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Regulation of Cell Cycle

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ABSTRACT
The cell cycle is a vital process in the life of every organism. Normally, the cell cycle results in cell division. Cell division consists of 2 main processes, namely DNA replication and chromosome division that has been duplicated into 2 daughter cells. Cyclin is expressed periodically so that the cyclin concentration varies at each phase of the cell cycle. Cdks are threonine or serine protein kinases which must bind to cyclin for its activation. The concentration of Cdks is relatively constant during the cell cycle. Cyclin-dependent kinase inhibitor (CKI) is a protein that can inhibit Cdk activity by binding to Cdk or the cyclin-Cdk complex

The cell cycle is a vital process in the life of every organism. Normally, the cell cycle results in cell division. Cell division consists of 2 main processes, namely DNA replication and chromosome division that has been duplicated into 2 daughter cells. In general, cell division becomes 2 stages, namely mitosis (M) (division of 1 cell into 2 cells) and interphase (the process between 2 mitoses). Interphase consists of phase 1 gap (G1), DNA synthesis (S), gap 2 (G2). Each stage in the cycle is strictly controlled by the cell cycle regulator, namely:

a. Cyclin. The main cyclin types in the cell cycle are cyclin D, E, A, and B. Cyclin is expressed periodically so that the cyclin concentration varies at each phase of the cell cycle. In contrast to other cyclin, cyclin D is not expressed periodically but is always synthesized as long as there is *growth factor* stimulation.
b. Cyclin-dependent kinases (Cdk). The main Cdk in the cell cycle are Cdk 4, 6, 2, and 1. Cdks are threonine or serine protein kinases which must bind to cyclin for its activation. The concentration of Cdks is relatively constant during the cell cycle. Cdks in the free state (unbonded) are inactive because of the catalytic site, where ATP and the substrate bind are blocked by the C-terminal ends of the CKIs. Cyclin will eliminate these blockages. When activated, Cdk will stimulate the *downstream* process by phosphorylating specific proteins.
c. Cyclin-dependent kinase inhibitor (CKI) is a protein that can inhibit Cdk activity by binding to Cdk or the cyclin-Cdk complex. Cyclin-dependent kinase inhibitors consist of two protein groups, namely

INK4 (p15, p16, p18, and p19) and CIP / KIP (p21, p27, p57). The INK4 family forms a stable complex with Cdk, thereby preventing the Cdk from binding to cyclin D. INK4 is responsible for preventing G1 phase progression. The CIP / KIP family regulates G1 and S phases by inhibiting the G1 cyclin-Cdk and cyclin B-Cdk1 complexes. The p21 protein also inhibits DNA synthesis by deactivating the *proliferating cell nuclear antigen* (PCNA). P21 expression is regulated by p53 because p53 is a transcription factor for p21 expression (Vermeulen et al., 2003).

Rb pathway

The cell cycle begins with the entry of cells from the G0 (*quiescent*) phase to the G1 phase due to a stimulus by *growth factors* **(Figure 1).** At the start of the G1 phase, Cdk 4 and or 6 are activated by cyclin D (cycD). The Cdk4 / 6 complex with cycD will initiate phosphorylation of the retinoblastoma protein family (pRb) during early G1. The effect of this phosphorylation, the function of deacetylated histone (HDAC) which is supposed to maintain the compactness of the chromatin structure is disturbed. As a result, the DNA structure loosens and the transcription factor that was initially bound to pRb is released and the transcription of the E2F *responsive genes* required for cell cycle progression to the S phase becomes active.

These genes include cycE, cycA, Cdc25, DNA polymerase, thymidylate kinase, thymidylate synthetase, DHFR, etc. (Satyanarayana and Kaldis, 2009; Vermeulen et al., 2003).

In the transition from the G1 phase to the S phase, Cdk2 is activated by binding to the cycE. The complex continues the pRb phosphorylation process (hyperphosphorylation status) so that the transcription process stimulated by E2F remains active and the restriction point (R) in the G1 / S phase limit can be exceeded. It is at this time that the cycA is transcribed (Satyanarayana and Kaldis, 2009). During G1 / S, the Cdk2-cycE complex also phosphorylates the p27 inhibitor so that p27 is degraded (Vermeulen *et al.,* 2003). When the cell cycle enters the S phase, the cycE will be degraded and the released Cdk2 will bind the cycA (*Cooper and Hausman*, 2004) (**Figure 2**).

