Cyclins and Breast Cancer

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Recent advances in the understanding of cell cycle control by cyclins and cyclin-dependent kinases provide a basis for delineating the molecular mechanisms of proliferation control by steroids and the development and progression of hormone-dependent cancers. Cyclin D1 is necessary, rate-limiting and sufficient for G1 progression in breast cancer cells and regulation of cyclin D1 expression or function is an early response to steroid and steroid antagonist regulation of proliferation. The cyclin D1 gene is amplified in ~15%, and its product overexpressed in 40–50%, of primary breast carcinomas. The strong evidence that cyclin D1 plays a major role in cell cycle control in breast epithelial cells suggests that its deregulated expression may have effects on disease progression and phenotype including sensitivity to endocrine therapies.

KEY WORDS: Cyclin D1; cell cycle; estrogen; progestins; steroid antagonists; 11q13 amplification.

INTRODUCTION

Evidence that steroid hormones are intimately involved in the pathogenesis of breast cancer (1) suggests that improved understanding of the steroidal control of cell proliferation may also yield insights into the initiation and progression of this disease. Since the majority of breast carcinomas retain some degree of steroid responsiveness, breast cancer cell lines have provided a frequently used model for addressing the mechanisms underlying control of proliferation by steroid hormones. These studies are given added impetus by the utility of specific antihormonal therapies in the treatment of breast cancer.

Progress through the cell cycle is regulated by the sequential activation of a series of enzymes comprising a cyclin and a cyclin-dependent kinase (CDK). These enzymes are regulated at multiple levels, providing a variety of possible means by which overall activity of the complex, and hence the rate of cell cycle progression, might be modulated. Recent studies have examined the interactions between steroid hormones and steroid antagonists and these components of the cell cycle machinery. These studies suggest that regulation of cyclin/CDK activity, particularly cyclin D1-associated kinase activity, is a universal response to steroidal regulation of proliferation, and in many cases may be a cause rather than merely a consequence of changes in the rate of cell proliferation.

Since cyclin/CDK function is central to cell cycle progression, it is not unexpected that loss of the normal regulation of these molecules has been implicated in oncogenesis. A number of cyclins are aberrantly expressed in human cancers and some evidence for transforming ability has emerged. Cyclin D1 is among the most commonly overexpressed oncogenes in breast cancer. Thus studies assessing the effects of alterations in its expression on cellular function may have major implications for understanding the development and progression of clinical breast cancer. This review summarizes current knowledge of the role of cyclins in breast cancer both in vitro and in vivo, focussing on cyclin D1.
MAMMALIAN CELL CYCLE CONTROL

Transient accumulation of cyclins, the consequent activation of CDKs and phosphorylation of specific substrates regulates passage through the eukaryotic cell cycle. The best characterized mammalian cyclins comprise four classes: A, B, D, and E. Cyclins A, B, and E appear to be universally expressed but the three D-type cyclins, cyclins D1, D2, and D3, are expressed in a cell-type specific manner (2). The functions of the D-type cyclins overlap but increasing evidence suggests that they are not entirely interchangeable (2). Changes in cyclin abundance govern much of the regulation of CDK activity as cells progress through the cell cycle (2, 3). The expression of cyclin D1 is strongly mitogen-inducible but does not oscillate throughout the cell cycle as dramatically as other cyclins (2, 3). Following mitogenic stimulation, cyclin D/Cdk4 or cyclin D/Cdk6 are the first active CDKs detected (2, 3). This is followed by cyclin E/Cdk2 activation near the G1/S phase boundary, cyclin A/Cdc2 activation upon S phase entry and cyclin B/Cdc2 activation during G2 and mitosis (2, 3). This suggests that cyclins D and E have G1 specific roles (Fig. 1).

Microinjection of cyclin D1 antibodies or antisense plasmids leads to cell cycle arrest when performed in early G1 phase cells but not in cells past the G1/S phase boundary, indicating that cyclin D1 is essential during G1 phase (4). Conversely, increased expression of cyclin D1 shortens G1 (2, 5–7). In breast cancer cells cyclin D1 is also sufficient for G1 progression: induction of ectopic cyclin D1 in cells arrested by serum deprivation leads to re-entry into the cell cycle with subsequent DNA synthesis and mitosis (7). The CDKs which associate preferentially with cyclin D1, Cdk4, and Cdk6, display an in vitro substrate preference for the retinoblastoma tumor suppressor protein, pRB (2). pRB is hypophosphorylated during early G1 but is hyperphosphorylated during the remainder of the cell cycle (8). Hypophosphorylated pRB is growth inhibitory, at least in part due to its ability to sequester transcription factors including E2Fs. Upon pRB phosphorylation, release of E2Fs allows the transcription of genes necessary for S phase entry (8). In breast cancer cells induction of cyclin D1 leads to pRB phosphorylation (9), while in serum-stimulated quiescent rodent fibroblasts premature expression of cyclin D1 advances the timing of pRB phosphorylation (10). Cells without functional pRB lose dependence on cyclin D1 for G1 progression but demonstrate an absolute requirement for cyclin D1 upon reintroduction of pRB (11). These data provide compelling evidence for the view that pRB is a critical physiological target for cyclin D1. However, it is likely that Cdk2 also phosphorylates pRB in vivo, perhaps contributing to the further phosphorylation of pRB as cells progress through S phase (Fig. 1) (8).

The catalytic activity of CDKs depends not only on cyclin association but also on appropriate phosphorylation of the CDK subunit (12). The CDKs are regulated post-transcriptionally by reversible phosphorylation at several residues, and the CDK-activating kinase (CAK) is itself a cyclin/CDK complex (cyclin H/Cdk7) regulated by phosphorylation. A further means of regulating cyclin/CDK function is provided by endogenous low molecular weight proteins which physically associate with the cyclins, CDKs or their complexes and inhibit CDK activity (13). A growing family of such inhibitors, for which p16INK4 is the prototype, selectively targets cyclin D-associated kinases. These share function but not structure with a second family of inhibitors, including p21 (WAF1, Cip1, Sdi1) and p27 (Kip1). The mechanism for inhibition of CDK activity is not well defined but recent data show that members of both inhibitor families prevent CAK phosphorylation (14). This does not appear to be mediated by direct interaction with CAK, suggesting that inhibitor binding might block CAK access to its substrate on the CDK.

Figure 1 presents a simplified model of interactions between some of the molecules involved in G1 phase progression. Activation of cyclin D-associated kinases, Cdk2 activation and phosphorylation of pRB are necessary for transit from G1 into S phase. Although it is clear that there are many additional complexities associated with G1 control, this model provides a framework for investigation of mechanisms underlying regulation of cell cycle progression in breast cancer cells.

REGULATION OF CELL CYCLE PROGRESSION AND CDK FUNCTION IN BREAST CANCER CELLS

Early studies of cell cycle control in hormone-responsive breast cancer cell lines showed that these cells are sensitive to growth factors, steroids and steroid antagonists during G1 phase (15, 16). The identification of the molecular targets mediating these cell cycle phase-specific actions, particularly those of ste-