Analysis of Cell-Cycle Checkpoint Pathways in Head and Neck Cancer Cell Lines

Implications for Therapeutic Strategies

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Objective: To determine the mechanism of action of paclitaxel (Taxol) and carboplatin in cell lines of head and neck squamous cell carcinoma (HNSCC).

Design: Four HNSCC cell lines were treated with paclitaxel and carboplatin, alone or in combination, and evaluated for cell-cycle position by means of flow cytometry, for molecular determinants of cell cycle by means of Western blotting and kinase analysis, and for anchorage-independent growth by means of soft-agar assays.

Results: Paclitaxel was more effective at inducing apoptosis and inhibiting anchorage-independent cell growth, compared with carboplatin. The activity of paclitaxel was correlated with an elevation of cyclin B1/CDC2 activity, prolonged mitotic arrest, and Bcl-2 phosphorylation. In contrast, carboplatin arrested cells before mitosis. Combination treatment with both agents, simultaneously or sequentially, was more effective at inhibiting cell growth than either single agent. Cellular outcome was the same regardless of which drug was used first. The order of addition of these 2 drugs differentially affected cell-cycle position. Paclitaxel pretreatment arrested cells in mitosis, whereas carboplatin pretreatment arrested cells before mitosis. Combination treatment with both agents, simultaneously or sequentially, resulted in premitotic arrest.

Conclusions: To our knowledge, this study is the first to explore how paclitaxel and carboplatin, alone or in combination, differentially affect cell-cycle checkpoint response and HNSCC cell growth. These results provide molecular validation for the current clinical use of both drugs in combination and set the stage for analyses of patient tumor specimens.


Strategies for the treatment of squamous cell carcinoma of the head and neck (HNSCC) have traditionally involved surgery and radiotherapy. For stages I and II cancer, this technique yields a 5-year survival rate of 70% to 95%. Unfortunately, more than 60% of HNSCC presents as locally advanced, stages III and IV cancer. Despite aggressive surgery and radiotherapy, disease-free survival is less than 30% at 3 years in this latter population. In patients with stages III and IV cancer, efforts have been made to provide better functional and oncological outcomes with various chemotherapeutic agents. Two agents used increasingly in the treatment of HNSCC are paclitaxel (Taxol) and carboplatin.

Paclitaxel is a natural product from the bark of the western yew tree and one of a new class of agents known as taxanes. The effects of paclitaxel are correlated with tubulin polymerization and stabilization and subsequent arrest of the cells in mitosis. Phase 2 studies have indicated a response rate to paclitaxel of 20% to 43% when it is used as a single agent in HNSCC, with a median survival of more than 9 months and a 1-year survival of 33%.

Carboplatin intercalates into DNA to form a bifunctional covalent link that interferes with DNA synthesis in the S phase of the cell cycle. Used as a single agent, carboplatin has yielded a response rate of 26% in initial phase 1 trials in HNSCC.

When combined in the treatment of HNSCC, paclitaxel and carboplatin have better overall clinical response rates ranging from 32% to 54%. In a study of advanced stages III and IV tumors, the combination of paclitaxel and carboplatin resulted in complete histological response in 28% of patients. Clinically, some of the most encouraging results have come from the use of radiotherapy administered concurrently with paclitaxel and carboplatin. In phase 2 trials in patients with advanced HNSCC, response rates of 90% to 100% have been reported with concomitant administration of paclitaxel, carboplatin, and radiotherapy, with histological response in as many as 66%.
patients with metastatic cervical adenopathy at presentation, this regimen has resulted in histologically proven negative findings in neck specimens in 63% to 71%.14-16 Although numerous clinical trials have evaluated the efficacy of chemotherapeutic agents used to treat HNSCC, little attention has been focused on drug mechanism, specifically as it relates to cell-cycle checkpoint signaling.

