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Review paper

Modulation of chemosensitivity through altered expression of cell cycle regulatory genes in cancer

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Alterations in the expression of genes affecting cell cycle progression occur in all human cancers. These may occur either by overexpression of genes such as cyclin D1, mutation of regulatory genes such as p16, or abrogation of checkpoints following DNA damage as in the cases of mutation or deletion of the p53 gene. Perturbation of the normal functions of these genes has a profound effect on cellular proliferation, differentiation and apoptosis. There is increasing evidence that such alterations may modulate the cellular response to treatment with chemotherapeutic agents. In many cases genetic alterations may induce resistance to drug treatment as in the case of mutations of the p53 gene. However, the deregulated expression of cell cycle genes may also increase sensitivity to treatment by directly altering the expression of the target for chemotherapeutic drugs as in the case of deletion of the retinoblastoma gene. It is crucial to understand the interactions between drug mechanisms of action and the genetic alterations in cancer to exploit potential areas in which the alterations found in tumors may constitute potential vulnerability.

Key words: Cell cycle, chemosensitivity, regulatory genes.

Factors modulating chemosensitivity in cancer

There has been dramatic progress in the identification of factors modulating the response of cancers to therapy. Several genes have been implicated in the development of acquired resistance. These include expression of P-glycoprotein (P-gp),[1] altered expression of topoisomerase II[2] (topo II) and increased DNA repair.[3] These factors may have important effects on intracellular drug concentration as in the case of P-gp or may alter expression of drug targets such as amplification of the dihydrofolate reductase (DHFR) gene conferring methotrexate resistance.[4] It has also been found that alterations in the expression of genes which mediate apoptosis may also be significant in chemosensitivity with increased expression of *bcl*-2 resulting in resistance to a variety of agents.[5]

Recent identification of many of the genes involved in cell cycle regulation has led to an appreciation that altered expression of these genes in cancer cells may be critical in determining drug sensitivity. There are two major pathways through which the altered expression of cell cycle genes found in human cancers may influence response to drug therapy. Altered expression or mutation of the p53 protein has a profound influence on cellular entry into the apoptotic pathway.[6,7] The predominant lesion triggering p53-mediated apoptosis is damage to DNA.[8] Another mechanism through which altered expression of cell cycle genes might result in changes in drug sensitivity is through changes in expression of the drug target directly which result from these genetic changes as in the case of overexpression of cyclin D1 and mutation or deletion of the retinoblastoma (pRb) gene which alter DHFR expression. Cells which proliferate more rapidly may be significantly more sensitive to drugs acting in S phase. It is thus crucial to assess the contribution of cell doubling time when investigating the specific effects of cell cycle gene expression on chemosensitivity.

The p53 pathway

Recent research has concentrated on the p53 gene which has an essential role in both cell cycle arrest and in the induction of apoptosis following DNA damage. p53 is a cell cycle checkpoint gene which monitors genomic integrity. Alterations in p53 either by mutation or deletion are among the commonest genetic alterations in human cancer. Following pro-

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duction of DNA strand breaks there is activation of p53.[9] This stimulates expression of the cyclin-dependent kinase inhibitor (CDKI) $p21^{WAF1}$/cip1,[10] which in turn prevents cell cycle progression primarily by inhibition of cyclin-dependent kinases (CDK4, CDK6 and CDK2) that are active at various points in the cell cycle[11] resulting in G_1 arrest. Other factors which may activate p53 include hypoxia,[12] irradiation[13] and reduced levels of intracellular nucleotide pools.[14] However, there is evidence that the cell cycle arrest mediated by $p21^{WAF1}$ induction may not be a necessary requirement for progression to apoptosis. For example, *myc*-induced apoptosis occurs in cells with wild-type (but not mutant) p53 despite inhibition of $p21^{WAF1}$ induction.[15] Other studies have also confirmed that $p21^{WAF1}$-induced cell cycle arrest in G_1 is not an absolute requirement for p53-dependent apoptosis.[16] In addition, the G_1 arrest induced by p53 does not necessarily result in apoptosis under certain circumstances and may delay cell cycle progression while DNA damage is repaired. It is possible that the extent of DNA damage sustained may be the critical determinant as to whether cell cycle arrest or apoptosis occurs. In hematopoietic cell lines the level of p53 may determine entry into specific pathways with lower levels of p53 inducing differentiation and higher levels resulting in apoptosis.[17]

The cell lineage may also be of major significance in determining the response to p53 induction. For example, a hematopoietic cell line expressing a temperature-sensitive mutant of p53 which results in a wild-type p53 genotype at the permissive temperature undergoes apoptosis with induction of wild-type p53.[18] In contrast, transfection of wild-type p53 into a colon cancer cell line using an inducible expression vector results in reversible G_1 cell cycle arrest but not apoptosis.[19]

Cyclin D1–pRb–p16 pathway

The major components of the eukaryotic cell cycle and their perturbation in cancer have been extensively reviewed[20,21] and only a brief outline will be provided here. Phosphorylation of the retinoblastoma gene (pRb) is the critical event in transition from G_1 to S phase.[22] This results in the dissociation of the complex of pRb with one of the E2F family of transcription factors (comprising E2F1, E2F2 and E2F3) which consequently stimulates transcription of several genes involved in DNA replication including DHFR and DNA polymerase.[23] Thus any process mediating the phosphorylation of pRb will result in accelerated progression through the G_1/S phase transition. In addition, deletions of pRb or mutations which affect the binding of pRb with E2F will result in an increase in 'free' E2F levels and cell cycle progression.

