Cdk1 and Cdk2 complexes (cyclin dependent kinases) in apoptosis: a role beyond the cell cycle

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Abstract
The family of cyclin-dependent kinase complexes (Cdks) are well known for their role in the cell division cycle. What is less well known, however, is that Cdks also participate in a subset of apoptosis programs. Evidence for the role of Cdks in apoptosis comes from a variety of experimental approaches, including studies using genetic mutants, protein inhibitors, and chemical inhibitors of protein kinase activity. The precise role of Cdks in apoptosis remains to be defined, although one promising approach to clarify this question is to identify Cdk protein substrates during apoptosis. Currently a number of Cdk inhibitors are being tested in clinical trials By understanding how Cdks function during apoptosis it may be possible to optimise the use of these inhibitors in treating human tumours by blocking proliferation but permitting apoptosis.

Keywords
Cyclin, Cdk, kinase, roscovitine, CYC202, flavopiridol

1. Introduction

The role of cyclin-dependent kinase complexes, Cdk1 and Cdk2, in the cell division cycle is well accepted based on compelling data from genetic and biochemical approaches. A role for Cdks in apoptosis has been less forthcoming, and it seems likely that Cdks might not be universally required for apoptosis in the same way that Cdks are universally required for the cell cycle in all eukaryotic species studied. In this review, different examples of Cdk1 activation during apoptosis and it relevance to cancer models are discussed. Despite the absence of a common model of Cdk participation in apoptosis, there are compelling experiment data that suggest that Cdks activity is required in a subset of apoptosis programs.

Cyclin dependent kinase complexes are composed of two proteins, one from the cyclin protein family and another from the Cdk1 catalytic subunit family [1]. Pairing between these two family members is specific, leading to distinct complexes composed of cyclin B1 and Cdk1, or cyclin E1 and Cdk2. With approximately 30 cyclins and 9 Cdks, the complete range of Cdk catalytic subunit and cyclin pairing is not yet fully described.

The majority of Cdk complexes play a role in the cell division cycle. Indeed, cyclin B was first identified because of its characteristic synthesis and destruction at each mitosis in fertilized sea urchin eggs [2]. Its partner, Cdk1 (also known as Cdc2) was originally identified by analysis of fission yeast with mutations in the Cdc2 gene, which arrest in late G2-phase of the cell cycle [3]. Other cyclins, such as cyclin D members and cyclin E members are important in the G1/S-phase transitions of the cell cycle, whereas cyclin A family members are required both in S-phase and in M-phase. The cell cycle can be defined by the activity of the Cdk subunits. Cdk1 is specifically required for mitosis, whereas Cdk2 and Cdk4 are required during G1/S-phase and pair accordingly with G1/S-phase cyclins. Other Cdk subunits such as Cdk5 and Cdk9 have roles outside of the cell cycle.

2. Cdk complexes in apoptosis

Cdk1 activity during apoptosis was first identified in experiments in which YAC lymphoma cells were treated with death inducing compounds, fragmentin-2 and perforin [4]. Co-incubations with these two agents in vitro mimics the cellular events of the release of granule serine proteases that naturally triggers apoptosis. After treatment, Cdk1 complexes were recovered by immunoprecipitation and tested for kinase activity. Within 15 minutes of stimulation, an increase of Cdk1 kinase activity could be detected which approached levels of that found in cells that blocked in mitosis, suggesting that a significant portion of the Cdk1 complexes were being activated. Analysis of the phosphorylation status of Cdk1 on tyrosine residue Y15 revealed that the loss of phosphate on this site corresponded with the increase in activity, suggesting that Cdk1 was being activated by the WEE1/Cdc25 pathway that is also operative during G2/M-phase transition. Further evidence for the role of Cdk1 in apoptosis came from studies in which the FT210 cultured cell line, which harbours a temperature sensitive mutation in the Cdc2 gene, was also tested under similar conditions to that of the YAC lymphoma cell line. At permissive temperature, these cells were sensitive to perforin and fragmentin-2 treatment, whereas at the restrictive temperature, where the Cdc2 protein is inactivated, only 25 % of the cells entered apoptosis. These results supported the initial observation that Cdk1 is required for apoptosis.