Normal eukaryotic cells progress through the cell cycle in a regulated manner owing to a cascade of biochemical events that coordinates the transition of cells from one phase to another. During a normal cell cycle, the completion of mitosis is followed by the G1 phase, in which a regulated series of events must take place before entry into the S phase. These events include elevations in D- and E-type signaling pathways. These checkpoint pathways monitor cellular integrity and ensure the completion of one phase of the cell cycle before initiation of the next phase. When activated by various forms of cellular or genotoxic stress, checkpoint signaling can halt cell-cycle progression if abnormalities such as DNA damage, aneuploidy, or mitotic spindle anomalies exist. At the G1/S-phase transition, p53, pRb, and a host of CDK inhibitors (p21Waf1/Cip1, p27Kip1, p57Kip2, and p16INK4A) are necessary for checkpoint function.19 Arrest of the G1 phase mediated by p53 depends on p21Waf1/Cip1 (p21) transactivation; embryonic fibroblasts from mice null for p21 (p21−/−) demonstrate a defective G1 checkpoint after genotoxic stress.20 In eukaryotic cells, progression from the G2 phase into mitosis depends on the activity of cyclin B1/CDC2,21 and is negatively regulated by CDC2 phosphorylation on threonine 14 and tyrosine 15 residues.22,23 Phosphorylation of CDC2 is regulated by the opposing effects of the activating CDC25C phosphatase and inhibitory protein kinases, Wee1 and Myt1.24 Phosphorylation of CDC2 appears to play a critical role in enforcing the G2-phase cell-cycle checkpoint after DNA damage. During mitosis, the spindle checkpoint monitors spindle microtubule structure and chromosome alignment.25

In the current study, we explored how the chemotherapeutic agents paclitaxel and carboplatin modulate normal eukaryotic cell proliferation and entry into mitosis specifically as it relates to cell-cycle checkpoint signaling.
cell-cycle events in HNSCC cell lines. A panel of HNSCC cell lines were treated with various combinations of paclitaxel and carboplatin, and the effects on cell-cycle progression, checkpoint signaling pathways, and cell growth were examined. Our primary objective was to gain insight into the mechanisms of drug action as they pertain to cell-cycle checkpoints in HNSCC. The ultimate goal is to use these preclinical molecular findings to devise improved clinical trials of paclitaxel and carboplatin and to provide clues to additional molecular mechanisms that can be targeted for the discovery of new drugs for the treatment of HNSCC.

RESULTS

EFFECTS OF PACLITAXEL AND CARBOPLATIN ON CELL-CYCLE PROGRESSION

To determine the effects of paclitaxel and carboplatin on cell-cycle progression, we initially treated 2 HNSCC cell lines, UNC-7 and UM-38, with each drug and observed the effects for 72 hours by means of flow cytometry (Figure 1). Treatment of the cells with paclitaxel (100 nmol/L) caused a significant increase in cells at the G2/M phase, with most cells arrested in mitosis by 24 hours. By 48 to 72 hours, a significant fraction of the UNC-7 cells had a subdiploid DNA content indicative of apoptosis (Figure 1). The increase in the percentage of the population with a subdiploid DNA content was accompanied by the appearance of cells with nuclear blebbing and chromatin condensation as visualized by staining with 4′,6-diamidino-2-phenylindole (data not shown). The UM-38 line underwent a similar arrest of the G2/M phase by 24 hours after paclitaxel treatment; however, most of the UM-38 cells maintained this arrest through 72 hours (Figure 1). Carboxatin (100 µmol/L) treatment of both cell lines caused an increase in the S-phase fraction between 18 and 24 hours and an eventual arrest of the UM-38 cells at the G2/M phase.

Since these 2 chemotherapeutics are administered concurrently in the clinical setting, we next examined the effect of simultaneous addition of paclitaxel (100 nmol/L) and carboplatin (100 µmol/L) to both cell lines in culture. The concurrent treatment of UNC-7 cells with both drugs resulted in a flow cytometric profile that was similar to that observed after treatment of the cells with paclitaxel alone; however, arrest of the G2/M phase was attenuated, with more cells remaining in the S-phase fraction (Figure 1). Simultaneous treatment of the UM-38 cells with both drugs also resulted in a similar cell-cycle profile to that observed with paclitaxel alone (Figure 1).