The Cdk2-cycA complex is needed by the cell to replicate DNA during the S phase. The Cdk2-cycA complex will phosphorylate the proteins needed for DNA replication to be active, for example, the CDC6 protein (Cell Division Cycle 6). The complex also prevents the *multiplicity* of DNA replication from occurring. At the end of the S phase, cycA will release Cdk2 and bind Cdk1 (Cdc2) which regulates the cell transition from S to G2 (Dhulipala et al., 2006). The cycA-Cdk1 complex will facilitate the condensation of chromatin required for cell multiplication *(Lapenna and Giordano*, 2009). In the G2 phase, cells also have the opportunity to perform a *repair* mechanism if there is an error in DNA synthesis (*Baumforth and Crocker*, 2003).

Entering the mitotic phase, cycA will be degraded and there is an increase in cycB expression which will bind Cdk1. The Cdk1-cycB complex actively promotes mitosis. The cycB-Cdk1 complex plays an important role in the control of *microtubule rearrangement* during mitosis (Dhulipala et al., 2006; Lapenna and Giordano, 2009).

Cdk1 can be deactivated by Wee1 and Myt1 by means of Wee1 and Myt1 will phosphorylate Cdk1 on tyrosine-15 and or threonin-14. The phosphorylation at these sites can be carried out by Cdc25 so that Cdk 1 becomes active again and the cell cycle continues (Vermeulen et al., 2003). At the end of the mitosis phase, the cycB will be degraded by the *anaphase promoting complex* (APC) through a proteolytic process. APC also functions to spur chromatids to separate and move to each pole to complete mitosis (anaphase) (Lapenna and Giordano, 2009).

Checkpoints on the cell cycle

To ensure that the DNA duplicates accurately and that separation from the chromosomes occurs properly, the cell cycle performs a checkpoint mechanism. The checkpoint is in charge of detecting DNA damage. If there is DNA damage, the checkpoint will trigger a

temporary cell cycle arrest for DNA repair or permanent cell cycle arrest so that the cells enter the senescent phase. If the *cell cycle arrest* mechanism is not sufficient to guarantee that the damaged DNA is duplicated, the cells will be eliminated by apoptosis (Siu et al., 1999).

The first checkpoint factor in mammalian cells is known as the *restriction point* (R) and appears towards the end of G1 (Cooper and Hausman, 2004). At this *checkpoint*, the stem cell DNA is checked for damage or not. If there is damaged DNA, the cell cycle is stopped until the damaged DNA repair mechanism has been completed. After surpassing R, the cell becomes *committed* (committed) to complete the entire cycle (*no return* point) and then the cell must be able to replicate DNA. If it does not go beyond R, the cell can return to phase G0. Loss of control of R will result in defective DNA survival.

p53 pathway

At the G1 / S *checkpoint*, DNA damage can lead to cell cycle arrest and this process is p53-dependent. In general, cell p53 levels are low due to negative regulation by mdm2 which targets p53 degradation, however DNA damage can rapidly induce p53 activity.

p53 is controlled by mdm2 and p19ARF. The p53 protein level is normally at low concentrations in the cells. However, once stimulated, the protein level will rapidly increase over its half-life, while the mRNA level remains relatively unchanged. Then, what can be concluded from this phenomenon? That the regulation of p53 occurs at the protein level (not DNA or RNA) is critical for its activation. The negative regulator of p53 is mdm2, which is a p53-responsive gene (gene expressed through the p53 transcription factor).

So you can imagine there is a negative feedback loop here. p53 is activated and then increases the mdm2 level. Mdm2 then inactivates p53 by binding to the complex or degrading p53. If cells want to increase the level of p53 protein, cells need to inhibit mdm2.

How do cells inhibit mdm2? It depends on the stimuli eg DNA destroying agents (radiation, UV, chemotherapy drugs). DNA damage agent will induce activation of kinases (such as ATM and DNA-PK) which can phosphorylate critical serine residues in the Mdm2-binding domain of p53.