One of the key steps in Cdk1 or in Cdk2 activation is binding of a Cdk subunit to its cyclin partner. Once the two proteins have formed a complex, the activity of the complex is held in check by a regulatory system that includes the WEE1 protein kinase, Cdc25 protein phosphatase and protease pathways [5]. It stands to reason then, that Cdk1 activation during apoptosis can only occur in cells that have already expressed either members of the cyclin A or of the cyclin B families of proteins. Shi and colleagues examined the specific Cdk1 kinase activities in Jurkatt cells that were programmed to enter apoptosis after treatment with

granzyme B [6]. Cells were first separated by centrifugal elution in order to test whether or not the cell cycle phase played a role in sensitivity to apoptosis. Tests for apoptosis showed that the cell cycle phase had little role in terms of sensitivity to apoptosis because the percentage of apoptotic cells varied not more that 15% between cell cycle phase, although the highest percentage was consistently found with cells in G2/M-phase. In this model system, the increase in Cdk1 kinase activity was associated with cyclin A/Cdk1 complexes, rather than cyclin B/Cdk1 complexes. These results showed that it is possible for cells to enter apoptosis at different phases of the cell cycle. In addition, the increase in activity found in cells undergoing apoptosis was associated with cyclin A complexes, which are more broadly expressed than cyclin B complexes. However, the expression levels of cyclin A or cyclin B were not directly examined.

3. Different experimental approaches have been used to test the role of Cdks in apoptosis

Whereas as there are limitations with any single experimental approach to address Cdk function in apoptosis, often results from several different approaches converge to permit the same conclusion. A role for Cdks in apoptosis has been supported by date from experiments that use both chemical and protein inhibitors as well as genetic approaches.

Cdk complexes and their role in apoptosis have also been tested in experiments in which cells express forms of the Cdk catalytic subunit that are inactive, known as dominant negative, even after binding to a cyclin partner. Harvey and colleagues examined Cdk2 activity in HeLa cells that entered apoptosis after treatment with staurosporine [7]. After expression of Cdk2D145N, a variant that is catalytically inactive [8], they noted that a reduced the number of cells that display condensed chromatin after treatment with staurosporine. In a complementary experiment, cells that expressed a dominant negative form of Cdk1 did not show a block in the apoptotic pathway, suggesting a specific role for Cdk2 in this system. Only some of the cellular events were blocked by Cdk2dn expression, such as chromatin condensation, cell rounding and loss of adherence, whereas caspase activation and annexin V binding were not changed in these cells. In this experimental model, the authors placed Cdk2 activity downstream to caspase activation, but necessary for completion of the apoptotic program. Dominant negative forms of Cdks including Cdk1, Cdk3, Cdk4, Cdk5 and Cdk6 have also been shown to block apoptosis in other experimental models [9-11].

During the cell cycle, Cdks are regulated in part by small proteins that interact directly with Cdk complexes [12]. Members of these functionally related proteins include p16ink, p21cip, and p27kip. In some cases, the loss of the gene encoding small protein inhibitors such as p16 is linked to a loss of control of the cell cycle and increase in susceptibility to tumour progression.

In the human gastric tumour model system, treatment of cells with TGF-B1 induces apoptosis starting at 6 to 12 hours, as detected by DNA degradation [13]. During the apoptotic program, p21 and p27 proteins are degraded by caspase 3, and this proteolysis can be blocked by incubation with caspase inhibitors. The degradation of p21 and p27 correlates with the increase in Cdk2 activity, which is also reduced in the presence of caspase inhibitors, presumably because of the forced presence of p21 and p27. These results suggest that small protein inhibitors of Cdks have a role in apoptosis, which is in addition to their role in regulating the cell cycle. Further evidence for a role of p21 and p27 in apoptosis has been reported in a liver injury model in rats [14] and evidence for a role of p16 in a cultured cell model in which p16 -/- U2OS cells are more sensitive to apoptosis than isogenic wild type cells [15].