Several cell cycle gene alterations promoting this effect occur in tumors including overexpression of cyclin D1 and mutation or deletion of the CDKI p16.[24] It has been found that in most tumors studied there may be an alteration in at least one component of the G_1 pathway.[25,26] For example, in a study of non-small lung cancer overexpression of cyclin D1 is frequent while mutation of pRb is less common.[27] In contrast, small cell lung cancers frequently have mutations or deletions of pRb while cyclin D1 alterations are uncommon. However, another study on small cell lung cancer found that cyclin D1 overexpression and pRb inactivation may occur concurrently; possibly the overexpression of cyclin D1 is an early event in oncogenesis while pRb alterations occur later.[28]

Cell cycle genes and response to therapy

p53 and chemosensitivity

It is clear that genetic alterations in cancer cells may also have profound effects on response to therapy; this has been particularly well studied in relation to expression of p53.[29] The induction of p53 following formation of DNA strand breaks is a potent stimulus for apoptosis. Germ cell tumors express large amounts of wild-type p53 which is transcriptionally inactive until the cell is exposed to DNA damaging agents.[30] These tumors are sensitive to a wide variety of drugs. Several investigators have shown that introduction of the p53 gene into cell lines with null p53 results in increased sensitivity to chemotherapeutic agents. For example, transfection of wild-type p53 into the p53 null HL-60 promyelocytic cell line resulted in a log increased sensitivity to etoposide, doxorubicin and 5-fluorouracil (5-FU).[31] There was no alteration in the expression levels of the drug target genes such as thymidylate synthase (TS) and DHFR but the presence of wild-type p53 modulated the downstream effects of drug exposure.

It is important to differentiate between whether the apparently improved prognosis in cancers such as colorectal cancer in cases which express wild-type p53 is secondary to the higher rate of tumor response or whether these tumors have a better prognosis *per se*. The response rate of colorectal hepatic metastases to 5-FU by portal vein infusion was not altered by p53 status as assessed by immunohistochemistry but overall survival was significantly improved in patients whose tumors expressed wild-type p53.[32] These

findings need to be confirmed in larger studies in a variety of tumor types.

However, not all chemotherapeutic agents show such a clear-cut relationship with p53 status. For example, the effects of p53 status are unclear with regard to response to both cisplatin and taxanes. In breast cancer cell lines where p53 function is abrogated by transfection of the papillomavirus E6 gene, there is increased sensitivity to cisplatin.[33] However, studies on clinical samples from patients with ovarian cancer found no correlation between p53 status and response to cisplatin treatment.[34] Furthermore, a study of response to cisplatin in patients with non-small cell lung cancer found expression of mutant p53 to be associated with lack of response to treatment.[35] There is also ambiguity with respect to paclitaxel and p53 status. Studies on p53 null and normal fibroblasts showed increased sensitivity to paclitaxel in p53 null fibroblasts possibly due to inappropriate cell entry into mitosis.[36] A similar result was obtained with cells transfected with E6. However, studies have not confirmed this relationship using cell lines of other lineages, and studies on clinical samples obtained from patients with non-small cell lung cancer have also found no correlation between p53 status and response to paclitaxel treatment.[37]

It appears, therefore, that these correlations may be cell-lineage specific and it will be important to analyze factors which influence sensitivity within particular cell types. As mentioned above, germ cell tumors, in which platinum is a key component in therapy, express wild-type p53. In contrast, small cell lung cancer, which expresses mutant p53 in the majority of cases,[38] is generally sensitive to chemotherapy with resistance developing as a result of expression of genes including the multidrug resistance protein. Another complicating factor is that specific mutations in p53 may be associated both with risk of relapse and with response to therapy. An interesting, albeit small, study of p53 mutations in breast cancer demonstrated that all patients with mutant p53 who progressed during treatment with doxorubicin had mutations affecting the zinc-binding domains of the protein involved in DNA binding.[39]

Apart from the direct effects of p53 on cell cycle arrest and apoptosis, there may be other mechanisms by which p53 alterations might affect chemosensitivity. Studies *in vitro* on the promoter region of the topo II gene, which is the cellular target for doxorubicin and VP-16, suggest that there may be an inhibitory effect of wild-type p53 on transcription.[40,41] This finding is difficult to reconcile with the increased sensitivity of cells expressing wild-type p53 to these drugs as an increased level of topo II is associated with sensitivity to topo II inhibitors.[42] Similar studies using reporter constructs suggest a direct effect on the promoter of P-gp by wild-type and mutant p53 might result in modulation of chemosensitivity.[43,44] These results have not been confirmed by clinical studies, e.g. in ovarian cancer, in which no correlation was found between p53 status and PGP expression.[45]

