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Minireview

The Role of Cell Cycle Regulators in Normal and Malignant Cell Proliferation

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Cell proliferation is governed by precise and orderly process the regulation of which involves many different proteins. The key enzyme for cell growth and arrest is cyclin dependent kinases (cdks). In human cells, several cdks orchestrate four distinct cell cycle phases (M, G_1, S and G_2) and they sequentially operate in an order of cdc1, cdk4, cdk6 and cdk2. The regulatory components of cdks consist of cyclins and two family of cdk inhibitors, INK4 (inhibitors of cdk4) and KIP (kinase inhibitor protein). G_1 regulatory molecules for cdk mainly respond to environmental cues of mitogenic and anti-mitogenic stimuli and therefore influence activities of G_1 cdks, namely, cdk4/6 and cdk2. G_1 inhibitors include $p21^{CIP}$ and $p27^{KIP1}$. Between them, $p27^{KIP1}$ has attracted attentions of many researchers because of its characteristic regulatory features and diverse functions. Besides, the role of $p27^{KIP1}$ in cancer development warrants further studies in the future. Therefore, this review will focus on the recent findings and especially on the complexity of regulatory mechanisms of $p27^{KIP1}$.

Key Words: Cell cycle, Arrest, cdk, $p27^{KIP1}$, Inhibitor

Binding of cell cycle inhibitors to cdk complexes

$p27^{KIP1}$ was first identified as a key mediator of TGF-β induced G_1 arrest (Polyak, 1994; Reynisdottir et al., 1995). It was found that during the response to TGF-β, $p27^{KIP1}$ moves out of cyclin D1-cdk4-$p27^{KIP1}$ complexes and binds to inhibit cyclin E-cdk2, leading to G_1 arrest. Along with p21, $p27^{KIP1}$ is a well-known inhibitor of cyclin E-cdk2 complexes. But in the case of cyclin D1-cdk4/6, $p27^{KIP1}$ function in assembly and activation of the cdk complex and thus an activator rather than an inhibitor. $p27^{KIP1}$ binding to cyclin D-cdk4/6 therefore may facilitate activation of cyclin E-cdk2 through sequestration of the inhibitory protein. The differential binding of $p27^{KIP1}$ to the distinct early and late cdks during G_1 cell cycle, cdk2 and cdk4/6, can be attributed to the phosphorylation status of $p27^{KIP1}$ (Ciarallo et al., 2002). Altered $p27^{KIP1}$ phosphorylation may thus switch $p27^{KIP1}$ from cyclin E-cdk2 complexes to cyclin D-cdk complexes, allowing resistance to antiproliferative signals. The cyclin E-cdk2 inhibitory activity of $p27^{KIP1}$ is maximal in G_0 and falls as cells move through G_1 into S phase. The cyclin D-cdk4/6 bound $p27^{KIP1}$ is maximal during early G_1. On the stimuli of anti-mitogenic signalling, $p27^{KIP1}$ dissociates from cdk4/6 complexes and accumulates in cyclin E-cdk2. The disrupted antiproliferative role of $p27^{KIP1}$ by its preferred binding status to cyclin D-cdk4/6 over cyclinE-cdk2 may cause human malignancy (Fig. 1).

Posttranslational modification determines the function

$p27^{KIP1}$ is a phosphoprotein and its phosphorylation is cell cycle regulated. Often phosphorylation is a signal for ubiquitination (Carrano, 1999; Montagnoli, 1999; Hara et al., 2001). $p27^{KIP1}$ is phosphorylated on serine by Erk1. This finding raises the question of whether and how phosphorylation by kinases is involved in the process of $p27^{KIP1}$ proteolysis. Cyclin E-cdk2 phosphorylates $p27^{KIP1}$ on Thr187, phosphorylation site of which is required for ubiqutin-dependent phosphorylation. This specific proteolysis