Phosphorylation of p53 to serine-15 and serine-37 by ATM or other protein kinases after DNA damage can prevent MDM2 binding with p53. Thus, when p53 is phosphorylated here (Figure 3), it can no longer bind to mdm2. Then, this is what is able to eliminate the mdm2-mediated inhibition of p53. Why does the DNA damage agent activate p53? Because if your DNA is damaged, you don't want to reproduce, do you? If you do, you can produce cells with destructive mutations that can then lead to cancer. Thus, p53 recognizes when a cell has undergone DNA damage and stops the cell cycle (cell cycle arrest) so that the cell can repair the damage, or in many cases, just tell the cell to commit suicide (apoptosis), namely by stimulating gene transcription such as p21 and Bax so that the cell cycle stops or apoptosis occurs (Siu *et al.,* 1999).

Another mechanism for inhibiting mdm2 is by oncogenes, a constitutive active mutant protein that continually tells cells to grow (E1A, Ras, c-Myc). Why do oncogens activate p53? Again, you don't want your cells to grow out of control, do you? So, p53 recognizes when this happens and stops the cell cycle. However, oncogenes did not lead to ATM or DNA-PK activation, in fact, oncogenes did not even lead to p53 phosphorylation in the MDM2-binding domain. So, how do oncogens inhibit mdm2? By inducing the expression of a tumor suppressor protein called p19ARF (Figure 5).

Therefore, it is easy to understand that p53 is the most frequently mutated gene in cancer. And from this, you can find out the importance of this gene. In normal cells, p53 is important at the main checkpoint control. He can recognize when no errors occur, for example DNA or cell damage stimulated by oncogenes, and immediately stop the cell cycle to prevent cells from becoming cancerous. Thus, if a cell loses p53, it will lose its important checkpoint function. Not only was the mutated p53 mutation found in cancer cells, but also mdm2 overexpression (which inhibits p53), as well as loss of p19ARF. Remember the Rb pathway, that

p16INK4a is also frequent in cancer. Yes, it turns out that p16INK4a and p19ARF (**a**lternate **r**eading **f**rame from the INK4a / ARF locus) are at the same locus, and in cancer this locus has deletions, so p16INK4a and p19ARF are missing.

The next *checkpoint* is in the S phase, which detects damage to replicated DNA. The *checkpoint* on G2 prevents initiation of mitosis before DNA replication is complete. The main *checkpoint* in the S / G2 / M phase is Chk1. When there is DNA damage, protein kinase ATR will phosphorylate Chk1, then Chk1 phosphorylate Cdc25C on serine-216. The phosphorylation causes the cycB-Cdk1 complex which is responsible for phase progression G2 / M is off.

In addition, Chk1 also phosphorylates Cdc25A which is responsible for activating the cycE-Cdk2 and cycA-Cdk2 complexes which play a role in S-phase progression. By phosphorylating Cdc25A by Chk1, the cyc-Cdk complex becomes inactive and S arrest occurs (Xiao et al., 2003; Beckerman et al., 2009).

The last checkpoint, called the spindle checkpoint, maintains the integrity of the genome towards the end of mitosis. If there is a failure to place the chromosome pair on the *spindle*, mitosis arrest will occur. In cancer cells, checkpoints do not function properly and the cell cycle takes place without control (Cooper and Hausman, 2004).

