The American Journal of Pathology, Vol. 185, No. 5, May 2015

The American Journal of
PATHOLOGY
ajp.amjpathol.org

REVIEW

Cyclin-Dependent Kinase Inhibitors and the Treatment of Gastrointestinal Cancers

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Accepted for publication January 13, 2015.

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The cell cycle is a highly conserved and tightly regulated biological system that controls cellular proliferation and differentiation. The cell cycle regulatory proteins, which include the cyclins, the cyclin-dependent kinases (CDKs), and the CDK inhibitors, are critical for the proper temporal and spatial regulation of cellular proliferation. Conversely, alterations in cell cycle regulatory proteins, leading to the loss of normal cell-cycle control, are a hallmark of many cancers, including gastrointestinal cancers. Accordingly, overexpression of CDKs and cyclins and by contrast loss of CDK inhibitors, are all linked to gastrointestinal cancers and are often associated with less favorable prognoses and outcomes. Because of the importance that the cell cycle regulatory proteins play in tumorigenesis, currently there is a broad spectrum of cell-cycle inhibitors under development that, as a group, hold promise as effective cancer treatments. In support of this approach to cancer treatment, the growing availability of molecular diagnostics techniques may help in identifying patients who have driving abnormalities in the cell-cycle machinery and are thus more likely to respond to cell-cycle inhibitors. In this review, we discuss the prevalence of cell-cycle abnormalities in patients with gastrointestinal cancers and provide a preclinical and clinical overview of new agents that target cell-cycle abnormalities with a special emphasis on gastrointestinal cancers. *(Am J Pathol 2015, 185: 1185—1197; http://dx.doi.org/10.1016/j.ajpath.2015.01.008)*

Unrestrained proliferation is a hallmark feature of gastrointestinal (GI) cancers.[1] The molecular pathogenesis of GI cancers is linked to oncogene activation such as RAS, dysfunction of tumor suppressor genes such as adenomatous polyposis coli, alternations in DNA repair pathways such as mismatch repair gene abnormalities, and cell-cycle dysregulation such as cyclin-dependent kinase (CDK) 4 overexpression.[1–3] Other events that play integral roles in the development of GI cancers include inflammation and immune dysregulation, and the interaction of these causative factors was recently reviewed.[3] Given the prevalence of cell-cycle abnormalities in GI cancers, there is growing interest in developing and testing inhibitors that target the cell cycle.[1]

Overview of the Mammalian Cell Cycle

The cell cycle is a highly structured and regulated system, composing of multiple regulatory, catalytic, and inhibitory proteins that act to direct normal mammalian cell proliferation and differentiation. It is not surprising, therefore, that the mechanisms that control normal cell division are frequently altered in many diseases, and aberrant cell-cycle control is a hallmark of most cancers.[4] Cell division is divided into two distinct stages, mitosis (M), in which the cell prepares for and undergoes cell division,[5] and interphase, which is further divided into three subphases, G_1, S, and G_2 (Figure 1). All phases of the cell cycle are controlled primarily through the cyclic expression of the regulatory cyclins and their catalytic partners, the CDKs, and inhibited by the CDK inhibitors (CDKis).[4,6] At least nine CDKs are

Supported by a Georgetown-Howard Universities Center for Clinical and Translational Science Pilot and Collaborative Program Clinical and Translational Science Award, US Department of Defense grant W81XWH-13-1-0327, and NIH grants R21 CA182050 and P30 CA51008-18 (Weiner) (C.A.).

