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Review

Cell Cycle and Cancer

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Cancer is frequently considered to be a disease of the cell cycle. As such, it is not surprising that the deregulation of the cell cycle is one of the most frequent alterations during tumor development. Cell cycle progression is a highly-ordered and tightly-regulated process that involves multiple checkpoints that assess extracellular growth signals, cell size, and DNA integrity. Cyclin-dependent kinases (CDKs) and their cyclin partners are positive regulators or accelerators that induce cell cycle progression; whereas, cyclin-dependent kinase inhibitors (CKIs) that act as brakes to stop cell cycle progression in response to regulatory signals are important negative regulators. Cancer originates from the abnormal expression or activation of positive regulators and functional suppression of negative regulators. Therefore, understanding the molecular mechanisms of the deregulation of cell cycle progression in cancer can provide important insights into how normal cells become tumorigenic, as well as how new cancer treatment strategies can be designed.

Keywords: Cancer treatment, CDK, CKI, Deregulation of cell cycle, Tumorigenesis

Introduction

Cancer cells differ from normal cells in many important characteristics. These include the loss of differentiation, self-sufficiency in growth signals, limitless replicative potential, increased invasiveness, and decreased drug sensitivity (Hanahan and Weinberg, 2000). These differences do not arise simply from uncontrolled cellular growth, but rather from a cellular evolution. The increased incidence of cancer as a function of age has long been interpreted to suggest that the progressive acquisition of mutations and epigenetic abnormalities in the expression of multiple genes that have highly diverse functions are required for tumorigenesis. An important group of these genes is involved in cell cycle checkpoints, which are positions of control that ensure the order of events in the cell cycle, and that integrate DNA repair with cell cycle progression.

Cell cycle transition is an ordered, tightly-regulated process that involves multiple checkpoints that assess extracellular growth signals, cell size, and DNA integrity. The somatic cell cycle is divided into four distinct phases (Fig. 1). During two of these phases, the cells execute the basic events in cell division like generation of a single and faithful copy of its genetic material (synthetic or S phase) and partitioning of all the cellular components between the two identical daughter cells (mitosis or M phase). The two other phases of cell cycle represent gap periods (G1 and G2), during which the cells prepare themselves for the successful completion of the S and M phases, respectively. When the cells cease proliferation, due either to specific antimitogenic signals or to the absence of proper mitogenic signaling, then they exit the cycle and enter a non-dividing, quiescent state, known as G0. In addition, the cell cycle may be arrested at the G1 or G2 checkpoints that assess cell size, extracellular growth signals, and DNA integrity.

The molecular analysis of human tumors has shown that cell cycle regulators are frequently mutated in human tumors, which underscores how important the maintenance of cell cycle commitment is in the prevention of human cancer. This review will focus on the abnormalities of the cell cycle control protein and their potential impact on cancer treatment. But, to understand the abnormalities of the cell cycle regulatory protein in cancer, we first need to consider their role in the normal cell cycle.

Control of Cell Cycle Progression

The molecular machinery of the cell cycle (the factors that control the various stages in the progression from G1 to M) has been substantially examined during the past decade

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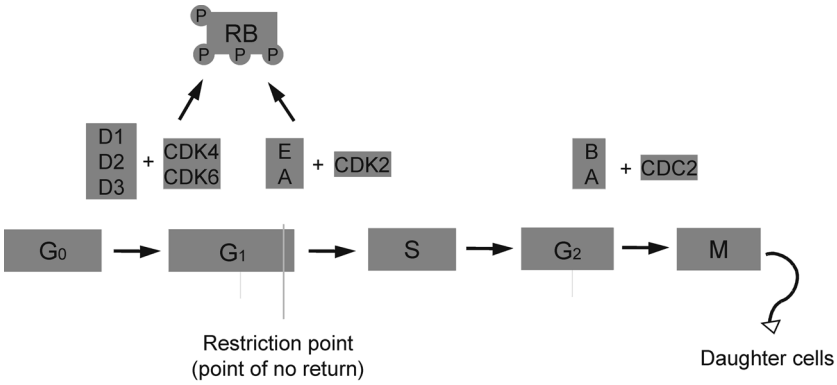

Fig. 1. Progression of cell cycle. Somatic cell cycle consists of four distinct phases: initial growth (G1), DNA synthesis (S), a gap (G2), and mitosis (M). The critical point of cell cycle control is the restriction point. After passing this point, the cell cycle is irreversibly committed to the next cell division.