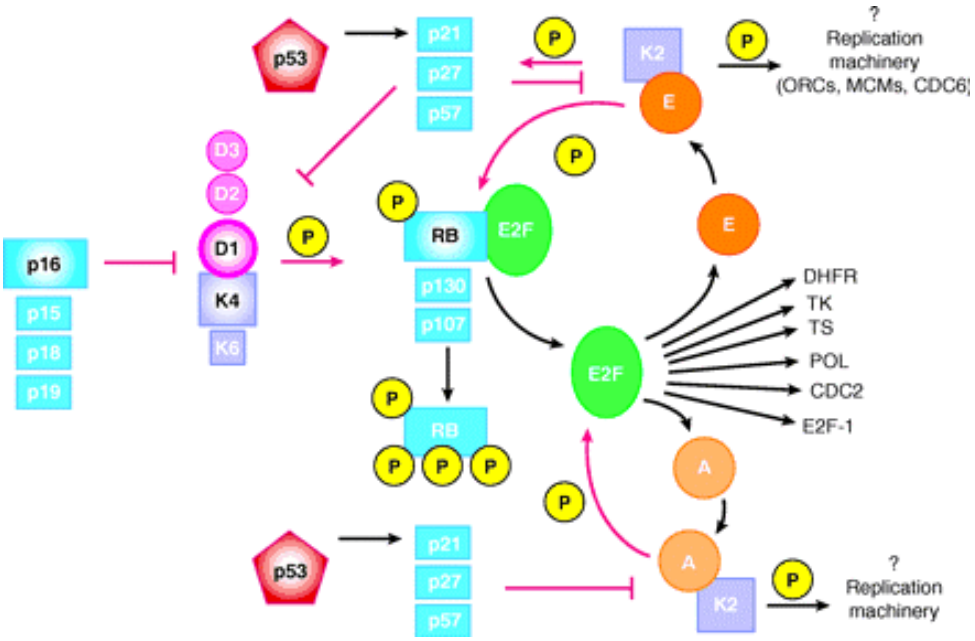

Figure 1.Cell cycle (Sherr, 1996)

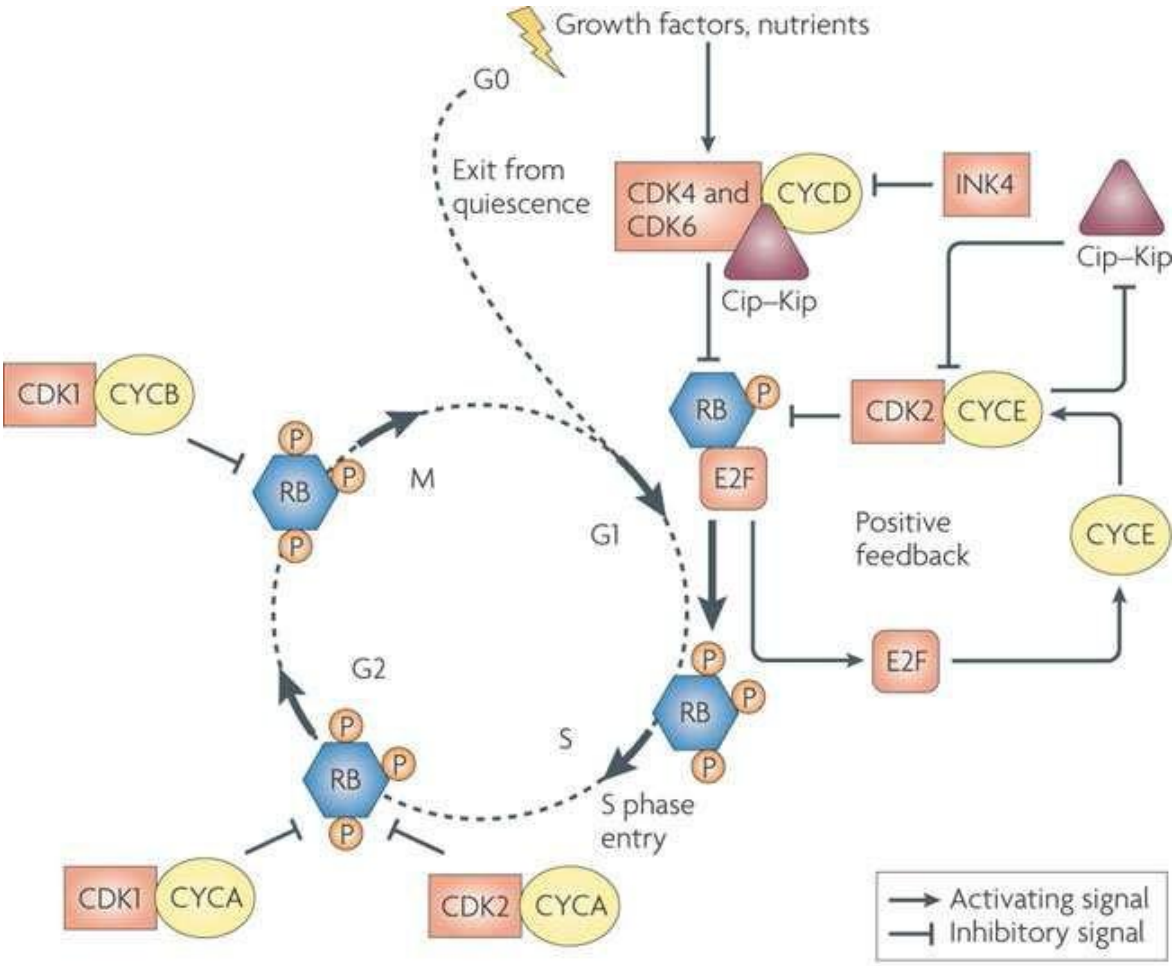

Figure 2. General illustration of the cell cycle. The cell cycle consists of 4 stages, namely G1, S, G2, and M. Cell cycle progression is controlled by cyclin (D, E, A, and B), cyclin-dependent kinases (4, 6, 2, and 1), and cyclin – dependent kinase inhibitors (eg Cip and Kip) (Lapenna and Giordano, 2009).

