

## Exploiting Cancer Cell Cycling for Selective Protection of Normal Cells

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### Abstract

**Chemotherapy of cancer is limited by its toxicity to normal cells. On the basis of discoveries in signal transduction and cell cycle regulation, novel mechanism-based therapeutics are being developed. Although these cell cycle modulators were designed to target cancer cells, some of them can also be applied for a different purpose, *i.e.*, to protect normal cells against the lethality of chemotherapy. Loss of sensitivity of cancer cells to cell cycle inhibitors can be exploited for selective protection of normal cells that retain this response. Indeed, inhibition of redundant or overactivated pathways (*e.g.*, growth factor-activated pathways) or stimulation of absent pathways in cancer cells (*e.g.*, p53, Rb, and p16) may not arrest cycling of cancer cells. But growth arrest of normal cells will then permit selective killing of cancer cells by cycle-dependent chemotherapy.**

### Introduction

Major research efforts are aimed at discovery of molecular targets that are specific for cancer cells, development of agents that are toxic to cancer cells, and at devising their synergistic combinations. In the last 20 years, molecular mechanisms of malignant transformation have been uncovered: autocrine secretion of growth factors (platelet-derived growth factor/*sis*, insulin-like growth factor, EGF,<sup>2</sup> and TGF- $\alpha$ ), overexpression of their receptors (*erbB/HER-2*), activation of signal transduction pathways (Ras, Src, Raf, and Bcr-Abl), overexpression of cyclins (cyclins D1 and E) and oncogenic transcription factors governing the cell cycle (*c-myc* and E2F), and loss of tumor suppressors (p53, p16, and BRCA1; Refs. 1–5). Identification of potential targets for therapeutic intervention fuels hope for a cure of cancer (6–9). Inhibitors of growth factor receptors, inhibitors Ras and phosphatidylinositol-3, Abl, Raf-1, and MEK kinases, and inhibitors of CDKs are currently undergoing clinical trials (6, 10–14). STI 571, an inhibitor of Abl, has already demonstrated high efficacy in the treatment of Ph(+) leukemia, a malignancy characterized by the expression of Bcr-Abl (15). Small molecule therapeutics have been designed to target mutant p53 (16–18). Telomerase, an enzyme required for maintenance of chromosome ends during cell division, is a promising target (19).

This brief overview shows that molecular mechanisms of cancer have been sufficiently elucidated to propose and develop molecular therapeutics. However, with a few exceptions, cancer cells lack sufficiently specific targets that would allow one to kill them selectively, and mechanism-based toxicities to normal cells are expected. Side effects of novel therapeutics include toxicity to bone marrow and the

gastrointestinal tract, dermatological toxicity, cardiotoxicity, and as-thenia (6, 13, 20–24). Do these toxicities reflect imperfections of currently used compounds? Will the next generation of mechanism-based drugs kill cancer cells exclusively?

Several problems were summarized previously (7): lack of cancer-specific targets, multiple genetic alterations in cancer, loss-of-function mutations, and development of drug resistance. Lack of cancer-specific targets is perhaps initially the most significant problem (25). In fact, except in rare instances, cancer cells may provide no exclusive target that is not essential for proliferation and survival of some normal cells. Although a therapeutic window (toxicity to cancer cells but not to normal cells) might be achieved, toxicity to normal cells will continue to limit chemotherapy of cancer, unless new treatment paradigms are developed.

Although the number of mutations that actually sustain the malignant phenotype is limited (7), characteristics of cancer cells from different patients may vary widely. For example, activators of the mitogen-activated protein kinase pathway arrest growth of cancer cells in a cell line-dependent manner (26). Because specific mutations and alterations in cancer cells of any particular patient are usually unknown, their identification as a basis for individual treatment will require extensive genetic/phenotypic mapping.

Independence from growth factors (autonomic growth) of cancer cells seemingly makes matters even worse. Many potential therapeutics are intended to target growth factor-activated pathways. Tumor cells can, however, enter and traverse the cell cycle in the absence of normal mitogenic signals. If downstream growth signaling pathways that drive the cell cycle are constantly activated in cancer cells, their upstream and parallel growth factor-activated pathways become non-essential for tumor growth. Also, loss of p53, p16, and Rb and overexpression of cyclins D and E abrogate breaks of the cycle. This is consistent with loss of sensitivity of cancer cells to growth inhibition (5).

