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REVIEW ARTICLE

A novel targeted therapy in breast cancer: Cyclin dependent kinase inhibitors

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Summary

Breast cancer is the most common cancer in women worldwide. Understanding the biology of this tumor is a prerequisite for selecting an appropriate treatment. Cell cycle alterations are seen in many cancers such as breast cancer. Newly popular targeted agent in breast cancer are cyclin dependent kinase inhibitors (CDKIs) which are agents inhibiting the function of cyclin dependent kinases (CDKs). They are categorized as selective and non-selective inhibitors of CDK. CDKIs have been tried as monotherapy and combination therapy. Palbocyclib is now a promising CDKI used in breast cancer. Nowadays palbocyclib is designed for a phase III trial for estrogen receptor (ER) positive breast cancer after showing favorable results in progression free survival in a phase II trial.

Key words: *breast cancer, cyclin dependent kinase inhibitors, targeted therapy*

Introduction

Cancer consists of immortal cells that can be fatal for patients. Ironically, these cells must die so that the patients survive. Cell division and cell death are the two predominant physiological processes that regulate normal tissue homeostasis. Alteration of these two physiological processes has a pivotal role in the pathogenesis of cancer [1]. Great efforts to ascertain components of the cell cycle are guiding to novel approaches for the treatment of cancer.

Genes encoding components of the cell cycle such as cyclin, CDKs and their endogenous inhibitors which are found in normal conditions are often impaired in many human cancers [2]. For example, CDKs are overactive in some cancers depending on cyclin overexpression or downregulation of endogenous CDKIs [3]. According to this data, researchers focus on whether the strategy of CDK inhibition is able to render cancer treatment more successful. Some studies suggest that inhibiting CDKs may be an effective therapy in many cancers including breast cancer [4]. In this review, we summarized the usage of CDKIs in breast cancer.

Cell cycle

Cell cycle is regulated by cyclins, CDKs, and CDKIs. These three key classes of regulatory molecules determine a cell's progress through the cell cycle [5]. Cell cycle is divided into 4 distinct phases (G_1, S, G_2, and M). G_0 represents exit from the cell cycle. Specific cyclin and CDKs complexes conduct cell cycle progression by regulating transition through G_0-G_1-S-G_2-M phases. Cell cycle is driven by CDKs, which are positively and negatively regulated by cyclins and CDKIs, respectively [6]. Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin [7].

Animal cells contain lots of CDKs. Some of them are directly involved in cell cycle regulation, such as CDK1, CDK2 and CDK4. For example, CDK1, with its partners cyclin A2 and B1, alone

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can drive the cell cycle in mammalian cells [8]. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Cyclin-CDK complexes in earlier cell-cycle phase help activate cyclin-CDK complexes in later phases [9]. In addition, a second group of CDKs are responsible for the regulation of cellular transcription. They have role of maintenance for cancer cells' survival. This group of CDKs consists of CDK7, CDK8, CDK9, CDK10, and CDK11.

A CDKI protein is an endogenous protein that interacts with a cyclin-CDK complex to block kinase activity, usually during G_1 or in response to signals from the environment or from damaged DNA. In the human body, there are two major CDKI protein families: the INK4a/ARF family and the Cip/Kip family. The INK4 family proteins are strictly inhibitory and bind CDK monomers. Crystal structures of CDK6-INK4 complexes show that INK4 binding twists the CDK to distort cyclin binding and kinase activity. The Cip/Kip family proteins bind both the cyclin and the CDK of a complex and can be inhibitory or activating. The Cip/Kip family proteins activate cyclin D and CDK4 or CDK6 complexes by enhancing complex formation [10].

To push the cell from G_1 to S phase the phosphorylation of retinoblastoma (Rb) protein by CDK4 or CDK6 in complex with their activating subunits, cyclin D1, D2 and D3 is necessary. The hyperphosphorylated Rb protein dissociates from the E2F/DP1/Rb complex to activate E2F. Activation of E2F results in transcription of various genes such as cyclin E, cyclin A, DNA polymerase, and thymidine kinase. For instance cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex that keeps up the progression through G_1-S phase. CDK2-cyclin A and CDK1-cyclin A regulate the completion of S phase. Then G_1/S progression initiates the G_2/M transition [11]. Finally, the cell cycle is completed and cell is going to divide.