Cyclin D1–pRb–p16 pathway and chemosensitivity

The effects of alterations in genes controlling progression through G_1 have been investigated in a variety of cellular models and, as with p53, it seems that the cell lineage is a critical determinant of effects on chemosensitivity. Furthermore, the relevance of these findings to the clinical situation is unclear. Alterations in pRb are particularly common in human cancer. Several studies have investigated the effect of pRb expression in protection from apoptosis and, conversely, the pro-apoptotic effects of pRb deletion or mutation.[22] Mice with homozygous deletions of pRb show defects in the hematopoietic and nervous systems associated with massive cell death, and are not viable beyond day 14 of gestation.[46] Abrogation of pRb function by expression of the adenovirus EIA oncogene may result in apoptosis;[6] this is prevented by ectopic expression of pRb.[47]

The increase in free E2F in the absence of functional pRb might also be expected to result in increased transcription of genes regulated by E2F including TS and DHFR which are important drug targets. To determine the effects of pRb expression on chemosensitivity, human sarcoma cell lines from pRb-positive and -negative genotypes were investigated for sensitivity to the antimetabolites methotrexate (MTX) and 5-FU. The major cellular target for MTX is DHFR which is transcriptionally regulated in part by E2F. Deletion or mutation of pRb results in an increase in 'free' E2F with consequently increased transcription of DHFR.[48] In sarcoma cell lines lacking functional pRb there is an increase in the level of free E2F as measured by a gel shift assay and this results in increased transcription of DHFR. There is also significant increased resistance to MTX, presumably secondary to the increased transcription of DHFR. Introduction of pRb into a line with truncated pRb results in increased sensitivity to MTX. These results suggest that the effect of alterations in cell cycle genes may be significant in directly affecting the level of drug target. In this comparison of pRb-positive and -negative cell lines it was found that the abrogation of pRb function also results in increased expression of TS, perhaps transcriptionally induced by E2F. This is associated with resistance to inhibitors of

TS such as 5-FU. Hence the pRb function within the cell may critically affect both the susceptibility to apoptosis and the response to specific drugs depending on the coincident p53 status.

Another frequent alteration in human tumors is increased expression of cyclin D1.[49] Several studies have indicated that an effect of cyclin D1 overexpression is to increase the proportion of cells in S and G_2 phase.[50,51] It does not appear that overexpression of cyclin D1 alone is not sufficient to induce transformation but cyclin D1 may cooperate with oncogenes such as *ras*.[52] A study of an esophageal cancer cell line in which an antisense strategy was used indicated that reversal of cyclin D1 overexpression may be sufficient to induce a regression of the neoplastic phenotype,[53] yet other studies suggest that overexpression of cyclin D1 in other cell types may itself induce cell cycle arrest.[54] Introduction of an antisense cyclin D1 plasmid into a human colon carcinoma cell line results in loss of tumorogenicity in nude mice even though there is a residual level of gene expression;[55] hence some tumors may rely on high levels of cyclin D1 in maintenance of the malignant phenotype.

To investigate directly the effect of cyclin D1 overexpression on drug sensitivity, an HT1080 fibrosarcoma cell line was transfected with an expression plasmid.[56] A 3-fold increase in cyclin D1 levels was achieved compared to the parental cell line and the increase in DHFR transcription was associated with an 8-fold increase in resistance to MTX. These results may be of clinical relevance as MTX is a widely used agent in head and neck cancer in which cyclin D1 overexpression is common and has been identified as a marker of poorer prognosis.[57,58] Tumors with high cyclin D1 expression may be resistant to MTX treatment.

However, down-regulation of cyclin D1 causing G_1 arrest may also result in resistance to chemotherapy following exposure of cells to agents inducing glucose related stress proteins (GRSP).[59] Treatment of A2780 human ovarian and HT-29 colon cancer cell lines with the calcium ionophore A23187 resulted in both p21 induction and decreased cyclin D1. Thus the effects of cyclin D1 may be paradoxical and determined by the cell lineage or by the quantity of protein expressed.