### Direct Targeting of Cancer Cells by Exploiting Uncontrolled Growth

Another strategy is to exploit the vulnerability of transformed cells to apoptosis, referred to as “Achilles heels” (Fig. 1). Under growth-limiting conditions, normal cells may undergo growth arrest, whereas transformed cells (with the activated “downstream” oncogenes: E2F, *myc*, and others) may undergo apoptosis (27–30). Oligopeptides that prevent interaction of E2F with cyclin A/CDK2 killed certain transformed but not normal cells (31, 32). However, apoptosis is often evaded in cancer (33, 34).

Loss of tumor suppressors such as p53, p16, and Rb (35–37) and overexpression of certain proteins such as HSI1X1 weaken the G<sub>1</sub> and/or G<sub>2</sub> checkpoints (38). This sensitizes cells to certain anticancer drugs (36, 39–41). Further disruption of cell cycle checkpoints in cancer cells may improve therapeutic index (42, 43). Pharmacological abrogation of G<sub>2</sub> checkpoints can increase sensitivity to chemotherapy in G<sub>1</sub> checkpoint-deficient cells (Fig. 2), whereas cells with normal checkpoints may take refuge in G<sub>1</sub>. After DNA damage, caffeine,

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<sup>2</sup>The abbreviations used are: EGF, epidermal growth factor; TGF, transforming growth factor; MEK, mitogen-activated protein kinase kinase; BHK, baby hamster kidney; CDK, cyclin-dependent kinase; Rb, retinoblastoma; PKC, protein kinase C; 5-FU, 5-fluorouracil.

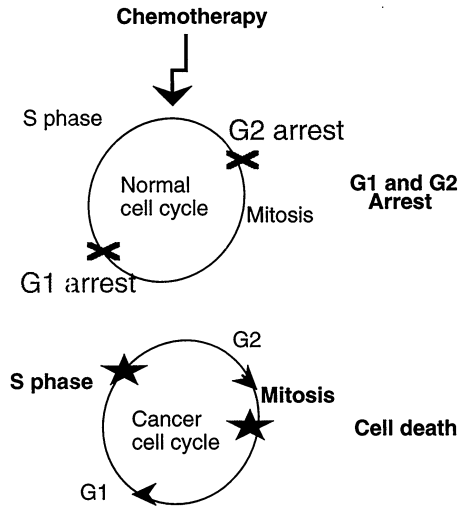


Fig. 1. "Achilles heel": enhanced cytotoxicity of chemotherapy to cancer cells lacking  $G_1$  and  $G_2$  checkpoints. As a desirable outcome of chemotherapy, normal cells undergo growth arrest in  $G_1$  and  $G_2$ , whereas cancer cells lacking  $G_1$  and  $G_2$  checkpoints enter S-phase and mitosis, where they are susceptible to chemotherapy.

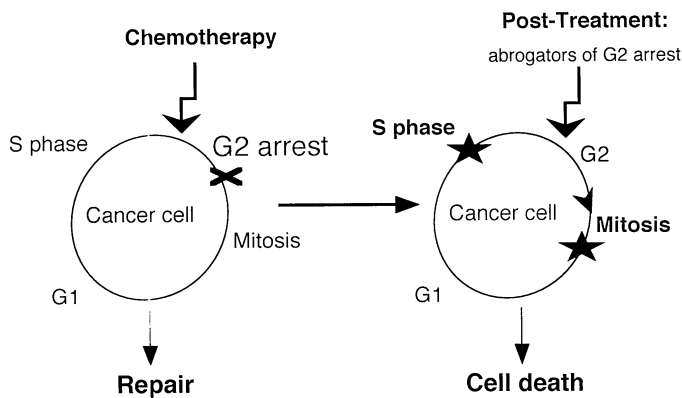


Fig. 2. Pharmacological abrogation of  $G_2$  checkpoint in cancer cells. In contrast to the optimal scenario in Fig. 1, many cancer cells may activate the  $G_2$  checkpoint in response to chemotherapy. Posttreatment with pharmacological abrogators of the  $G_2$  checkpoint results in cell death. In this scenario, normal cells take refuge in  $G_1$  arrest.

pentoxifylline, and UCN-01 can abrogate the  $G_2$  checkpoint, leading to mitotic catastrophe (44–51). Posttreatment with pentoxifylline selectively enhanced the cytotoxicity of DNA-damaging agents to cancer cells (47). Because loss of cell cycle checkpoints is the most universal alteration in human cancer (52, 53), common strategies might be developed against a wide variety of cancers as a more promising approach than unspecific attempts to block the cancer cell cycle (54). Importantly, sensitization of cancer cells by abrogating their checkpoints mirrors desensitization (protection) of normal cells by activating their checkpoints (Fig. 2 versus Fig. 3).