(Pardee *et al*., 1989; Xiong *et al*., 1991; Sherr *et al*., 1993; Morgan *et al*., 1995; Hwang *et al*., 1998; Zhan *et al*., 1999; Raleigh *et al*., 2000)

The heart of the regulatory apparatus during the cell cycle progression is a family of enzymes, called the cyclin-dependent kinases (CDKs). The active forms of CDKs are a complex of at least two proteins, a kinase and a cyclin (Table 1). They often contain other proteins with poorly understood functions. These complexes undergo changes in the kinase and cyclin components that are believed to drive the cell from one stage of the cell cycle to another (Pardee *et al*., 1989; Xiong *et al*., 1991; Sherr *et al*., 1993; Morgan *et al*., 1995; Hwang *et al*., 1998; Zhan *et al*., 1999; Raleigh *et al*., 2000). According to this paradigm, the cell cycle is determined by the constellation of proteins that are activated or inactivated by phosphorylation, a result of the activity of the CDKs during that stage (Fig. 1). In mammalian cells, a succession of kinase subunits (CDK4, CDK6, CDK2, and CDC2) is expressed along with a succession of cyclins (cyclin D, E, A, and B), as the cells progress from G1 to mitosis. CDK4 and CDK6 complexed with one of several D-type cyclins functions early in the G1 phase, probably in response to growth factors. CDK2 that complexed with cyclin E, cyclin A, or both is essential for the G1 S transition and DNA replication, respectively. CDC2 that complexed with cyclin A and cyclin B is essential for mitosis. Additional CDKs and cyclins will be added to this list (Table 1).

The passage of cells from one stage of the cell cycle to another is tightly regulated by a wealth of controls that act on the transcription of cyclin genes, the degradation of cyclins, and modification of the kinase subunits by phosphorylation. A number of positive or negative feedback loops also contribute to the cell cycle progression (Fig. 2). CDK activity is positively regulated by the association with the cyclins, and by phosphorylation of the T-loop threonine by the CDK-activating kinase (CAK), a serine/threonine kinase that is also involved in transcription and DNA repair (Nigg, 1996). Inhibitory phosphorylation of adjacent threonine and tyrosine residues (T14/Y15 in CDC2) is mediated by dual specific kinases (Wee1 and MYT1). This inhibition is relieved when the CDC25 phosphatases dephosphorylate these residues, which triggers entry into mitosis (Morgan, 1997; Ekholm and Reed, 2000). CDKs and their cyclin partners are positive regulators or accelerators that induce cell cycle progression; whereas, important negative regulators, such as cyclin-dependent kinase inhibitors (CKIs), act as brakes to stop the cell cycle progression in response to regulatory signals (Fig. 2). By direct association with CDK, CKIs can negatively regulate CDK activity. There are two types of CKIs. The four members of the INK family, INK4A (p16), INK4B (p15), INK4C (p18), and INK4D (p19), exert their inhibitory activity by binding to CDK4 and CDK6, and preventing their association with D-type cyclins. The three members of the CIP/KIP family, CIP1 (p21), KIP1 (p27), and KIP2 (p57), form heterotrimeric complexes with the G1/S CDKs. CKIs are induced in response to different cellular processes (Sherr and Roberts, 1999; Sherr, 2000). For instance, in quiescent cells, the KIP1 levels are generally high. CIP1 is one of the effectors of p53, a tumor suppressor that is important in the DNA damage checkpoint.

The critical point of cell cycle control is the restriction

Table 1. Mammalian cyclin-dependent kinases (CDKs) and their regulatory cyclins

CDKs	Cyclins
Cdc2	Cyclin A & B
Cdk2	Cyclin A ,E & D
Cdk3	Cyclin E
Cdk4	Cyclin D1,D2,D3
Cdk5	p35
Cdk6	Cyclin D1,D2,D3
Cdk7	Cyclin H
Cdk8	Cyclin C
Cdk9	Cyclin T