The downstream effects of cyclin D1 overexpression and pRb alterations may not be identical although either of these genetic alterations (as well as changes in p16 and overexpression of CDKs) may result in progression through G_1. However, there are documented cases of small cell lung cancer in which both overexpression of cyclin D1 and alteration of pRb occur,[28] and this may indicate that there are not the same resultant genotypes from these two events. This might be expected as there are several functions of pRb not directly related to cell cycle progression such as interaction with c-*abl* and modulation of transcription through binding of RNA polymerase 1.[21,22]

The p16 gene is a CDKI and abrogation of its function results in increased cell proliferation. Introduction of p16 into pRb-positive, but not -negative, cell lines results in cell cycle arrest.[60] To determine the effects of p16 on chemosensitivity an inducible plasmid was transfected into the HS294T melanoma cell line.[61] Induction of p16 results in reversible cell cycle arrest. Cells held in G_0/G_1 during this induction were resistant to a variety of agents including MTX, cisplatin and vincristine. It is, however, unclear as to whether the major contribution to drug resistance is decreased cellular proliferation or a direct effect of p16 on apoptosis; this may be a factor in the relative resistance of normal tissues to the effects of chemotherapy.

As in the case of p16 there is some evidence that alteration of other CDKIs in cancer may affect response to treatment. The key gene modulating p53 activity is $p21^{WAF1}$. Interestingly, mutations in the $p21^{WAF1}$ gene appear to be rare in human cancer cell lines and tumors.[62] Furthermore, mice with homozygous deletion of $p21^{WAF1}$ show a normal phenotype.[63] As discussed above, colon cancer cells with deletions of $p21^{WAF1}$ show abnormal response to treatment with doxorubicin with progression to G_2; inactivation of $p21^{WAF1}$ in HCT 116 colon carcinoma cells resulted in a G_2 block with cells unable to undergo mitosis.[64] The cells with inactivated $p21^{WAF1}$ were more sensitive to doxorubicin than the parental line. There was repeated entry into S phase and development of aneuploidy indicating uncoupling of S and M phase. Interestingly, studies on colon cancer cells with mutated p53 which effectively showed an attenuated $p21^{WAF1}$ response to DNA damage suggested a similar response to drug exposure with repeated S phases. Therefore, the ability of cells to induce $p21^{WAF1}$ and thereby arrest in the cell cycle is a critical factor in preventing apoptosis following treatment with some DNA damaging agents. The role of $p21^{WAF1}$ may be important both in induction of G_1 arrest and regulation of the mitotic checkpoint.

The involvement of $p21^{WAF1}$ in DNA repair may also be significant in drug sensitivity. A study of the $p21^{WAF1}$-negative HCT116 cells demonstrated decreased proficiency in repair of a cisplatin damaged plasmid as compared to the $p21^{WAF1}$-positive counterparts.[65] This may be due to the need for $p21^{WAF1}$ in the nucleotide excision repair (NER) pathway which is involved in repair of cisplatin damage; however, the significance of $p21^{WAF1}$ in NER remains unclear. This result was confirmed by experiments in which an inducible $p21^{WAF1}$ plasmid was

transfected into the p53-deficient DLD1 colorectal carcinoma cell line.[66] However, induction of $p21^{WAF1}$ did not result in any alteration of sensitivity to doxorubicin; the repair of doxorubicin DNA damage is not mediated through the NER pathway.

The action of $p21^{WAF1}$ in mediating cell cycle arrest depends on the presence of an intact pRb. To investigate the potential significance of a second control point for $p21^{WAF1}$, the osteosarcoma cell line SaOs-2, which lacks both functional p53 and pRb, was transfected with an inducible $p21^{WAF1}$ plasmid.[67] In this study expression of $p21^{WAF1}$ is associated with increased sensitivity to a variety of drugs including doxorubicin, MTX and tomudex. There was an increased level of functional E2F as a result of E2F hypophosphorylation. Hypophosphorylation of E2F1 may be modulated through the binding to and inhibition of the cyclin A/cdk2 complex by $p21^{WAF1}$ and it is known that the hypophoshorylation of the E2F-1/DP1 heterodimer increases the level of DNA binding. Thus the effects of $p21^{WAF1}$ expression may be dependent on the cellular lineage and the genotype, in particular with pRb status.

p27 and chemosensitivity

The CDKI $p27^{kip1}$ gene is related to p21 and, similarly, genotypic mutations have been found rarely in human tumors. However, loss of $p27^{kip1}$ expression is a common finding in solid tumors including breast cancers.[68] The expression of $p27^{kip1}$ is increased in cells which have adhesive contact with other cells;[69] this is also associated with resistance to chemotherapeutic agents perhaps partially due to the reduced proliferative capacity of cells at confluence. Alteration of cell growth from monolayer cultures to three-dimensional spheroids results in marked up-regulation of $p27^{kip1}$. The use of antisense oligonucleotides to down-regulate $p27^{kip1}$ results in loss of aggregation. Interestingly, this loss of $p27^{kip1}$ expression was associated with significant cellular sensitization to the alkylating agent 4-hydroperoxycyclophosphamide. This suggests both that $p27^{kip1}$ may be important as a cause of acquired drug resistance and that agents which reduce $p27^{kip1}$ expression might act as chemosensitizers.