A strategy designed to protect normal cells from cycle-dependent lethal agents by exploiting differences in cell cycles of normal and cancer cells was proposed in 1975 (55). Because we cannot inhibit cycling of cancer cells as easily as cycling of normal cells, we can turn this disadvantage to a therapeutic advantage. We can target normal cells without affecting cancer cells. At that time, this approach was limited by insufficient knowledge of cell cycle regulation and by the lack of molecular therapeutics that target the cell cycle. The way of history is a spiral, ever repeating itself. This time, the molecular tools have become available to exploit even the tiniest differences between normal and cancer cell cycles. Initially unforeseen, remarkable advances in mechanism-based therapeutics may enable exploitation of

loss of sensitivity of cancer cells to cell cycle inhibitors for selective protection of normal cells that retain this response.

### The Restriction Point of the Cell Cycle: Initial Observations

Even before the discovery of oncogenes and growth factors, malignant growth was characterized by uncontrolled cell cycle. This observation offered a basis for selective protection of normal cells against lethal anticancer agents that are cell cycle phase specific (55). In initial work, normal BHK cells but not tumorigenic polyoma virus-transformed BHK cells (PyBHK) were arrested by physiological deprivation of serum or by metabolic inhibitors (55). Such pretreatments prevent normal cells from entering S or M phase and thus protect them against S-phase- and M-phase-specific agents. In contrast, polyoma virus-transformed cells were not killed under these conditions. Growth arrest and protection of cells was achieved by using nonselective cell cycle inhibitors (cycloheximide, an inhibitor of translation, and L-histidinol, a structural analogue of the essential amino acid L-histidine and a reversible inhibitor of protein biosynthesis; (56–58). However, nonspecific cell cycle inhibitors (e.g., cycloheximide and actinomycin D) are clinically too toxic. Furthermore nonspecific inhibitors of metabolism may arrest growth of cancer cells. For example, pretreatment of human breast cancer cells with fluorouracil antagonized effects of paclitaxel *in vitro* (59). This perhaps precluded the development of therapeutic regimes to protect normal cells. However, in sequence-dependent clinical trials, less bone marrow toxicity was found when DNA-damaging drugs were administered before the M-phase-specific agent paclitaxel (60, 61). Retrospectively, we can speculate that the antagonism between DNA-damaging drugs and paclitaxel was translated in the clinic as a decrease in side effects on normal cells.

### Mechanism-based Selective Protection of Normal Cells

**Exploiting Loss of p53-dependent Checkpoints.** DNA damage results in induction of wild-type p53 and p21, a CDK inhibitor, which

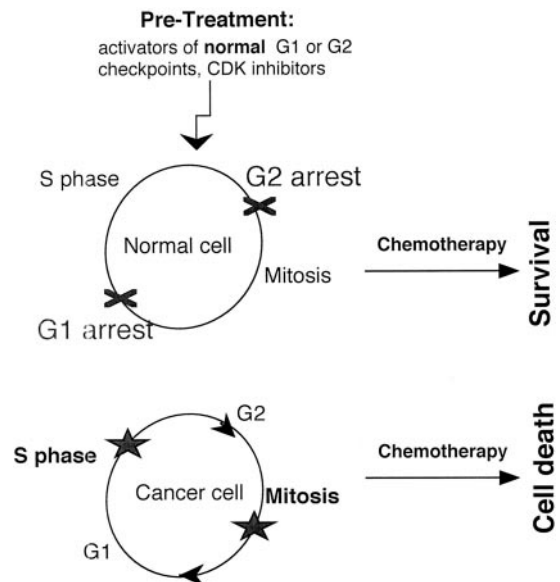


Fig. 3. Selective protection of normal cells by pharmacological pretreatment. *Top cycle*, the pretreatment of a normal cell with modulators of growth regulatory pathways causes  $G_1$  and/or  $G_2$  growth arrest. Thereby, the cell become less sensitive to chemotherapy. *Bottom cycle*, the pretreatment has no effect on a cancer cell, because of absent (e.g., Rb  $-/-$ ) and dispensable targets or resistance to pharmacological pretreatment. Chemotherapy then still kills the cancer cell.