A complementary approach to inhibiting apoptosis by genetic methods is the reduction of Cdk activity and inhibiting apoptosis by small chemical inhibitors. When both of

these methods give the same result in an experimental model, it strongly supports the hypothesis that Cdks play a role in apoptosis. Treatment of cells with small molecule inhibitors of Cdks such as roscovitine and related derivatives result in a reduction of the number of cells that enter apoptosis. Although one must always be conscious that the specific target of a chemical inhibitor might not be the presumed target, there are a number of experimental approaches available that can strengthen the interpretation of any chemical inhibition experiment.

In one example, roscovitine, an isopropylpurine derivative that inhibits Cdk 1 and Cdk2 reduces apoptosis in human hepatoma cell lines. Hsu and colleagues used an experimental model in which the p53 negative cell line Hep3B and p53 wild type cell line HepG2 were treated with CD437, which induces a S-phase cell cycle block followed by apoptosis [16]. The increase in the number of cells that entered apoptosis correlated with an increase in expression of cyclin B and cyclin A protein levels, as well as increases in associated protein kinase activity. Once cells were induced to enter apoptosis by CD437 treatment, they were then treated with two structurally related Cdk inhibitors roscovitine, olomoucine, and an inactive isomer of olomoucine, iso-olomoucine [17]. Cells that were treated with either roscovitine or olomoucine were more frequently viable (resistant to apoptosis induced by CD437) as compared to non treated cells, and as compared to cells treated with the inactive compound iso-olomoucine. These results suggest that Cdks are activated during apoptosis and that their activity is essential for completion of apoptosis.

Similar results have been reported in other experimental systems using roscovitine and other Cdk inhibitors. In a model using cultured cortical neurons treated with proteasome inhibitors, subsequent treatment with flavopiridol, Cdk inhibitor that is chemical distinct from roscovitine, protects cells from apoptosis [10]. Cells from leukemic models can also be protected from apoptosis when treated with roscovitine [11]. In an example using HL60 human promyelocytic leukaemia cells, analysis of cyclin B1-Cdk1 complex during apoptosis showed an increase in Cdk1 kinase activity shortly after treatment with a variety of apoptosis inducing agents [18]. Other cell lines in which Cdk inhibitors have been used to block apoptosis include FaO hepatoma cells [19] and HUT78 cell lines [20]. Additional support for the specificity of inhibition of Cdks by chemical approaches comes from studies in which apoptosis can be inhibited by compounds that are not structurally related, yet still inhibit Cdks when tested in vitro. In addition to the examples cited above, another compound, purvalanol, which is structurally related, yet distinct from roscovitine, can also protect cells from apoptosis under certain conditions [21].

4. Cdk inhibitors can also induce apoptosis

Although at first view it may seem contradictory the results described above, there are several reports that describe experimental conditions in which Cdk inhibitors induce apoptosis, rather than protect cells from apoptosis. Understanding the molecular mechanisms by which this occurs is less obvious than in examples by which Cdk inhibitors block apoptosis. One reason for this, is that many studies have been done in which complementary approaches that all block Cdk activity give the same result. For example, Cdk activity can be reduced by non-chemical means, such as use of dominant negative cDNAs that encode Cdk1 or other Cdks. These non-chemical approaches have not been used as frequently in studies that address Cdk inhibition in promoting apoptosis, therefore the interpretation of the mechanism of the result more difficult than the interpretation of experiments in which multiple methods were used. Nonetheless, even if the mechanism might not be understood, under certain conditions, Cdk inhibitors induce apoptosis.