E2F alterations and chemosensitivity

The downstream effect of down-regulation of genes controlling G_1 progression is the release of 'free' E2F which may then activate promoters of E2F-inducible genes through binding to a consensus DNA sequence.[70] There are five E2F genes of which E2F1, E2F2 and E2F3 are known to bind to pRb. E2F1 overexpression has been found to result in cellular transformation in an immortalized cell line and can transform primary cells in co-operation with the *ras* oncogene.[71,72] Overexpression of E2F allows cells to enter S phase even in the absence of growth factors, resulting in apoptosis.[73] A study in which E2F1 was overexpressed in a myeloid cell line demonstrates that E2F1 can induce apoptosis following treatment with etoposide and doxorubicin, but not with other classes of chemotherapeutic agents.[74] This effect is p53 independent and is prevented when topo II is pharmacologically depleted by treatment with the stoichiometric inhibitor ICRF-193, which does not bind DNA.

As the effects of the changes in G_1 status discussed above presumably increase levels of free E2F, it might be expected that mutation or overexpression of E2F might occur in tumor cells. However, with the exception of the HEL leukemia cell line in which E2F amplification occurs,[75] no other abnormalities of E2F have thus far been identified in cell lines or tumors. In fact, mouse knockouts of the E2F1 gene showed an increased incidence of tumors, mainly sarcomas, suggesting a role for E2F as both a tumor suppressor gene and an oncogene.[76]

G_2 checkpoint and chemosensitivity

An important area is in the role of the cyclins and associated proteins involved in mitosis and apoptosis. DNA damage following drug exposure often results in pre-G_2 arrest and alteration of this checkpoint in tumors might also be expected to have implications for chemosensitivity.[77] It has been shown, for example, that the topo I inhibitor camptothecin (CPT) induces G_2 arrest; resistance to CPT in colon cancer cell lines may be due to the ability of cells to down-regulate cyclin B/Cdc2 kinase activity. Resistant lines are able to overcome the S phase blocking action of CPT and can complete DNA elongation, in contrast to sensitive lines which arrest in S phase and cannot complete DNA synthesis.[78] Transient activation of Cdc2 may occur at an early stage of drug-induced apoptosis. However, there is an intriguing suggestion that the status of the Cdc2 protein kinase may determine apoptotic sensitivity. A study using a murine mammary carcinoma cell line which expresses a temperature-sensitive Cdc2 mutant gene found that increased apoptosis occurred in cells exposed to mitoxantrone when Cdc2 levels were depleted at the

restrictive temperature.[79] This occurred despite the presence of the same amount of DNA damage in both parental and mutant cell lines. Moderate levels of DNA damage might result in Cdc2-induced G_2 arrest while higher drug concentrations may lead to cell death in the absence of cell cycle arrest. Cells with mutant p53 may be dependent on a G_2 checkpoint to repair DNA damage. Abrogation of this G_2 checkpoint by using a protein kinase C inhibitor in breast cancer cell lines sensitizes cells with mutant, but not wild-type p53, to the effects of mitomycin.[80]

Conclusion

It is therefore clear that alterations in several cell cycle genes may sensitize cells to apoptosis following treatment with chemotherapeutic agents. The downstream effects of these treatments are not non-specific responses to DNA damage but depend on the exact type of DNA lesion produced by the agents. It is therefore crucial to investigate the factors which modulate the action of drugs to determine future approaches to therapy. For example, the loss of p53 function in tumors may constitute a point of vulnerability in treatment with agents such as paclitaxel.[36] Furthermore, it is clear that the effects of modulating cell cycle gene expression may also be profoundly affected by the cellular context. The strategies for novel cancer therapies by developing agents which selectively damage specific DNA sequences and investigating mechanisms for reversing the effects of deregulated cell cycle genes in tumors should therefore be seen as complementary rather than alternative approaches to treatment. It will be increasingly important to delineate the ways in which these pathways interact.