in turn results in Rb-dependent growth arrest in G<sub>1</sub> and/or G<sub>2</sub> phases (62–64). In contrast, certain p53- or p21-deficient cancer cells undergo mitosis (36, 35). This small difference could be exploited therapeutically. At low concentrations, DNA-damaging drugs such as doxorubicin or etoposide can induce p21-dependent growth arrest without cell death, thereby protecting cells from the cytotoxicity of microtubule-active drugs such as paclitaxel and vincristine, which kill mitotic cells. Specifically, pretreatment with 25–50 ng/ml doxorubicin for 16 h caused p53- and p21-dependent arrest in HCT116 cells. Treatment with 50 ng/ml of paclitaxel for 2–3 days resulted in selective killing of checkpoint-deficient cells lacking p53 or p21 (65). Overexpression of p21 alone was less protective than treatment with DNA-damaging drugs, indicating that p21 was necessary but not sufficient for cytoprotection. Unlike p21, low doses of doxorubicin caused efficient G<sub>2</sub> arrest (65). However, even at moderate doses, doxorubicin may cause a senescence-like cell phenotype and terminal growth arrest in HCT116 cells (66). The task will be to identify p53-inducing compounds that are less toxic and are more reversible than doxorubicin. For example, ribonucleotide depletion induces p53-dependent reversible cell cycle arrest in the absence of detectable DNA damage (67–69). Another problem is that DNA-damaging drugs can induce G<sub>2</sub> arrest by p53-independent mechanism involving the Chk1 kinase (70, 71), and therefore some cancer cells lacking p53 may also become arrested in G<sub>2</sub> because of activation of Chk1 (71). This arrest, however, can be abrogated by low doses of UCN-01, which inhibits Chk1.

**Exploiting Growth Factor-activated Pathways.** Tumor cells can cycle in the absence of normal mitogen stimuli. Inhibition of normal cells' growth factor-activated pathways (growth factors and their receptors and Ras/mitogen-activated protein kinase) may provide a promising strategy for selective protection of normal cells. For example, dependence from EGF is often lost in malignant transformation (72). Thus, EGF-dependent immortalized MCF-10A breast cells undergo G<sub>0</sub>-G<sub>1</sub> arrest after EGF withdrawal, unlike EGF-independent MCF-7 cancer cells. Low concentrations of AG1478, an inhibitor of the EGF receptor kinase, therefore, arrested MCF-10A but not MCF7 cells. As a result, AG1478 abrogated the lethality of paclitaxel to MCF-10A cells but not to MCF-7 cells (73).

MEK inhibitors such as PD098059 are designed to exert cytostatic or preferably cytotoxic activity. This inhibition was demonstrated in normal fibroblasts and in cells transformed by oncogenes, the activity of which strictly depends on MEK (11). As compared with normal cells, PD 098509 inhibited MEK without arresting growth of some cancer cells (74).

Inhibitors of PKC $\alpha$  are being developed for killing cancer cells. Targeting PKC $\alpha$  can also be considered for selective growth arrest and protection of normal cells, because PKC $\alpha$  seems to be dispensable for growth of many cancer cell lines. UCN-01 kills cancer cells at doses that are higher than those necessary to inhibit PKC $\alpha$ , by affecting other targets (49, 75). But less toxic and reversible compounds should specifically protect normal cells. For example, GF 109203X inhibits PKC $\alpha$  and is only weakly cytostatic to cancer cells, even at high doses (75). Although both UCN-01 and GF 109203X inhibit PKC $\alpha$ , UCN-01 was selected as an anticancer drug because of its higher cytotoxicity (76). Except for a selective growth arrest of PKC-dependent normal cells, one would choose the less toxic GF 109203X. The protein kinase inhibitor staurosporine, at low concentrations, had no effect on the cell cycle progression of tumor cells but arrested normal cells in G<sub>1</sub> phase (77, 78). It has been proposed that low concentrations of staurosporine can be used to selectively protect normal cells against the cytotoxic drugs that target proliferating cells (77–79). In fact, protection of normal proliferating cells against chem-

otherapy by staurosporine-mediated, selective, and reversible G<sub>1</sub> arrest has been demonstrated (80).