Hahntow and colleagues examined the effects of roscovitine treatment of leukemic cells taken from patients with chronic lymphocytic leukaemia [22]. Cells were isolated from

patients, and then treated with increasing concentrations of roscovitine and examined for apoptosis. At 10 µM concentration, which is a concentration obtainable in plasma, the number of viable cells decreased from 72.4 % to 46.9% and continued to decrease with increasing concentrations of roscovitine. Normal B cells and PBMNCs were not sensitive to roscovitine, suggesting that cancer cells might be more sensitive to a Cdk inhibitor. Under these conditions, it was concluded that roscovitine induced apoptosis because the reduction in viability could be avoided by co-incubation with zVAD.fmk, a caspase inhibitor, indicating that caspases participate in Cdk inhibitor induced cell death. Besides an increase in annexin V staining, incubation with roscovitine also induced an increase in the protein levels of Bak and a low molecular weight form of Bax, two members of the pro-apoptotic family of Bcl-like proteins.

Consistent with results that have been reported in examples in which Cdk inhibitors protect from apoptosis, compounds from different chemical families also drive cells into apoptosis. In experiments using cultured cell lines from head and neck cancers, roscovitine induces apoptosis in 11/11 cell lines tested. The parameters that were measured included a reduction in protein kinase activity after treatment with roscovitine, generation of cells with subG1 quantity of DNA and direct visualisation of fragmented DNA. The authors proposed that the cellular response to roscovitine includes an increase in the expression of members to the BCL protein family. The authors went on to use similar approaches in testing flavopiridol in oral squamous cell carcinoma [23]. Flavopiridol, a synthetic flavone, also induced cells to enter apoptosis in this model. One striking result from this study was that the protein levels of cyclin A and cyclin B decreased during the time of treatment. Thus the inhibition of Cdks eventually led to the absence of certain Cdk complexes, which suggests that Cdks that require either of these cyclins do not participate in this type of apoptosis, or that the complete shutdown of Cdk activity via the absence of cyclins induces apoptosis.

A novel Cdk inhibitor, SU9516, a member of the indolinone chemical family that inhibits Cdk1 and Cdk2 at 40 and 22 nM respectively, is another example of a Cdk inhibitor that induces apoptosis [24]. The authors tested the compound in RKO colon cancer cells and found a dose dependent and time dependent increase in apoptosis. The reduction in Cdk2 activity was determined by a reduction in Rb protein phosphorylation, which is a substrate of Cdk2/cyclin E complexes. These result suggests that indeed, Cdk2 activity is reduced in cells by this compound and that the reduction in Rb activity might be related to entry into apoptosis.

The conflicting role of Cdks in either protecting cells from apoptosis or inducing apoptosis can be perhaps modelled in certain experimental conditions. Park and colleagues used the neural PC12 cell line and tested the effect of olomoucine and flavopiridol upon apoptosis when the cells were either differentiated or proliferation competent [25]. They reported that PC12 cells in proliferation were not protected from apoptosis by Cdk inhibitor, and in fact, inhibitors promoted apoptosis. By contrast, when cells were cultured under conditions so that they differentiated (post-mitotic), flavopiridol and olomoucine were able to protect cells from conditions that would lead to apoptosis. Based on these results, it appears that the cellular context is a key element in determining whether or not apoptosis signals require Cdk activity.

5. Cdks in apoptosis and mitosis

The Cdk1-cyclin B complex is absolutely required for entry into mitosis in all species studied. During mitosis, the subcellular organisation of a cell changes dramatically as the nuclear envelope disassembles, chromosomes condense, and a mitotic spindle separates sister chromatids to opposite poles of the cell before cytokinesis. Many of these events can be traced to the increase in the specific activity of Cdk1-cyclin B complex and phosphorylation

of protein substrates such as nucleolin, histones H1 and H3, motor proteins (Eg5), and the anaphase promoting complex (APC). The changes in the nuclear organisation that superficially resemble each other during apoptosis or mitosis, coupled with the measured increases in Cdk1 activity under some conditions have lead some laboratories to propose that apoptosis is a failed mitosis (mitotic failure) and hence it is logical that Cdk activity increase during apoptosis because cells are entering mitosis before dying. Discussions of the subject are made more complicated by a different cellular event known as mitotic catastrophe. Therefore, a clear understanding of the role of Cdks in apoptosis is hampered by unclear definitions of cellular events that can lead to different outcomes such as cell death and cell division (for review see [26].