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References

1. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993; **62:** 385-427.
2. Hochhauser D, Harris AL. The role of topoisomerase IIα and β in drug resistance. *Cancer Treat Rev* 1993; **19:** 181-94.
3. Chu G. Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. *J Biol Chem* 1994; **269:** 787-90.
4. Bertino JR. Ode to methotrexate. *J Clin Oncol* 1993; **11:** 5-14.
5. Reed JC, Miyashita T, Takayama S, *et al.* BCL-2 family proteins: regulators of cell death involved in the pathogenesis of cancer and resistance to therapy. *J Cell Biochem* 1996; **60:** 23-32.
6. Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993; **74:** 957-67.
7. Lowe SW, Bodis S, McClatchley A, *et al.* p53 status and the efficacy of cancer therapy *in vivo*. *Science* 1994; **266:** 807-10.
8. Fan S, El-Deiry WS, Bae I, *et al.* p53 gene mutations are associated with decreased sensitivity of human lymphoma cells to DNA damaging agents. *Cancer Res.* 1994; **54:** 5824-30.
9. Nelson WG, Kastan MB. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. *Mol Cell Biol* 1994; **14:** 1815-23.
10. el-Deiry WS, Tokino T, Velculescu VE, *et al.* WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993; **75:** 817-25.
11. MacLachlan TK, Sang N, Giordano A. Cyclins, cyclin-dependent kinases and cdk inhibitors: implications in cell cycle control and cancer. *Crit Rev Eukaryot Gene Expr* 1995; **5:** 127-56.
12. Graeber TG, Osmanian C, Jacks T, *et al.* Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 1996; **379:** 88-91.
13. Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T. p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 1993; **362:** 847-9.
14. Sherley JL. Guanine nucleotide biosynthesis is regulated by the cellular p53 concentration. *J Biol Chem* 1991; **266:** 24815-28.
15. Wagner AJ, Kokontis JM, Hay N. Myc-mediated apoptosis requires wild type p53 in a manner independent of cell cycle arrest and the ability of p53 to induce $p21^{waf1/cip1}$. *Genes Dev* 1994; **8:** 2817-30.
16. Attardi LD, Lowe SW, Brugarolas J, Jacks T. Transcriptional activation by p53, but not induction of the p21 gene, is essential for oncogene-mediated apoptosis. *EMBO J* 1996; **15:** 3693-701.
17. Ronen D, Schwartz D, Teitz Y, Goldfinger N, Rotter V. Induction of HL-60 cells to undergo apoptosis is determined by high levels of wild-type p53 protein whereas differentiation of the cells is mediated by lower p53 levels. *Cell Growth Different* 1996; **7:** 21-30.
18. Ramqvist T, Magnusson KP, Wang Y, Szekely L, Klein G, Wiman KG. Wild-type p53 induces apoptosis in a Burkitt lymphoma (BL) line that carries mutant p53. *Oncogene* 1993; **8:** 1495-500.
19. Yang B, Stambrook PJ, Markowitz SD. Wild-type p53 demonstrates functional dominance in a human colon carcinoma cell line in which it induces reversible growth arrest. *Clin Cancer Res* 1996; **2:** 1639-47.
20. Sherr CJ. Cancer cell cycles. *Science* 1996; **274:** 1672-8.
21. Wang JYJ, Knudsen ES, Welch PJ. The retinoblastoma tumor suppressor protein. *Adv Cancer Res* 1994; **64:** 25-85.
22. Herwig S, Strauss M. The retinoblastoma protein: a master regulator of cell cycle, differentiation and apoptosis. *Eur J Biochem* 1997; **246:** 581-601.
23. Schilling LJ, Farnham PJ. Transcriptional regulation of the