Normal bone marrow and epithelial cells are particularly vulnerable to chemotherapy. Damage to the normally replacing tissues of the body, specifically the gastrointestinal tract, limits the treatment and, hence, cure rate of cancer patients. TGF- $\beta$  inhibits proliferation of hematopoietic progenitor cells and normal epithelial cells and protects these cells from the toxicity caused by vinblastine, vincristine, etoposide, paclitaxel, Ara-C, methotrexate, or 5-FU (81). The protected cells can then reenter the cell cycle. Furthermore, TGF- $\beta$  protected mice from the lethal doses of 5-FU and doxorubicin (82) and reduced the severity and duration of oral mucositis induced by 5-FU *in vivo*. TGF- $\beta$  protected small intestinal clonogenic stem cells from radiation damage, reducing diarrhea and animal mortality (83). Administration of topical TGF- $\beta$  prior to chemotherapy with 5-FU significantly reduced the severity of mucositis and chemotherapy-associated weight loss and increased survival (84). The sensitivity to TGF- $\beta$  is lost during transformation of epithelial cells. Although serving as a good illustration of the idea of selective protection, problems with its distribution may decrease TGF- $\beta$  usefulness, because it is a protein, not a small molecular therapeutic. Searches for compounds that similarly reversibly inhibit proliferation of hematopoietic and epithelial cells will be especially valuable.

**Exploiting CDK Inhibitors.** p16 and p21, CDK inhibitors, can prevent drug-induced apoptosis by arresting growth (9, 85). It has been shown that overexpression of p16 or p21 caused reversible growth arrest and resistance to methotrexate, cisplatin, vinblastine, and paclitaxel (86–88). Therefore, p16-inducing agents may selectively arrest growth of normal cells, because loss or inactivation of p16 is common in tumors (89). (To avoid a confusion, it is important to emphasize that therapeutic reactivation of p16 in cancer cells is intended to correct defects in cancer cells. Once corrected, however, the loss of p16 could not be exploited for selective cytoprotection of normal cells.) Although p21 is not lost in cancer cells, the sensitivity to p21 may be decreased in cancer cells (26, 90). This also can be used for a therapeutic advantage. In contrast to p16 and p21, another CDK inhibitor, p27, does not seem to be a suitable candidate for cytoprotection (88). p21 and p27 may also act as positive activators of proliferation (91), thereby potentially providing a means for selective lack of arrest of cancer cells. Inhibition of CDK2 may represent a therapeutic strategy for preventing of chemotherapy-induced alopecia by arresting the cell cycle and reducing the sensitivity of the epithelium to many cell cycle-active anticancer agents (92).

Rb is often directly or indirectly inactivated in cancer. Rb acts as a central mediator of the proliferative block induced by a diverse range of DNA-damaging stimuli (93) and kinase modulators. Its loss can prevent growth arrest by CDK inhibitors (62, 94, 95).

**Exploiting Drug Resistance.** The development of multidrug resistance is a canonical example of limitations of cancer therapy. Numerous cytostatic drugs are pumped out from multidrug resistance cells; therefore, they continue to proliferate, whereas normal cells undergo growth arrest. But this phenomenon can be exploited for selective protection of normal cells against cytotoxicity caused by the cytotoxic drug that target proliferating cells (96). After treatment with a drug that arrests normal but not the resistant cancer cells, a second drug to which multidrug-resistant cells are not cross-resistant should kill only the proliferating cells (96).

## Conclusion

Diverse strategies in cancer therapy are emerging, which are often based on opposite approaches. As examples, pharmacological inactivation of wild-type p53 can protect host tissues against lethal DNA

damage, without affecting tumor cells lacking p53 (97). In contrast, slight activation of wild-type p53 by low levels of DNA damage can protect cells with normal checkpoints against subsequently added microtubule-active drugs. This diversity of approaches may be proved useful. Importantly, they may share therapeutic agents; the same drug can be used either to induce growth arrest, to abrogate a checkpoint, or to induce apoptosis, depending on cell type, drug dose, and its combinations with other drugs. For example, UCN-01 was initially viewed as a drug to inhibit growth and kill cancer cells (98). However, further utility is indicated by its ability to inactivate checkpoint kinases and thus sensitize cells to cycle-dependent chemotherapy (50). The notion that it is easier to arrest the growth of normal cells than cancer cells is also the basis for the therapy aimed at angiogenesis by targeting endothelial cells (99).

Toxicity to normal cells is unavoidable, unless drugs have a highly specific cancer cell target. The idea of exploiting defective control of cancer growth for selective protection of normal cells may help to decrease side effects. As we have presented here, pretreatment with a cytostatic drug can be designed to selectively block cycling of normal cells. Searches for such compounds will probably often lead to mechanism-based therapeutics that were initially considered to target cancer cells.

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