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Mechanisms of therapeutic CDK4/6 inhibition in breast cancer

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ABSTRACT

Cyclin dependent kinase (CDK) 4/6 inhibitors have advanced the treatment of metastatic breast cancer by targeting the cell cycle machinery, interrupting intracellular and mitogenic hormone signals that stimulate proliferation of malignant cells. Preclinical evidence demonstrated that derangements of cyclin D1, CDK4/6, and retinoblastoma expression are common in breast cancer, and suggested a therapeutic benefit from interrupting this axis required for cell cycle progression. Studies of cell lines and animal models of breast cancer have demonstrated the complex interplay between the cell cycle and estrogen receptor and human epidermal growth receptor 2 signaling, which informs our understanding of synergistic use of CDK4/6 inhibitors with endocrine therapy, as well as mechanisms of resistance to endocrine therapy. Interestingly, estrogen receptor activity leads to upregulation of cyclin D1 expression, but the estrogen receptor is also in turn activated by cyclin D1, independent of estrogen binding. Early CDK inhibitors were nonspecific and limited by systemic toxicities, while the current generation of CDK4/6 inhibitors have shown promise in the treatment of hormone receptor-positive breast cancer. Preclinical investigations of the three CDK4/6 inhibitors approved by the US Food and Drug Administration (palbociclib, ribociclib, and abemaciclib) lend further insight into their mechanism of action, which will hopefully inform the future use and refinement of these therapies. Finally, we summarize evidence for additional novel CDK4/6 inhibitors currently in development.

1. Background

One in eight women will develop breast cancer, representing an estimated 255,000 new cases in the US in 2017 [1]. Despite screening for early detection and advances in multimodality treatment, breast cancer is responsible for over 40,000 deaths annually (Figure 1) [1]. Despite favorable outcomes for early stage breast cancers, women with metastatic breast cancer have a 5-year overall survival of just 22% [2].

Breast cancer is a heterogeneous disease, and histologic and molecular characterization has clarified major subtypes with important prognostic and predictive value. Breast cancer is classified by hormone receptor (HR) expression, including estrogen receptor (ER) and progesterone receptor, as well as human epidermal growth factor receptor 2 (HER2). In the US, approximately 70% of new breast cancers are ER+/HER2 non-amplified, also categorized as luminal A subtype, while an additional 10% are ER+/HER2+, or luminal B subtype [3]. Fewer than 5% of new breast cancers are ER-/HER2-amplified, while the remaining 15% are triple negative breast cancer, most commonly basal histologic subtype. Targeting these proteins and their downstream growth pathways represents a cornerstone of breast cancer therapy, including aromatase inhibitors, selective ER modulators, and ER antagonists, as well as anti-HER2 monoclonal antibodies. Unfortunately, most patients with advanced disease progress on endocrine therapy within 1 to 2 years [4,5].

Further investigation into the cellular mechanisms driving cancer cell growth and interaction with these hormone-signaling pathways has identified additional therapeutic targets to prolong survival in these patients [6]. In recent years, the advancement of cyclin-dependent kinase (CDK) 4/6 inhibitors palbociclib, ribociclib, and abemaciclib is changing outcomes for patients with advanced or recurrent HR+ breast cancer. These therapies demonstrate encouraging outcomes with limited toxicities, and represent a promising new avenue in breast cancer care.

2. Action of CDKs 4 and 6 in health and disease

Cell division is a tightly regulated cellular process that relies on multiple checkpoints to prevent unrestricted proliferation. Loss of this cell cycle regulation is a hallmark of cancer, and thus these pathways are a primary target of rational therapeutic design [7].