dihydrofolate reductase/rep-3 locus. *Crit Rev Eukaryot Gene Expr* 1994; **4:** 19-53.
24. Cordon-Cardo C. Mutation of cell cycle regulators; biological and clinical implications for human neoplasia. *Am J Pathol* 1995; **147:** 545-60.
25. Lukas J, Aagaard L, Strauss M, Bartek J. Oncogenic aberrations of p16INK4/CDKN2 and cyclin D1 cooperate to deregulate G1 control. *Cancer Res* 1995; **55:** 4818-23.
26. Bartkova J, Lukas J, Guldberg P, *et al.* The p16-cyclin D/Cdk4-pRb pathway as a functional unit frequently altered in melanoma pathogenesis. *Cancer Res* 1996; **56:** 5475-83.
27. Schauer IE, Siriwardana S, Langan TA, Sclafani RA. Cyclin D1 overexpression vs retinoblastoma inactivation: implications for growth control evasion in non-small cell and small cell lung cancer. *Proc Natl Acad Sci USA* 1994; **91:** 7827-31.
28. Shapiro GI, Edwards CD, Kobzik L, *et al.* Reciprocal Rb inactivation and p16INK4 expression in primary lung cancers and cell lines. *Cancer Res* 1995; **55:** 505-9.
29. Harris CC. Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. *J Natl Cancer Inst* 1996; **88:** 1442-55.
30. Lutzker SG, Levine AJ. A functionally inactive p53 protein in teratocarcinoma cells is activated by either DNA damage or cellular differentiation. *Nat Med* 1996; **2:** 804-10.
31. Banerjee D, Lenz H-J, Schnieders B, *et al.* Transfection of wild-type but not mutant p53 induces early monocytic differentiation in HL60 cells and increases their sensitivity to stress. *Cell Growth Different* 1995; **6:** 1405-13.
32. Belluco C, Guillem JG, Kemeny N, *et al.* p53 nuclear protein overexpression in colorectal cancer: a dominant predictor of survival in patients with advanced hepatic metastases. *J Clin Oncol* 1996; **14:** 2696-701.
33. Fan S, Smith ML, Rivet DJ, *et al.* Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. *Cancer Res* 1995; **00:** 1649-54.
34. Righetti SC, Della Torre G, Pilotti S, *et al.* A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. *Cancer Res* 1996; **56:** 689-93.
35. Rusch V, Klimstra D, Venkatraman E, *et al.* Aberrant p53 expression predicts clinical resistance to cisplatin-based chemotherapy in locally advanced non-small cell lung cancer. *Cancer Res* 1995; **55:** 5038-42.
36. Wahl AF, Donaldson KM, Fairchild C, *et al.* Loss of normal p53 function confers sensitization to Taxol by increasing G_2/M arrest and apoptosis. *Nat Med* 1996; **2:** 72-9.
37. Safran H, King T, Choy H, *et al.* p53 mutations do not predict response to paclitaxel/radiation for nonsmall cell lung carcinoma. *Cancer* 1996; **78:** 1203-10.
38. Miller CW, Simon K, Aslo A, *et al.* p53 mutations in human lung tumors. *Cancer Res* 1992; **52:** 1695-8.
39. Aas T, Borresen A-L, Geisler S, *et al.* Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat Med* 1996; **2:** 811-4.
40. Sandri MI, Isaacs RJ, Ongkeko WM, *et al.* p53 regulates the minimal promoter of the human topoisomerase II alpha gene. *Nucleic Acids Res* 1996; **24:** 4464-70.
41. Wang Q, Zambetti GP, Suttle DP. Inhibition of DNA topoisomerase II alpha gene expression by the p53 tumor suppressor. *Mol Cell Biol* 1997; **17:** 389-97.
42. Fry AM, Chresta CM, Davies SM, *et al.* Relationship between topoisomerase II level and chemosensitivity in human tumour cell lines. *Cancer Res* 1991; **51:** 6592-5.
43. Chin K-V, Ueda K, Pastan I, Gottesman MM. Modulation of the activity of the promoter of the human MDR1 gene by Ras and p53. *Science* 1992; **255:** 459-62.
44. Goldsmith ME, Gudas JM, Schneider E, Cowan KH. Wild type p53 stimulates expression from the human multidrug resistance promoter in a p53-negative cell line. *J Biol Chem* 1995; **270:** 1894-8.
45. Schneider J, Rubio MP, Barbazan MJ, Rodriguez-Escudero FJ, Seizinger BR, Castresana JS. P-glycoprotein, HER-2/*neu*, and mutant p53 expression in human gynecologic tumors. *J Natl Cancer Inst* 1994; **86:** 850-5.
46. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, Weinberg RA. Effects of an Rb mutation in the mouse. *Nature* 1992; **359:** 295-300.
47. Haas-Kogan DA, Kogan SC, Levi D, *et al.* Inhibition of apoptosis by the retinoblastoma gene product. *EMBO J* 1995; **14:** 461-72.
48. Li WW, Fan J, Hochhauser D, *et al.* Lack of functional retinoblastoma protein mediates increased resistance to antimetabolites in human sarcoma cell lines. *Proc Natl Acad Sci USA* 1995; **92:** 10436-40.
49. Bartkova J, Lukas J, Strauss M, Bartek J. Cyclin D1 oncoprotein aberrantly accumulates in malignancies of diverse histogenesis. *Oncogene* 1995; **10:** 775-8.
50. Quelle DE, Ashmun RA, Shurtleff SA, *et al.* Overexpression of mouse D-type cyclins accelerates G_1 phase in rodent fibroblasts. *Genes Dev* 1993; **7:** 1559.
51. Resnitsky D, Gossen M, Bujard H, Reed SI. Acceleration of the G_1/S phase by expression of cyclins D1 and E with an inducible system. *Mol Cell Biol* 1994; **14:** 1669.
52. Hinds PW, Dowdy SF, Eaton EN, Arnold A, Weinberg RA. Function of a human cyclin gene as an oncogene. *Proc Natl Acad Sci USA* 1994; **91:** 709.
53. Arber N, Doki Y, Han EK, *et al.* Antisense to cyclin D1 inhibits the growth and tumorigenicity of human colon cancer cells. *Cancer Res* 1997; **57:** 1569-74.
54. Han EK, Sgambato A, Jiang W, *et al.* Stable overexpression of cyclin D1 in a human mammary epithelial cell line prolongs the S-phase and inhibits growth. *Oncogene* 1995; **10:** 953-61.
55. Zhou P, Jiang W, Zhang YJ, *et al.* Antisense to cyclin D1 inhibits growth and reverses the transformed phenotype of human esophageal cancer cells. *Oncogene* 1995; **11:** 571-80.
56. Hochhauser D, Schnieders B, Ercikan-Abali E, *et al.* Effect of cyclin D1 overexpression on drug sensitivity in a human fibrosarcoma cell line. *J Natl Cancer Inst* 1996; **88:** 1269-75.
57. Bartkova J, Lukas J, Muller H, Strauss M, Gusterson B, Bartek J. Abnormal patterns of D-type cyclin expression and G_1 regulation in human head and neck cancer. *Cancer Res* 1995; **55:** 949-56.
58. Michalides R, van Veelen N, Hart A, Loftus B, Wientjens E, Balm A. Overexpression of cyclin D1 correlates with recurrence in a group of forty-seven operable squamous cell carcinomas of the head and neck. *Cancer Res* 1995; **55:** 975-8.
59. Tomida A, Suzuki H, Kim HD, Tsuruo T. Glucose-regulated stresses cause decreased expression of cyclin D1 and hypophosphorylation of retinoblastoma protein in human cancer cells. *Oncogene* 1996; **13:** 2699-705.