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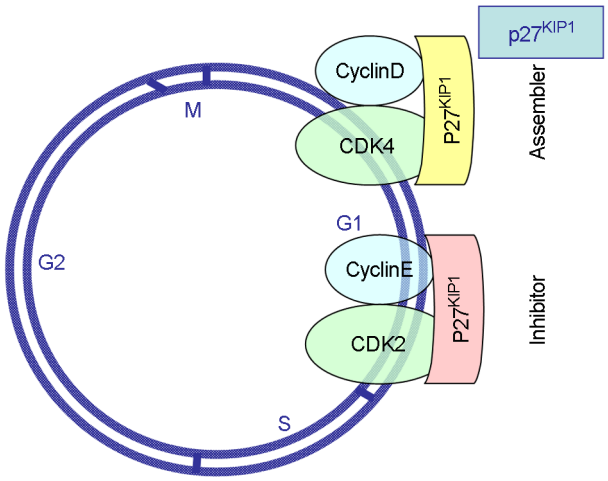

Fig. 1. Differential roles of cell cycle regulator $p27^{KIP1}$ on cdk activities. As several cdks operate four distinct cell cycle phases (M, G_1, S and G_2), their regulatory components of cdks consist of cyclins and cdk inhibitors. The G_1 regulatory molecule, $p27^{KIP1}$, can help and block activities of cdk4 and cdk2, respectively. In early G_1, $p27^{KIP1}$ can help assembly of cdk4 and cyclin D but the later G_1 $p27^{KIP1}$ may become a bona fide cdk inhibitor toward the cyclin E-cdk2 complex. During this transition, $p27^{KIP1}$ undergoes modification of phosphorylation or dephosphorylation by signalling kinases or phosphatases.

of $p27^{KIP1}$ is involved in the pathway of activation of Cdks. Recent findings show that PKB can phosphorylate $p27^{KIP1}$ directly without affecting stability of $p27^{KIP1}$ on Threonine 157, which maps within the nuclear localization signal of $p27^{KIP1}$ (Liang, 2002; Viglietto et al., 2002). The Akt-induced T157 phosphorylation causes retention of $p27^{KIP1}$ in the cytoplasm, precluding $p27^{KIP1}$-induced G_1 arrest. Akt is also shown to down-regulate $p27^{KIP1}$ transcription by phosphorylation-dependent inhibition of the Forkhead family of transcription factors. $p27^{KIP1}$ needs to be transported into the nucleus to exert CDK inhibitory action, phosphorylation at Ser10 was recently reported to increase nuclear export of $p27^{KIP1}$ through binding to CRM1. Therefore, several kinases regulate degradation and cytoplasmic localization of $p27^{KIP1}$ through phosphorylation on various sites.

Localization by phosphorylation status

$p27^{KIP1}$ phosphorylation by PKB impairs its nuclear import and leads to $p27^{KIP1}$ accumulation in the cytoplasm. In human breast cancers, cytoplasmic mislocalization of $p27^{KIP1}$ is associated with PKB activation, loss of differentiation and poor patient outcome. Altered cell cycle regulation and defective checkpoint controls are a characteristic of cancer cells (Liang, 2002; Viglietto et al., 2002). Mislocalization of $p27^{KIP1}$ can also lead to TGF-β resistance and gives a growth advantage during malignant cancer progression. Loss of the antiproliferative effects by $p27^{KIP1}$ mislocalization in cytoplasm during TGF-β induced arrest signal can result in alterations in cell cycle controls. PI3K inhibitors such as wortmannin or LY294002, as well as overexpression of PTEN, result in upregulation of $p27^{KIP1}$ and conversely, $p27^{KIP1}$ is downregulated in the cells lacking PTEN (Kurose et al., 2001). The upregulation of $p27^{KIP1}$ in response to decreased PIP_3 levels occurs by protein stabilization. $P27^{KIP1}$ is destabilized by phosphorylation on T187 and subsequent recognition by ubiqutin-dependent proteasome machinery. Two kinases, CDK2 and Jab1, have been implicated in $p27^{KIP1}$ destabilization and it was suggested that PKB might also be responsible for this destabilization. However, PKB is involved in mislocalization of $p27^{KIP1}$ with its action of destabilization. PI3K inhibitors reduce the lifespan of cells (Fujita et al., 2002). Then, elevated $p27^{KIP1}$ due to inhibition of PI3K signaling contributes to senescence. In the case of human tumors, $p27^{KIP1}$ protein levels are often significantly diminished particularly in their more advanced stages. The downregulation of $p27^{KIP1}$ in tumors occurs principally by protein destabilization, although it remains to be established whether this is a direct reflection of the balance between the activities of PTEN and PI3K. Relation of $p27^{KIP1}$ mislocalization to cancer has been a focus and the biochemical pathways responsible for $p27^{KIP1}$ cytosolic accumulation are under extensive investigations.