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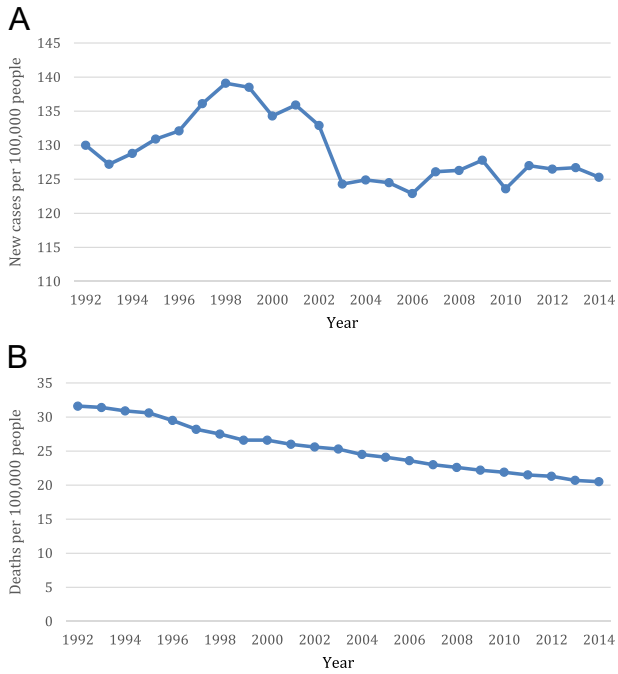

Figure 1. (A) New breast cancer cases in the US, SEER 1992 to 2014. (B) Breast cancer deaths in the US, SEER 1992 to 2014.

CDKs 1–6 play a key role in coordinating cell cycle progression, described here, while CDKs 7, 8, and 9 have downstream effects as transcriptional regulators [8]. Cyclins act as a regulatory subunit to control the kinase activity of CDKs, and when partnered together these complexes initiate signals allowing progression of the cell cycle through phases of first growth (G1), synthesis (S), second growth (G2), and mitosis (M) [9,10] (Figure 2). Three interphase CDKs, cyclin D-specific kinases CDK4 and CDK6 and cyclin E-specific CDK2,

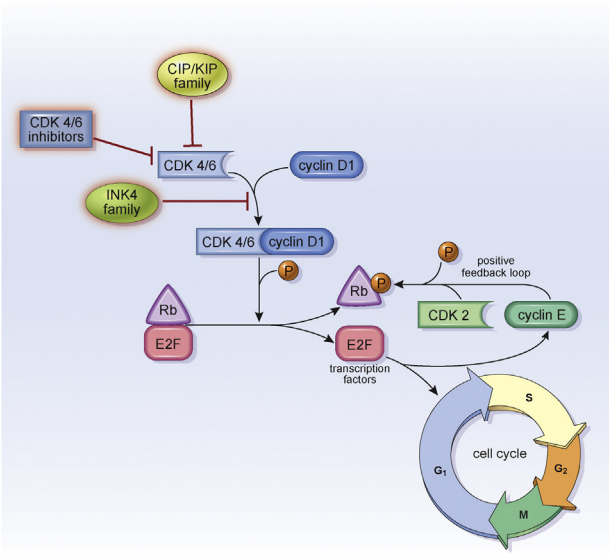

Figure 2. CDK4/6 in the cell cycle. Cyclin D1 binds the catalytic site of CDK4 or CDK6 and serves to activate its kinase function. The cyclin D1-CDK4/6 complex then phosphorylates the Rb protein, releasing its inactivation of G1 to S phase transcription factors, including the E2F family proteins. This disinhibition promotes transcription of gene products essential to cell cycle progression, including CDK2. CDK2 is activated by cyclin E, which further phosphorylates Rb, creating a positive feedback loop to allow progression past the restriction point of the cell cycle and initiation of DNA synthesis (S phase). Cyclin-dependent kinase inhibitors prevent unchecked cell division by blocking CDK4/6 binding to cyclin D1 or kinase activity, and include the CIP/KIP and the INK4 protein families.