60. Medema RH, Herrera RE, Lam F, Weinberg RA. Growth suppression by $p16^{ink4}$ requires functional retinoblastoma protein. *Proc Natl Acad Sci USA* 1995; **92:** 6289-93.
61. Stone S, Dayananth P, Kamb A. Reversible, p16-mediated cell cycle arrest as protection from chemotherapy. *Cancer Res* 1996; **56:** 199-202.
62. Terry LA, Boyd J, Alcorta D, *et al.* Mutational analysis of the p21/WAF1/CIP1/SDI1 coding region in human tumor cell lines. *Mol Carcinog* 1996; **16:** 221-8.
63. Deng C, Zhang P, Harper JW, Elledge SJ, Leder P. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G_1 checkpoint control. *Cell* 1995; **82:** 675-84.
64. Waldman T, Lengauer C, Kinzler KW, Vogelstein B. Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21. *Nature* 1996; **381:** 713-6.
65. McDonald ER, III, Wu GS, Waldman T, El-Deiry WS. Repair defect in p21 WAF1/CIP1 −/− human cancer cells. *Cancer Res* 1996; **56:** 2250-5.
66. Sheikh MS, Chen YQ, Smith ML, Fornace AJ Jr. Role of p21Waf1/Cip1/Sdi1 in cell death and DNA repair as studied using a tetracycline-inducible system in p53-deficient cells. *Oncogene* 1997; **14:** 1875-82.
67. Li WW, Fan J, Hochhauser D, Bertino JR. Overexpression of $p21^{waf1}$ leads to increased inhibition of E2F-1 phosphorylation and sensitivity to anticancer drugs in retinoblastoma-negative human sarcoma cells. *Cancer Res* 1997; **57:** 2193-9.
68. Porter PL, Malone KE, Heagerty PJ, *et al.* Expression of cell-cycle regulators p27*Kip*1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients. *Nat Med* 1997; **3:** 222-5.
69. St Croix B, Florenes VA, Rak JW, *et al.* Impact of the cyclin-dependent kinase inhibitor p27*Kip*1 on resistance of tumor cells to anticancer agents. *Nat Med* 1996; **2:** 1204-10.
70. Nevins JR. E2F: a link between the Rb tumor suppressor protein and viral oncoproteins. *Science* 1992; **258:** 424.
71. Johnson DG, Cress WD, Jakoi L, Nevins JR. Oncogenic capacity of the E2F1 gene. *Proc Natl Acad Sci USA* 1994; **91:** 12823-7.
72. Singh P, Wong SH, Hong W. Overexpression of E2F-1 in rat embryo fibroblasts leads to neoplastic transformation. *EMBO J* 1994; **13:** 3329-38.
73. Qin XQ, Livingston DM, Kaelin WG, Adams PD. Deregulated transcription factor E2F-1 expression leads to S phase entry and p53-mediated apoptosis. *Proc Natl Acad Sci USA* 1994; **91:** 10918-22.
74. Nip J, Strom DK, Fee BE, Zambetti G, Cleveland JL, Hiebert SW. E2F-1 cooperates with topoisomerase II inhibition and DNA damage to selectively augment p53-independent apoptosis. *Mol Cell Biol* 1997; **17:** 1049-56.
75. Saito M, Helin K, Valentine MB, *et al.* Amplification of the E2F1 transcription factor gene in the HEL erythroleukemia cell line. *Genomics* 1995; **25:** 130-8.
76. Yamasaki L, Jacks T, Bronson E, Goillot E, Harlow E, Dyson NJ. Tumor induction and tissue atrophy in mice lacking E2F-1. *Cell* 1996; **85:** 537-48.
77. Lock RB, Ross WE. Inhibition of $p34^{cdc2}$ kinase activity by etoposide or irradiation as a mechanism of G_2 arrest in Chinese hamster ovary cells. *Cancer Res* 1990; **50:** 3761-6.
78. Goldwasser F, Shimizu T, Jackman J, *et al.* Correlations between S and G_2 arrest and the cytotoxicity of camptothecin in human colon carcinoma cells. *Cancer Res* 1996; **56:** 4430-7.
79. Ongkeko W, Ferguson DJ, Harris AL, Norbury C. Inactivation of Cdc2 increases the level of apoptosis induced by DNA damage. *J Cell Sci* 1995; **108:** 2897-904.
80. Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA, O'Connor PM. UCN-01: a potent abrogator of G_2 checkpoint function in cancer cells with disrupted p53. *J Natl Cancer Inst* 1996; **88:** 956-65.

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