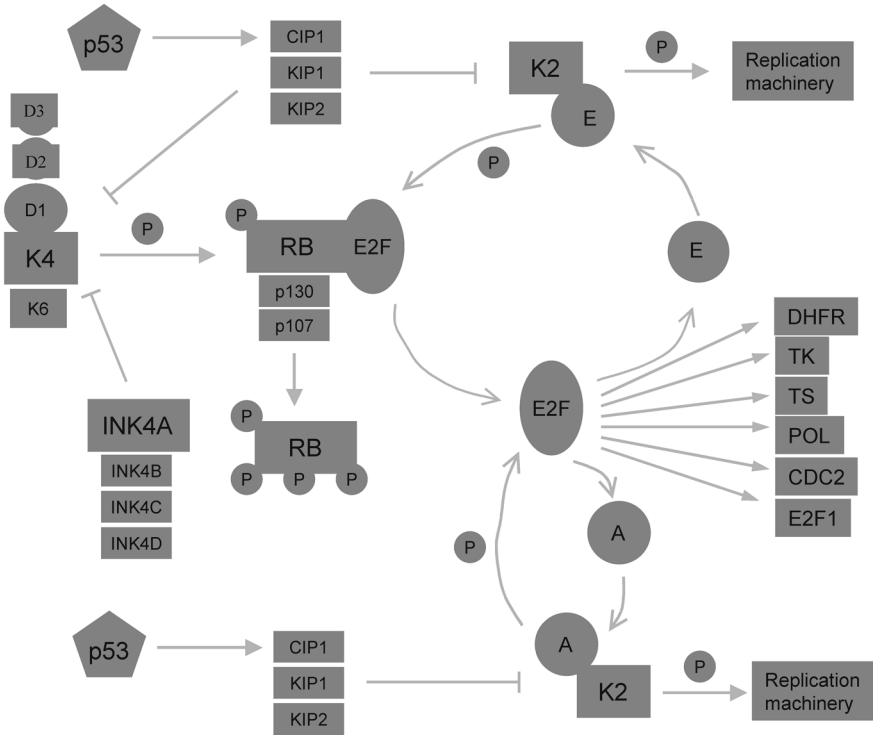

Fig. 2. Regulation of G1/S cell cycle progression. The kinase activity of cyclin D-CDK4/6 and cyclin E-CDK2 complexes are negatively-regulated by two cyclin-dependent kinase inhibitor (CKIs) families the INK4 (inhibitor of CDK4) and CIP/KIP (CDK interacting protein/CDK inhibitor) proteins.

point. After passing this point, the cell is irreversibly committed to the next phase of the cell cycle (Fig. 1). The restriction point control is mediated by the cyclin D and cyclin E-dependent kinases. The primary substrates of CDK4/6 and CDK2 in G1 progression are the members of the retinoblastoma protein family pRB, p107, and p130 (Fig. 2) (Morgan, 1997; Adams, 2001). These molecules function as negative regulators at the restriction point. One important target of pRB for regulating the early G1 cell cycle progression is the E2F family of transcription factors (Harbour and Dean, 2000). E2Fs regulate the expression of a host of genes that mediates both restriction point transversals, such as cyclin E, and the S phase progression, such as dihydrofolate reductase and thymidylate synthase (Fig. 2). Moreover, pRb has some CDK-independent functions, such as the repression of some promoters and RNA pol III activity (Adnane *et al*., 1995; Sellers *et al*., 1995; Weintraub *et al*., 1995; Chow and Dean, 1996; Chow *et al*., 1996; White *et al*., 1996). Therefore, the re-introduction and expression of pRb into tumor cells may arrest growth by mechanisms that are independent of the CDK complex formation.