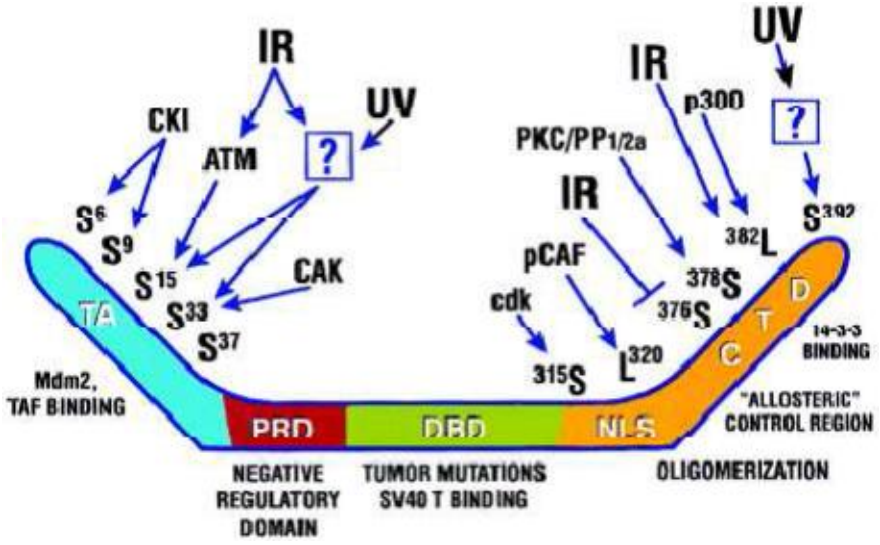

Figure 3. Schematic of the p53 domain including post translational modification sites (phosphorylation and acetylation) involved in stabilization, activation, or suppression (Giaccia, and Kastan, 1998)

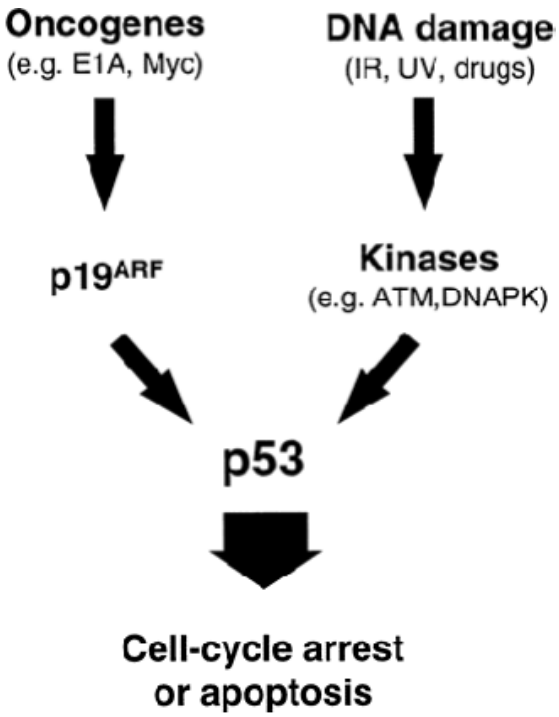

Figure 4. Oncogenes and DNA damage agents activate p53 through different mechanisms. p19ARF acts as an intermediary in the activation of p53 by mitogenic oncogens such as E1A, Ras, c-myc. In contrast, p53 activation due to DNA damage agent involves de novo phosphorylation of serine 15 in the p53 domain (and other residues) by kinases such as DNA-dependent protein kinase (DNA-PK) or the product of the ataxia-telangiectasia (ATM) gene. Activation of p53 by oncogenes does not involve phosphorylation on serine-15, and both serine-15 phosphorylation (not shown) and p53 activation following DNA damage are unimpaired in the absence of ARF. Therefore, the two upstream signal paths to p53 are fundamentally different (de Stanchina et al, 1998).