Mitotic catastrophe can be induced experimentally by treating cells with agents that bypass DNA damage checkpoints or by manipulating key cell cycle genes. Premature chromosome condensation, which can lead to mitotic catastrophe, can be induced by fusing cells that are in mitosis with cells that are in S-phase. One of the first reports of premature chromosome condensation came from studies in which mitotic HeLa cells were fused with cells that were in one of the three other phases of the cell cycle (G1, S, or G2 phases) [27]. Under these experimental conditions, chromosome were formed, and, the results eventually led to the discovery of maturation promoting factor (MPF) which is partly composed of the Cdk1-cyclin B1 complex. Mitotic catastrophe can also be induced by genetic manipulation of key enzymes that regulate the cell division cycle, such as ablation of Cdc25 gene in yeast [28]. The interpretation of entry into mitosis as compared to entry into apoptosis lies also within the definition of condensed chromosomes, which are distinct from condensed chromatin. Chromosomes are easy to visualise by microscopy and they form only during mitosis. Chromatin condenses during apoptosis and it is difficult to visualise because the DNA is not organised, yet, nuclei appear differently in cells that are in apoptosis when compared to cells that are not. The confusion in defining condensed chromosomes or condensed chromatin has led to the conclusion that a failed mitosis precedes apoptosis when Cdks are activated.

One example in which cells must pass through mitosis before entering apoptosis can be found in an experimental model in which HeLa cells that are modified to express viral proteins are fused and then treated with compounds that block DNA damage checkpoint response. Under these conditions, Cdk activity is elevated, the cell then enter mitosis and die (mitotic catastrophe and apoptosis) [29]. In this model, condensed chromosomes can be seen by microscopy, thus confirming that cells enter mitosis. Apoptosis is mediated by caspase 2, and further treatment of cells with caspase inhibitors (Z-VAS.fmk) blocks cells from apoptosis and permits cytokinesis. From these data, the authors propose a role for checkpoint kinase 2 (Chk2) in generating aneuploid cells as well as a molecular mechanism that links mitotic catastrophe to apoptosis. It remains to be shown that this mechanism exists outside of these experiment conditions.

In the absence of data on whether or not chromosomes are formed in cells that are in an apoptosis program, it remains unclear if cells must enter mitosis to enter apoptosis. The specific events for which Cdks are required in order for cells to enter apoptosis are currently not known. It stands to reason that one approach to clarify the role of Cdks in apoptosis, outside of a mitotic catastrophe model, would be to identify the proteins that are phosphorylated during apoptosis. The identification of these substrates and the pathways in which they participate might help link Cdk activity to that of apoptosis in the same manner in which the identification of the phosphorylation of nuclear lamins led to the link of nuclear membrane disassembly to Cdk1 [30].

One substrate of Cdk1 during apoptosis has been identified in an experimental model studying post-mitotic neurons in rat cerebella [31]. In a first step, the expression of Cdk1

(Cdc2) was confirmed by immunofluorescence detection with specific Cdk1 antibodies in rat brain slices. When brain slices were treated with low KCl solutions to induce apoptosis, Cdk1 expression and activity levels increased, even though the cells were not dividing. Similar to results described above in experiments using cultured cells, the Cdk inhibitor roscovitine blocked the induction of apoptosis. This supported the notion that Cdk1 activity was required for apoptosis. The authors then examined the phosphorylation status of potential Cdk phosphorylation sites in the proapoptotic protein BAD. They characterised serine 128 (S128) of BAD as potential Cdk1 phosphorylation site in apoptotic cells. In further experiments to test the significance of BAD phosphorylation, they reported that the phosphorylation status of BAD is important for its interaction with14-3-3 proteins, which sequesters it away from its targets at the mitochondria [32]. When BAD is phosphorylated on S128, it disrupts its interaction with 14-3-3 proteins and permits the interaction with protein partners at the mitochondrial surface.