All cancers activate cell cycle to sustain their survival. Selecting an appropriate agent for the appropriate tumor type is very hard, because, first of all, it should be identified which regulator of the cell cycle is responsible for the cell cycle downstream of an oncogenic event. Therefore, mouse models have been used to understand what kind of the cell cycle inhibitor is against which cancer type. In many cancers CDKs are overactive or CDK-inhibiting proteins are dysfunctional. For example, upregulation of CDK4 or downregulation of a naturally occurring inhibitor of CDK4, called p16INK4A, lead to loss of proliferative control of cell through enhanced CDK4 activity, resulting in hyperphosphorylation of Rb protein and in carcinogenesis [12]. According to this information, it is rational to target CDK function to prevent overproliferation of cancer cells and to use CDKIs to treat human cancers.

CDK inhibitors in breast cancer

Breast cancer is the most common cancer in women worldwide [13] and some alterations of the cell cycle have been detected in this disease. Checkpoint deregulations play a key role in some breast cancers. Alterations of pathways that include cyclin, CDK, endogenous CDKI and Rb protein are seen in nearly all cancers, including breast cancer. Cyclin D1 and cyclin E overexpression, decreased expression of CDKI p27Kip1 are some of them in human breast cancer [14,15]. Cyclin D1 amplification is seen in nearly 60% of breast cancers. Estrogen uses cyclin D1 as one of its target genes to mediate its mitogenic effects. Some studies suggested that among patients with high tumor expression of cyclin D1, overexpression of HER2 was associated with reduced recurrence-free survival and tamoxifen responsiveness [16]. Overexpression of cyclin D1 changes the antagonistic effect of tamoxifen to an agonistic effect. Therefore tamoxifen resistance might be predicted with cyclin D1 overexpression [17]. However, this data has not been exactly verified and the prognostic significance of cyclin D1 overexpression is not completely understood.

There are a lot of CDKIs that have gone through or are currently tested in ongoing clinical trials in cancer treatment [18-21]. Most of them are targeting multiple CDKs, but some are targeting specific CDKs. Selective inhibition of CDKs is much better than non-selective, because more adverse and toxic effects have been seen with non-selective inhibitors. For instance, palbocyclib, a selective CDKI, exerts its killing effect on tumor cells rather than on normal cells. Various types of cancers including leukemia, melanoma, liposarcoma, hepatocellular carcinoma and breast cancer are being tested for palbocyclib effectiveness [18].

Understanding the biology of a tumor is a prerequisite for selecting an appropriate treatment. It is well known that CDK4/6 binds cyclin D1 for phosphorylation of Rb protein and activa-

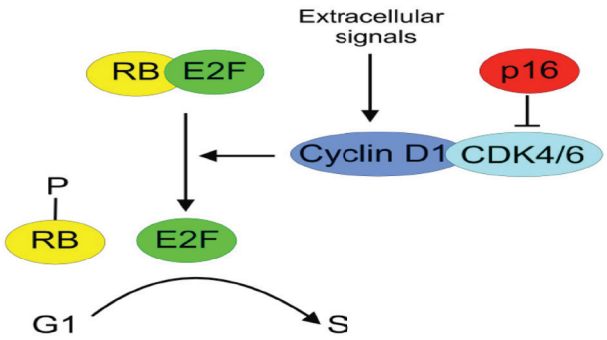

Figure 1. Cyclin D1/ CDK4 and CDK6/ Rb/ E2F pathway for G_1 to S transition.

tion of E2F transcription factors to progress cell cycle. This pathway is shown in Figure 1. If this well known pathway is blocked somewhere, the cell cycle progression will be arrested [22]. However, this mechanism is not adequate for someone to hypothesize that cyclin D1-overexpressing tumors will respond to any blockage in this pathway. For instance, mantle cell lymphoma, which is a high grade tumor, overexpresses cyclin D1 in 90%, yet CDK4/6 inhibitor achieves only 18% response rate. Mantle cell lymphoma cells may be dependent on cyclin D1 for their proliferation but not for their survival or any other resistance mechanisms may occur [23]. In particular, studies in HER2-induced mice mammary cancer models suggest that CDK4 and cyclin D1 are required to grow and to maintain tumor cells. In low-grade and cyclin D1-overexpressing malignancies, such as ER positive breast cancer, CDK4/6 inhibitor may have therapeutic potential. Cytoxic agents or targeted agents prevent tumor enlargement rather than tumor shrinkage. Palbocyclib, an oral CDK4/6 inhibitor breaks the above-described pathway, blocks Rb phosphorylation and subsequently induces G_0/G_1 arrest in sensitive cell lines.