Deregulation in cancer

In many cancers, accelerated proteolysis causes reduced $p27^{KIP1}$ protein. Less often, primary tumours may exhibit strong cytoplasmic $p27^{KIP1}$ expression. Cytoplasmic $p27^{KIP1}$ has been observed in some advanced cancer-derived cell lines. Thus, some cancers may express a stable but inactivated $p27^{KIP1}$. Mechanisms of mitogenic of anti-

mitogenic signalling pathways alter $p27^{KIP1}$ inhibitor function are a focus in the field. Ras activation can lead to activation of PKB. PKB can increase cyclin D1 levels and down-regulate $p27^{KIP1}$ expression. Thus, activation of the PI3K/PKB pathway may play a key role in loss of responsiveness to antiproliferative signals and cancer progression *in vivo* at least in part through impaired $p27^{KIP1}$ localization and function. PKB mediates resistance to IL-6 or TGF-mediated G_1 arrest (Yue, 1999; Hideshima et al., 2001). The balance between PTEN and PI3K activities controls the levels of PIP_3, which serves as anchor site for proteins containing the pleckstrin-homology (PH) domain. The downstream target is the PH-containing Ser/Thr-kinases PDK-1 and PKB. Loss of PTEN occurs in a variety of human cancers and, its inactivation can lead to loss of cell cycle regulation (Li and Sun, 1998). PI3-kinase has many components and mutations in those components can cause dangerous human cancers. In c-Myc or MAPK activated cancer-derived lines, cyclin D1-cdk4/6 complexes sequester $p27^{KIP1}$, the cyclin E-cdk2-inhibitory action of $p27^{KIP1}$ is impaired. c-Myc inhibits p15 induction by TGF-β and may also induce a factor that inactivates $p27^{KIP1}$ (Sandhu, 2000; Gstaiger et al., 2001). Up to 50% of human cancers show loss of $p27^{KIP1}$ through accelerated $p27^{KIP1}$ proteolysis. In certain primary tumors, $p27^{KIP1}$ is mislocalized to the cytoplasm away from nuclear targets and PKB actvation might be responsible. A link between Ras and the cdk inhibitor $p27^{KIP1}$, where Ras causes down-regulation of $p27^{KIP1}$ expression, has also been observed in a variety of cell types (Sheng et al., 2001). Down regulation of $p27^{KIP1}$ levels in response to mitogenic stimuli in cancers are via a Ras-dependent mechanism. Mitogen activation of Ras and Ras-mediated downregulation of $p27^{KIP1}$ in late G_1 involves both suppression of protein synthesis and enhancement of protein degradation in NIH 3T3 cells. Inhibition of PI3K, but not Erk, was found to block growth factor-induced downregulation of $p27^{KIP1}$. Ras also regulates $p27^{KIP1}$ function by modulating its association with different cdk-cyclin complexes. There is a report that induction of activated MEK does not cause downregulation of $p27^{KIP1}$ while it promotes the sequestration of $p27^{KIP1}$ by cyclin D1 (Cheng et al., 1998). Another example is that the inducible activation of estrogen receptor fusion proteins of Raf-1 or MEK1 caused downregulation of $p27^{KIP1}$ protein levels in NIH 3T3 cells (Villalonga et al., 2000). Both PKB and Erk can phosphorylate $p27^{KIP1}$ and phosphorylated $p27^{KIP1}$ is impaired in binding to cdk2 (Lee et al., 2009).

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