This work lead to a number of important questions about the physiological role of Cdks in apoptosis [33]. For example, it appears that Cdk1 expression can be induced in cells that are outside of the cell cycle, suggesting that it may have a specific role in apoptosis. The expression level of cyclins was not determined in this study, in which it would be interesting to note if and how cyclin expression was also regulated. It would be also be important to know also if the phosphorylation of “apoptotic” substrates occurs during a normal cell cycle, or if there is a level of regulation that separates these two signalling pathways. For example, during mitosis, is BAD phosphorylated or does phosphorylation only occur during apoptosis? In many models of neuronal apoptosis, both developmental and injury dependent, activation of Cdks has been reported [33]. In some cases, Cdk activation is believed to be linked to cell cycle entry but not proliferation because cell number does not increase and cells appear to die before entering mitosis. Although this has been named “aberrant cell cycle entry”, it remains to be formally shown if the cell cycle is really engaged, or if apoptosis is engaged using enzymes that are typically assigned to the cell cycle. Further characterisation of substrates will likely aid in resolving this question.

6. Clinical relevance of apoptosis and Cdk inhibitors

Understanding the links between Cdk inhibitors and induction or prevention of apoptosis is important because Cdk inhibitors are currently being tested in clinical trials as anticancer agents. The development of compounds such as roscovitine, flavopiridol and SU9516, have been motivated by the goal to reduce the proliferation rate of tumours by stopping the cell cycle via the inhibition of Cdk1 and Cdk2. In tests in vitro, roscovitine (known as CYC202 in clinical trials) reduced the proliferation index in 19 different cultured cells that have been isolated from different tumour types [34]. The compound was also active in animal models bearing Lovo human colorectal tumours and in models with human uterine xenografts. In tests of non-proliferating cells and animal MTD tests, the compound was tolerated orally at very high concentrations. Currently, roscovitine/CYC202 is being tested by the biotechnology company Cyclacel in phase II trials.

If data from in vitro cellular models are correct, then it would appear that under certain condition Cdks are also required for cells to engage apoptosis. In terms of clinical relevance, this property of Cdk activity is essential for treating tumours because it is necessary that tumour cells die during treatment in order to reduce tumour burden. It is possible then, that indiscriminate use of Cdk inhibitors might in fact protect certain tumours cells from apoptosis rather than causing them to die. Currently, this question remains hypothetical because data are lacking or incomplete from animal models and from ongoing treatments in clinical trials. In addition, the role of Cdks in apoptosis in cellular models is still poorly understood because one cannot yet predict under which conditions and why Cdks are required for apoptosis.

It will be difficult to understand how Cdk inhibitors function in man unless the question is tested directly with biomarkers for cell division and for apoptosis during clinical trials. In the absence of direct evidence, the measure of Cdk inhibitor activity will reflect more clinical benefit (complete or partial response in patients) versus potential toxicity, rather than a measure of apoptosis induction. Some evidence from animal models suggests that added benefit might be possible in the clinic when Cdk inhibitors are used in combination with cytotoxic treatments. Abal et colleagues [35] have shown that a combination of roscovitine (CYC202) with a topoisomerase I inhibitor (camptothecin) shows greater activity in a human carcinoma model in vivo as compared to when either compound is used alone. The reason for choosing a Cdk inhibitor in combination with camptothecin came from tests in cell based assays in which they remarked that Cdk1 expression increases when cultured cells are cultivated with camptothecin. Based on their results in vitro and in vivo, they propose the use of combination therapy for cancer treatment in man.

The examination of cyclin B1 protein levels in samples taken from human tumours suggests that cyclin expression not always be related to cell cycle phase. In two studies, the protein levels of cyclin B were compared to the DNA content of cells in order to identify the position of the cell cycle [36, 37]. By flow cytometry and by Western blotting analysis, it was found the cyclin B1 protein was present in cells in G1 phase, which is surprising because cyclin B1 is normally expressed in cells in G2 or in M-phase. This observation was confirmed in samples taken from different tumour types including breast cancer and lymphocytes isolated from leukaemia patients. Additional studies by confocal microscopy were used to report that cyclin B1 was expressed in the nucleus, which is the location for activated forms of the Cdk1/cyclin B1 complexes in cells in mitosis [38, 39] and in cultured cells in apoptosis [40]. In the case of the samples from human tumours, however, the activity of the Cdk complexes were not studied either directly or indirectly by examination of the phosphorylation status of Cdk1 substrates. In addition, the cellular status in terms of apoptosis was not reported. Despite that the significance of the expression of cyclin B1 outside of its normal cell cycle phase was not examined, this observation, taken from human tumours, shows that our understanding of cyclin expression is not complete.