The efficacy of palbocyclib was first tried in mouse models. Palbocyclib alone was found to be active and inhibited cell progression in *in vitro* studies [24]. ER-positive cell lines, including those with HER2 amplification, were most sensitive to growth inhibition by palbocyclib while nonluminal/basal subtypes were most resistant. Analysis of variance in both sensitive and resistant cells suggested that Rb protein and cyclin D1 were elevated and CDKN2A, which encodes p16,an endogenous inhibitor of CDK4 and CDK6, was decreased in most sensitive lines. Cell cycle analysis showed G_0/G_1 arrest in sensitive cell lines [24]. We know that tamoxifen and transtuzumab are more efficacious in ER-positive and HER2-amplified breast cancers, respectively. In an *in vitro* study that Finn et al. conducted, they identified a subgroup of patients most likely to benefit from palbocyclib: the ER-positive luminal subtype [24]. They also identified potential synergy with standard therapies, like tamoxifen and transtuzumab. Another result of this study was that elevated cyclin D1 and Rb expressions and decreased p16 expressions in tumor tissue were indicators of response of palbocyclib.

To understand the efficacy of palbocyclib Dean et al. conducted a study in which surgically resected breast tumors were cultured with or without palbocyclib [25]. Regardless of ER or HER2 status, only Rb-positive tumor cell showed growth inhibition in response to palbocyclib. Tumors lacking Rb were completely resistant. This result characterizes Rb as the predominant target of CDK4/6 and the primary marker of drug response in breast cancer cells. This study also suggested the importance of direct screening of tumors for RB expression to select patients appropriate for palbocyclib treatment.

In order to understand whether combination therapy of palbocyclib with any chemotherapeutic agent is effective, palbocyclib was used with carboplatin [23]. Although carboplatin is an agent not used for first-line treatment in breast cancer, it is used in the metastatic setting. Palbocyclib 150 mg/kg/day was combined with carboplatin in mouse models with metastatic mammary cancer and this combination achieved statistically better results than carboplatin alone; however, palbocyclib was not found to be efficacious in Rb-deficient mice. In addition, no extra myelosuppression with the combination of chemotherapy and palbocyclib vs chemotherapy alone was observed.

Another study [27] was carried out to investigate the efficacy of palbocyclib in combination with doxorubicin in triple-negative breast cancer cell lines. Again, Rb expression was of paramount importance in determining response to either monotherapy with palbocyclib or combination treatment . In Rb-deficient cancer cells, CDK4/6 inhibition had no antitumor effect. Although in Rb-expressing cancer cells palbocyclib and doxorubicin provided a synergistic cytotoxic effect, doxorubicin-induced cytotoxicity was substantially reduced when combined with palbocyclib.

Palbocyclib was tried in combination with letrozole in a phase 1 study to assess tolerability and safety [28]. The combination was well tolerated and it was safe in 12 postmenopausal patients with ER-positive, HER2-negative breast cancer

patients. Antitumor activity was seen in this trial. In combination with letrozole 2.5 mg per day, the recommended dose of palbocyclib was determined as 125 mg per day for 3 weeks followed by 1 week off (schedule 3/1). Based on this phase 1 trial palbocyclib was desinged for a phase 2 clinical trial [28].

The combination of palbocyclib and letrozole was compared against letrozole alone in a phase 2 study in165 patients with advanced breast cancer. The study consisted of two parts: part 1 enrolled patients with ER-positive and HER-2 negative disease without other selection criteria; part 2 enrolled postmenopausal ER-positive, HER2-negative patients with cyclin D1 amplification and/or loss of p16 by fluorescent *in situ* hybridization. The addition of palbocyclib to letrozole prolonged median time to disease progression to 26.1 months compared with 7.5 months for letrozole alone. Palbocyclib and letrozole combination provided surprising improvement in progression free survival in this population. According to measurable response, 45% receiving the combination had measurable response, while in the letrozole group only 31% had measurable response. After 6 months follow-up period tumor shrinkage and/or stable disease rates were 70% in the combination group and 44% in the letrozole alone group. Eventually, palbocyclib prolonged median progression free survival by over 18 months [29].

Conclusion

Targeting CDK or CDKI is a popular issue in oncology. Although specifying CDKI in a specific cancer is obscure, ongoing clinical studies about these agents seem to shed adequate light in this setting. Palbocyclib is now a promising therapeutic agent in breast cancer. Nowadays palbocyclib is planned for a phase 3 trial for ER-positive breast cancer after having shown favorable results in progression free survival in phase 2 trials. If the results of studies about palbocyclib are confirmed in a large phase 3 trial, palbocyclib will be a novel important targeted agent for advanced ER-positive breast cancer.

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