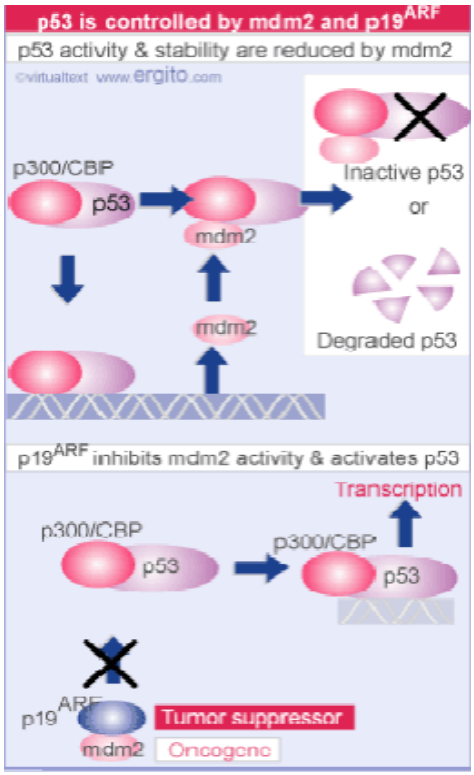

Figure 5. P53 regulation is carried out by mdm2 and p19ARF (alternate reading frames from the INK4a / ARF (CDKN2A) locus)

Referensi

1. Baumforth and Crocker, 2003, Molecular and Immunological Aspects of Cell Proliferation, in Molecular Biology in Cellular Pathology, Wiley (http://onlinelibrary.wiley.com/doi/10.1002/0470867949.ch6/summary)

2. Cooper GM and Hausman RE, 2004, The Cell: A Molecular Approach, Fifth Edition, ASM Press and Sinauer Associates, Inc.

3. de Stanchina E, McCurrach ME, Zindy F, Shieh SY, Ferbeyre G, Samuelson AV, Prives C, Roussel MF, Sherr CJ, Lowe SW., E1A signaling to p53 involves the p19(ARF) tumor suppressor., Genes Dev. 1998 Aug 1;12(15):2434-42.

4. Dhulipala, V.C., Welshons, W.V., and Reddy, C.S., 2006, Cell Cycle Proteins in Normal and Chemically Induced Abnormal Secondary Palate Development: a Review, Human Exp. Toxicol. 25: 675-682.

5. Giaccia, A.J. and Kastan, M.B, 1998, The complexity of p53 modulation: emerging patterns from divergent signals, Genes and Development 12: 2973-2983.

6. Lapenna, S., and Giordano, A., 2009, Cell Cycle Kinases as Therapeutic Targets for Cancer, Nat. Rev. Drug Discov. 8(7): 547-566.

7. Reed SI, 2011, Chapter 6: Cell Cycle, in DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology, 9e, LWW

8. Satyanarayana, A., and Kaldis, P., 2009, Mammalian Cell-cycle Regulation: Several Cdks, Numerous Cyclins, and Diverse Compensatory Mechanisms, Oncogene, 28: 2925-2939.

9. Schwartz GK, 2005, Development of Cell Cycle Active Drugs for the Treatment of Gastrointestinal Cancers: A New Approach to Cancer Therapy, Journal of Clinical Oncology, July 10, 2005 vol. 23 no. 20 4499-4508

10. Sherr CJ., Cancer cell cycles., Science. 1996 Dec 6;274(5293):1672-7

11. Siu, W.Y., Yam, C.H., and Poon, R.Y.C., 1999, G1 versus G2 Cell Cycle After Adriamycin-induced Damage in Mouse Swiss3T3 Cells, Left. 461: 299-305.

12. Vermeulen, K., Berneman, Z.N., and Van Bockstaele, D.R., 2003, Cell Cycle and Apoptosis, Cell Prolif. 36(3): 165-175.

13. Xiao, Z., Chen, Z., Gunasekera, A.H., Sowin, T.J., Rosenberg, S.H., Fesik, S., and Zhang, H., 2003, Chk1 Mediates S and G2 Arrest Through Cdc25A Degradation in Response to DNA-Damaging Agents, J. Biol. Chem. 278(24): 21767-21773.