Abnormalities in the Cell Cycle Control Proteins in Cancer

Abnormalities in Cyclins and CDKs: In recent years, it has become apparent that tumorigenesis is frequently associated with mutations or abnormalities in the expression of various cyclins, CDKs and CKIs in several types of human cancers (Weinstein and Zhou, 1997; Sgambato *et al*., 1998). In 1991, a presumptive oncogene that is composed of the parathyroid hormone gene (PRAD1) that fused to the gene that encodes cyclin D1 was identified in a human parathyroid adenoma (Heichman and Roberts, 1994; King *et al*., 1994). This observation provided the first clue that cyclins might be directly involved in some human cancers. Subsequently, evidence was presented for the involvement of cyclins in other human cancer cells. These included B cell lymphoma and breast, gastric, colon and esophageal carcinomas, as well as several other types of cancers (Hunter and Pines, 1994). Indeed, the increased expression of cyclin D1 is one of the most frequent abnormalities in human cancer, since it occurs in ~60% of breast cancers, 40% of colorectal cancers, 40% of squamous carcinoma of the head and neck, and 20% of prostate cancers (Weinstein *et al*., 1997; Weinstein and Zhou, 1997; Han *et al*., 1998; Sgambato *et al*., 1998). Consistant with these results, cyclin D1 substitutes or partially substitutes for certain oncogenes in the cellular transformation assay (Hinds *et al*., 1994; Lovec *et al*., 1994). The cyclin E gene, which acts in late G1, is also overexpressed and dysregulated in a variety of human cancers (Malumbres and Barbacid, 2001). The amplification and overexpression of CDKs and its regulators have rarely been described in human cancers. Some

Table 2. Alterations of INK4A (p16) in human cancers

Tumor	Type of alteration	Frequency (%)
Melanoma	Mutation	25
Adult T cell leukemia	Deletion	28
T cell acute lymphocytic leukemia	Deletion	59
Oral squamous cell carcinoma	Mutation	9
Gliosarcoma	Deletion	37
Bladder carcinoma	Deletion	20
Upper track urotherial carcoinoma	Deletion	31
Head and neck	Mutation	13

reports demonstrated that the CDK4 gene is overexpressed in certain tumor cell lines (Hunter and Pines, 1994). As with cyclins, however, a general involvement of CDKs in cancer has yet to be demonstrated.

Abnormalities in CKIs

Two families of CKIs have been characterized, based on their specificity. The first is the INK4 family, which includes p15 (INK4B), p16 (INK4A), p18 (INK4C), and p19 (INK4D) (Sherr and Roberts, 1995). The INK4 family of proteins specifically targets the cyclin D-dependent kinases (CDK4 and CDK6) (Sherr and Roberts, 1995; Ruas and Peters, 1998). The INK4A (p16) protein, which was originally identified as a CDK4-interacting protein that inhibits CDK4 kinase activity (Serrano *et al.*, 1993), has been mapped to chromosome 9p21 (Kamb *et al.*, 1994). Due to a hot spot of genomic alterations in cancers, intense studies have focused on the role of INK4A in tumorigenesis. Mutations and deletions of INK4A genes are frequently found in a variety of human malignancies and transformed cells (Table 2) (Kamb *et al.*, 1994; Nobori *et al.*, 1994). These include melanoma, acute lymphocytic leukemia, osteosarcoma, lung, brain, breast, head and neck, bladder, and ovarian cancers. The frequent inactivation of INK4A in these tumors suggests that the loss of INK4A provides a selective cellular growth advantage.

INK4B is encoded immediately adjacent to INK4A at the INK4 locus, 9p21. Its expression is induced in response to transforming-growth factor beta (TGF-β) treatment (Hannon and Beach, 1994). The INK4B sequence is very similar to INK4A, about 70% at the amino acid level. Its nearly identical biochemical behavior, as an inhibitor of CDK4/6, supports the notion that INK4B might be a player in tumor suppression. Although evidence for the tumor suppressor role of INK4B is abundant, the INK4B role in tumor suppression is unclear. No, or rare, point mutations have identified INK4B in tumor cell lines. The majority of homozygous deletions affect either both the INK4A and INK4B loci, or INK4A alone in most tumors (Rocco and Sindransky, 2001). Specific deletions of INK4B have been found in only a few cases of leukemia and lymphomas (Nguyen *et al.*, 2000; Vidal and Koff, 2000; Rocco and Sindransky, 2001). In contrast, the hypermethylation of INK4B seems to be frequent in several cancers (Cameron *et al.*, 1999; Nguyen *et al.*, 2000; Wong *et al.*, 2000; Chim *et al.*, 2001). This suggests that the silencing of the INK4B promoter by methylation plays an important role in tumor development.