sequentially phosphorylate the retinoblastoma (Rb) protein [11]. In its physiologic role, Rb acts as a tumor suppressor by stalling cell cycle progression to S phase, and loss of Rb is associated with tumorigenesis in multiple tumor types [12]. After phosphorylation by the cyclin D1-CDK4/6 complex, the phosphorylated Rb protein (pRb) releases multiple transcription factors integral to the G1- S phase transition, including the E2 family transcription factors (E2F). E2F binds DNA to promote transcription of genes including cyclin E, and thus promotes continued progression through the cell cycle [13] (Figure 2). Following G1 to S phase transition, action of cyclin E-CDK2 complex stimulates preparation for DNA synthesis, and CDK1 is responsible for initiation of mitosis [8]. Negative regulators of the G1 to S phase transition key to preventing unchecked proliferation include tumor suppressors in the INK4 family of proteins (p16, p15, p18, and p19) that specifically bind to CDK4 and CDK6, preventing activation by cyclin D1 and leading to G1 arrest in Rb-proficient cells [14]. On the other hand, cyclin inhibitory proteins (CIPs) or kinase inhibitory proteins (KIPs), including p21 and p27, bind all CDKs to varying degrees, and can be either inhibitory or activating at different levels of expression [15].

In breast cancer and other malignancies, deregulation of the key players in the cyclin D1-CDK4/6-Rb signaling cascade promotes unchecked cell proliferation [15,16]. Evidence suggesting CDK4/6 and cyclin D1 as potential therapeutic targets in breast cancer are summarized in Table 1 [17–25]. *In vitro*, breast cancer cell lines overexpressing cyclin D1 demonstrated increased rates of cell cycle progression, and *in vivo,* transgenic mice overexpressing cyclin D1 have increased rates of mammary cancer [17,18]. Similarly, knock-in mice with constitutively active CDK4 develop multiple tumor types, including breast cancer [20]. The role of cyclin D1 and CDK4/6 in breast cancer tumorigenesis is further supported by resistance to Neu/ErbB2-driven tumors in mice deficient in either cyclin D1 or in CDK4 [19,21–23]. Importantly, loss of function mutation of cyclin D1 had minimal effect on normal mammary growth and development [24]. Furthermore, ablation of cyclin D1 in adult mice with HER2-mediated mammary tumors ceased tumor proliferation and induced senescence, without prominent effects on the overall health of the animal, suggesting it as a potentially more cancer-selective target [25].

The cyclin D1-CDK4/6-Rb axis appears to be most active in ER+ and HER2+ breast cancers. The *CCND1* gene encoding cyclin D1 is amplified in 15% to 20% of all breast cancers, though cyclin D1 protein is overexpressed in more than 50% of cases (Table 2) [26–34]. The overexpression of cyclin D1 is associated with malignant lesions and is less common in benign or premalignant tissues [30], and cyclin D1 protein overexpression has also been associated with ER positivity in breast cancer [31]. Cyclin D1 gene amplification occurs most commonly in ER+ luminal B or ER-/HER2-enriched histologic subtypes, and about 30% of luminal A tumors, while CDK4 gene expression is also increased in these subtypes, albeit to a lesser degree (14%–25%) [32,34]. Furthermore, ER+ and HER2+ cell lines also tend to have functional phosphorylated Rb, whereas basal type breast cancers are more likely to have Rb loss and are driven by alternative pro-mitogenic signals [33–35] (Table 2). Similarly, cyclin E is more commonly overexpressed in basal tumors and is associated with poor prognosis, as compared with the overexpression of cyclin D1 in luminal subtypes [34,36]. In this manner, cyclin E may be an alternate driver of Rb phosphorylation to promote S phase transition, despite inhibition of the cyclin D1-CDK4/6 complex [37].

The majority of breast cancers are driven by hormone signaling, primarily estrogen, which acts as a mitogen to promote progression through the cell cycle at the G1 to S phase transition, and evidence suggests multiple simultaneous pathways converge on the cyclin D1-CDK4/6 axis [38]. Through these signaling cascades, cyclin D1 is responsive to mitogens and growth signals extrinsic to the cell,