factor receptor 2; PCR, polymerase chain reaction; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

plays a pivotal role in detecting specific mutations crucial for personalized treatment.[46] The presence of PIK3CA mutations in HR+ breast cancer can significantly impact treatment decisions. Targeted therapies that inhibit the PI3K pathway, such as the PIK3CA inhibitor alpelisib, have been developed to address resistance associated with these mutations. Clinical trials such as the SOLAR-1 trial have shown significantly improved progression-free survival in postmenopausal women and men diagnosed with HR+, HER2-, PIK3CA-mutated advanced breast cancer when treated with a combination of alpelisib and fulvestrant, an endocrine therapy.[23,25] However, PIK-3CA inhibitor therapy is not recommended for early breast cancer.[47] The use of alpelisib in combination with fulvestrant exemplifies personalized medicine in breast cancer treatment, allowing oncologists to tailor therapy based on the specific genetic and molecular characteristics of the tumor. Molecular profiling of breast cancer tumors by pathologists, including the identification of PIK3CA mutations, HER2 amplification, and other molecular alterations, is essential for selecting appropriate targeted therapies.[10,24]

Conclusions

Personalized therapy has been developed for breast cancer treatment. Therefore, it is important to have molecular classification for every breast cancer patient. The discovery of PIK3CA inhibitors has opened a new way to treat advanced breast cancer that is HER2-negative. It can be used in combination with SERD therapy for hormone receptor-positive breast cancer. Detection of PIK3CA mutations is necessary to determine which patients benefit from PIK3CA inhibitor therapy.

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Conflict of interest

The authors declare that there is no conflict of interest related to this publication.

Author contributions

Writing of the main concept of this article (WS), writing of the details of article (LPG); technical support and manuscript revision (LT). All authors have made a significant contribution to this study and have approved the final manuscript.

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