7. Perspectives and conclusions

The role of cyclins in the cell division cycle have been well characterised biochemically and genetically. Their role in apoptosis is less clear, although there are ample experimental data that link Cdk activity to apoptosis. A key different between Cdk activity in apoptosis and Cdk activity in the cell cycle is that there are many examples of apoptosis without changes in Cdk activity, whereas during the cell cycle, Cdk1 activity is essential under any condition tested and in all eukaryotic species. Therefore, Cdks do not have a universal role in apoptosis as they do in the cell division cycle.

Experimentally it might be possible to use new mouse models to better understand how and if Cdks participate in apoptosis. Mice that are null homologous for some of the genes that have been described here such as Cdk2 [41] and BAD, a potential Cdk substrate during apoptosis [33] have been recently generated. The normal development and behaviour of these mice suggest that Cdk2 complex proteins and Cdk1 substrates are not required for homeostatic apoptosis. The role of pathological apoptosis in these mice has yet to be reported.

As Cdk inhibitors go into clinical trials for cancer treatment, it becomes increasingly important to understand how these inhibitors function to ensure that they are used to cause maximum tumour cell death and not, eventually, protect cells from death. Data from experiments in cellular systems, although removed from clinical samples, suggest that cyclin expression is linked to apoptosis under certain conditions. If we were to understand the

conditions in which Cdks were required for apoptosis, it might be possible to use Cdks inhibitor to an advantage in the clinic.

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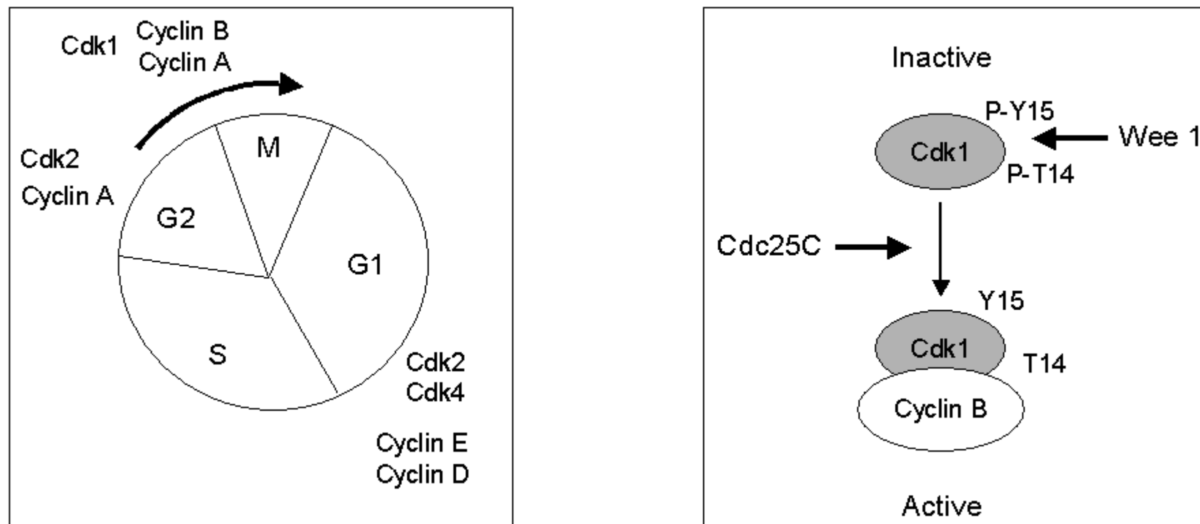

Figure 1. Panel A. In a normal dividing cell, the cell cycle is composed of four phases, G1, S, G2 and M-phase. The transition between each phase is determined by the synthesis of specific cyclins and the activity of a corresponding Cdk complex.