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2015.01.008

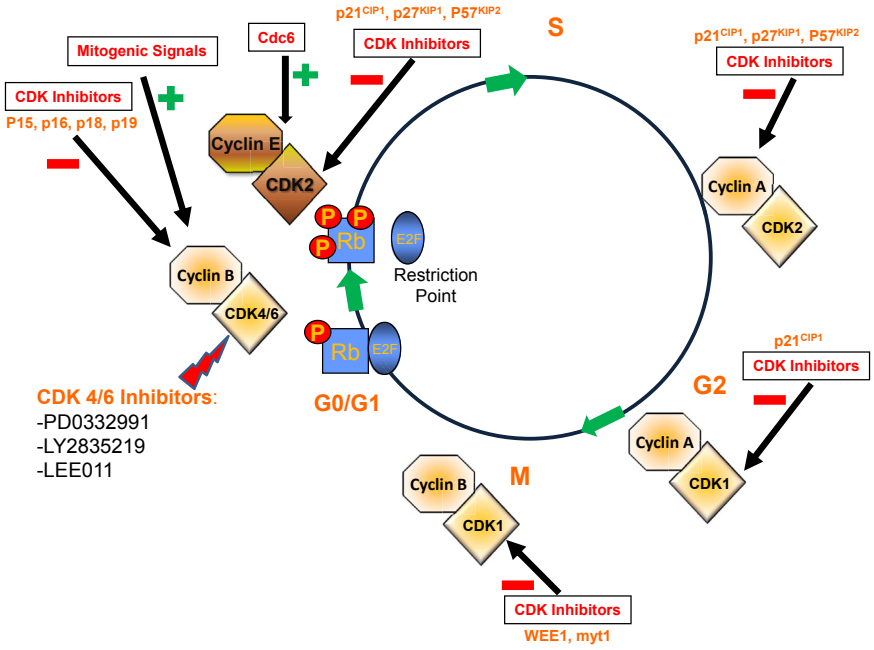

Figure 1 Key regulators of the mammalian cell cycle. The **green plus signs** represent positive regulators of cell cycle progression, whereas the **red minus sign** are cell cycle inhibitory proteins. The yellow P represent phosphorylation events on the Rb. Also shown are three CDK4/6 inhibitors that are currently in various stages of clinical development: PD-033299 (Pfizer, New York, NY), LY2835219 (Eli Lilly, Indianapolis, IN), and LEE011 (Novartis, Basel, Switzerland). CDK, cyclin-dependent kinase; Rb, retinoblastoma protein.

described, although only five of them have defined roles in the cell cycle[6] (Table 1). CDK4/6 associates with the D-type cyclins (D1, D2, and D3) to regulate cell-cycle progression in the G_1 phase.[4,6] Similarly, the cyclin E/CDK2 complex regulates the late G_1 phase and the induction of DNA synthesis in early S phase. CDK2 also associates with cyclin A to control proper DNA replication and synthesis in the S phase.[7] As the cell-cycle progresses, cyclin A then associates with CDK1 to promote cell entry into the M phase. This function is also aided by the activity of CDK7 and cyclin H. CDKis include several families that differ on the basis of their structure and target.[8] Finally, CDK1 and cyclin B function as the key mediators of mitotic entry.[8]

The Cell Cycle

The G_1 or Gap 1 phase was originally described as the period of time that occurred before the onset of DNA synthesis (the S phase).[9] Normal cells deprived of the proper growth conditions arrest in a resting or G_0 part of G_1.[7] On stimulation by mitogenic signals, these quiescent cells begin to progress toward a major G_1 checkpoint termed the restriction (R) point in G_1. The R point is regulated by the retinoblastoma 1 (*Rb1*) tumor suppressor gene. In its hypophosphorylated state Rb binds to members of the E2F family of transcription factors, most notably E2F1.[7] This Rb/E2F interaction suppresses transcription of critical E2F-regulated cell-cycle genes through the recruitment of chromatin remodeling enzymes such as histone deacetylases.[10]

Early G_1 progression is controlled by the D-family of cyclins and their catalytic protein partners, CDK4 or CDK6. Cyclin D (*CCND1*) gene expression and protein amounts are low in quiescent cells and are rapidly induced on stimulation by growth-supportive conditions or through the activity of many oncogenes.[7] Cyclin D then dimerizes with CDK4 or CDK6 to form a catalytically active protein complex. One of the major substrates for the cyclin D/CDK4 or CDK6 complex is Rb. The phosphorylation of Rb by the cyclin D/CDK complex begins the stochastic inactivation of Rb and allows the cell cycle to progress toward the R point of the cell cycle. Once past the R point, cells become committed to entering the S phase. The inactivation of Rb relieves its inhibitory action on the transcription factor E2F, which thereby supports further progression through the cell cycle.[7] E2F directs the synthesis of cyclin E and CDK2 which further inactivates Rb. It is now evident that E2F regulates the expression of a variety of genes that mediate DNA replication, nucleotide biosynthesis, and DNA repair activities such as DNA polymerase α, thymidine kinase, thymidylate synthase, ribonucleotide reductase, and RAD51.[9]

To maintain proper early G_1 regulation, the activity of the cyclin D1/CDK4 or CDK6 complex is antagonized by members of the INK4 family of CDKis. The INK4 family is composed of $p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$, which specifically inhibit the catalytic subunits of CDK4 and CDK6.[9] Similarly, the CDK interacting protein/kinase inhibitory protein (CIP/KIP) family of CDKis ($p21^{Cip1}$, $p27^{Kip1}$, and $p57^{Kip}$) are potent inhibitors of the E- and A-type cyclins and their catalytic partners, CDK2 and CDK1, and to a lesser extent of the CDK1/cyclin B complex in G_2.[3,8] Progression through late G_1 and the induction of DNA synthesis in S phase are induced by the increased cyclin E protein amounts, and the resultant association with CDK2, the primary catalytic partner of cyclin E. The activity of the cyclin E/CDK2 complex is potentiated by