The second family of CKIs is the CIP/KIP family, which shares homology at the N-terminal CDK inhibitory domain. This includes p21(CIP1/WAF1), p27 (KIP1), and p57 (KIP2). The $p21^{CIP1}$ binds and inhibits several CDK-cyclin complexes, including CDK2-cyclin E and CDK4-cyclin D1. The $p21^{CIP1}$ expression is directly induced by p53 (Sherr, 1994). This immediately focuses attention on $p21^{CIP1}$ as a potential mediator of p53-dependent tumor suppression. Furthermore, the overexpression of $p21^{CIP1}$ can cause G1 arrest. So far, however, there have been no reports of $p21^{CIP1}$ alterations in tumors or cell lines. If $p21^{CIP1}$ is an important mediator in the p53-dependent tumor suppression, then the mutations of $p21^{CIP1}$ might be expected in some fraction of tumors and cell lines. Therefore, the genetic evidence of $p21^{CIP1}$ as a general tumor suppressor is still undetermined.

The $p27^{KIP1}$ was identified as CDK-binding proteins that are activated by TGF-β, contact inhibition of cell growth (Polyak *et al.*, 1994; Toyoshima and Hunter, 1994). The $p27^{KIP1}$ protein sequence bears some similarities to p21, and also inhibits several CDKs. No mutations in the $p27^{KIP1}$ gene have been reported in tumor and cell lines. However, the reduced expression of $p27^{KIP1}$ is frequently detected in human cancers. These include the breast, prostate, gastric, lung, skin, colon, and ovarian cancers (Catzavelos *et al.*, 1997; Esposito *et al.*, 1997; Mori *et al.*, 1997; Loda *et al.*, 1997; Porter *et al.*, 1997; Cordon-Cardo *et al.*, 1998; Florenes *et al.*, 1998). Surprisingly, however, a relatively high expression of $p27^{KIP1}$ is found in a series of human esophageal cancer cell lines (Doki *et al.*, 1997). Furthermore, several human colon and breast cancer cell lines also express high levels of $p27^{KIP1}$, but low levels in three normal human mammary cell lines. It is also overexpressed in the small-cell carcinomas of the lung, despite their high degree of malignancy (Yatabe *et al.*, 1997). The increased expression of $p27^{KIP1}$ in cancer cells seems paradoxical, because mutations of this gene have not been found or are extremely rare in various cancers (Sgambato *et al.*, 2000). A possible expression for the increase of $p27^{KIP1}$ in some cancer cells is that they have become refractory to the inhibitory effects of this protein. Further studies will be required to establish the role of $p21^{CIP1}$ and $p27^{KIP1}$ in cancer development.

Cell Cycle Control and Cancer Treatment

The frequent loss of cell cycle regulation in human cancer has revealed targets for possible therapeutic intervention. Indeed,

restoring proper restriction point control to cancer cells might allow them to return to a quiescent state. Alternatively, it could take advantage of their uncontrolled proliferation to facilitate apoptotic death, or to specifically exposure cancer cells to cytotoxic treatments (Chen *et al*., 1999).

CDKs are actively being targeted, due to their central role in the control of cell cycle progression. Designing inhibitors that block CDK activity are the most direct and promising strategy. Substantial efforts from many groups have led to the discovery, optimization, and characterization of potent CDK inhibitors. Three properties make CDK inhibitors attractive as anti-tumor agents. First, they are potent anti-proliferative agents, arresting cells in G1 or G2/M (Damiens *et al*., 2000; Soni *et al*., 2001). Second, they trigger apoptosis, alone or in combination with other treatments (Edamatsu *et al*., 2000). Third, in some instances, the inhibition of CDKs contributes to the cell differentiation (Matushansky *et al*., 2000). Only reports on the clinical trials of flavopiridol and UCN01 (7-hydroxystaurosporine) are available, but several other CDK inhibitors are currently being researched.

Potential gene therapeutic strategies are also being established, based on the negative regulators of cell cycle progression (such as INK4A, $p21^{CIP1}$, and $p27^{KIP1}$) to inhibit cell transformation and cancer growth. The use of gene therapy continues to be a promising, yet elusive, alternative for the treatment of cancer. The origins of cancer must be well understood so that the therapeutic gene can be chosen that has the highest chance of successful tumor regression. The gene delivery system must be tailored for optimum transfer of the therapeutic gene to the target tissue. In the near future, new drug compounds and gene therapy protocols will be available. They will help the fight against cancer, since they will broaden our understanding of the cell cycle and cancer.

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