Panel B. During the G2-phase of the cell cycle, Cdk1 associates with cyclin B but it is maintained in an inactive state by phosphorylation at tyrosine 15 and threonine 14 by Wee1 kinase. To enter M-phase, the Cdk1 complex is dephosphorylated by Cdc25 phosphatase. The regulation of Cdks during apoptosis is currently an active field of study.

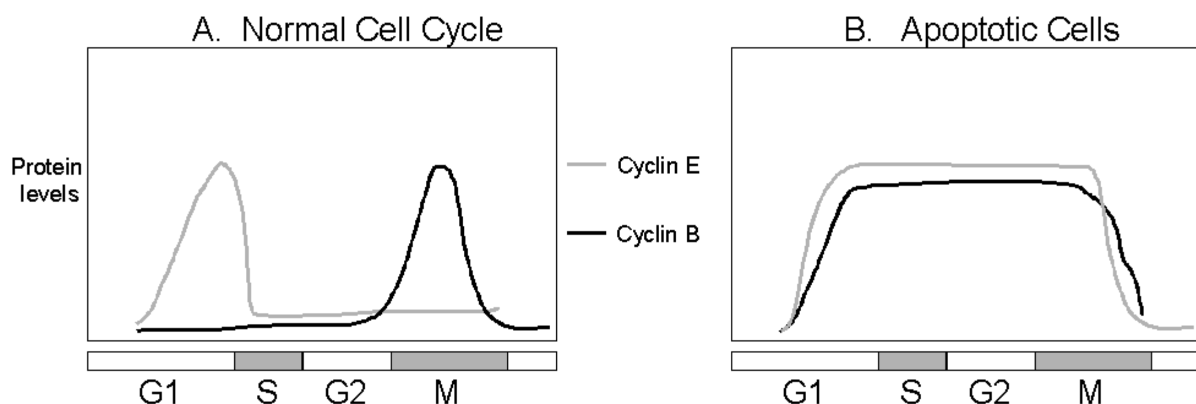

Figure 2. Panel A. The relative protein levels of cyclins changes as cells progress to a specific phase of the cell cycle. Cyclin E (grey) is highly expressed in G1/S-phase where as cyclin B (black) is highly expressed in M-phase. In apoptotic cells, however, (panel B) cyclin B can also be found in G1 cells, which is never seen in normal cells and other cyclins can be detected outside of their normal cell cycle phase (cyclin E is shown here). These observations suggest that cyclins and Cdks may have a role in apoptosis that is independent of their role in the cell cycle.

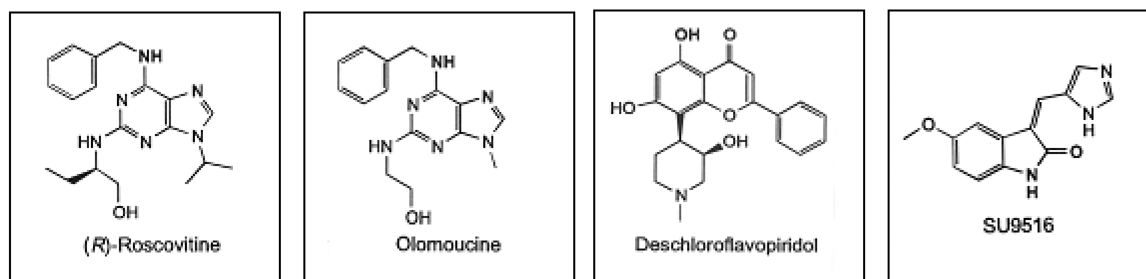

Figure 3. The chemical structures of four different Cdk inhibitors are shown. Two of the structures, roscovitine and olomoucine are from the same chemical family. These compounds can block apoptosis in a number of differently experimental conditions, and in some cases can induce apoptosis when used alone. A complete summary of Cdk inhibitors is described in [17].

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