EFFECTS OF PACLITAXEL AND CARBOPLATIN ON CHECKPOINT SIGNALING PATHWAYS

To gain insight into the molecular mechanisms behind the differential cell-cycle responses and outcomes observed after paclitaxel and carboplatin treatment, alone or in combination, we analyzed the drug-induced modulation of cell cycle and cell viability relative to the levels and activity of proteins involved in cell-cycle checkpoint signaling. For all subsequent experiments, we analyzed the following 4 HNSCC cell lines: UNC-7, UNC-10, UM-14C, and UM-38. All 4 cell lines were treated with paclitaxel (100 nmol/L), carboplatin (100 µmol/L), or a combination of both drugs. Cell-cycle and molecular analyses were performed at 6, 12, 24, and 36 hours after treatment. Paclitaxel induced an accumulation of cells in the G2/M phase by 24 hours in all cell lines. Carboplatin treatment stimulated an increase in the fraction of S-phase cells in all cell lines when used as a single agent. When given in combination with paclitaxel, carboplatin uniformly attenuated arrest of the G2/M phase. Thus, regardless of the differing genetic alterations present in the 4 tumor cell lines under examination, modulation of cell-cycle position was relatively similar after a given drug treatment. These observations prompted an examination of the levels, phosphorylation status, and activity of selected cell-cycle regulatory proteins.

Cellular response to genotoxic agents and microtubule inhibitors has been linked to p53-mediated signaling. Thus, we examined p53 protein levels and activity by means of Western blotting. We assessed p53 activity by looking for increased levels of the p53 downstream target gene product, p21. We observed a modest increase in p53 levels after 36 hours of carboplatin treatment, alone or in combination with paclitaxel, in the UNC-7 cells only (Figure 2A). However, the increase in p53 level was not sufficient to induce expression of the downstream target gene p21, suggesting that the p53 signaling pathway is altered in this cell line. Levels of p53 were significantly elevated and not altered when compared with controls in the remaining lines, consistent with the presence of a mutant p53 protein (Figure 2B-D).
To further analyze the p53 status of the 4 cell lines, we compared, side by side, the basal p53 and p21 protein levels in all 4 HNSCC cell lines under control conditions (Figure 2E). Levels of p53 protein were elevated in the UNC-10, UM-14C, and UM-38 cells compared with the UNC-7 cells, consistent with the presence of mutant
p53 in the first 3 lines. In parallel, UNC-7 cells were treated with doxorubicin, an anticancer agent that induces stabilization of only wild-type p53 protein and elevation of p21 protein levels in cells containing functional, wild-type p53. After doxorubicin treatment, we performed Western blotting to compare the relative levels of p53 and p21 proteins under control and treated conditions with those observed in a well-characterized isogenic set of colon carcinoma epithelial cell lines that contains wild-type p53 (HCT116) or is null for p53 (HCT116 p53−/−) owing to homologous recombination at the p53 locus. As seen in Figure 2E, relative to the robust elevation in p53 and p21 protein levels in the HCT116 cells, the elevation in p53 and p21 protein levels in the UNC-7 cells was minimal. Furthermore, the very low level p53-independent elevation of p21 observed in the HCT116 p53−/− cells after doxorubicin treatment was similar to that observed in the UM-14C and the UM-38 cells after paclitaxel and carboplatin treatment (Figure 2C-D). The doxorubicin-induced, p53-independent elevation of p21 levels was not sufficient to inhibit CDK activity in the HCT116 p53−/− cells as previously shown by Flatt et al, and it was not sufficient to induce cell-cycle arrest in the UNC-7 and UNC-10 cells.

Several studies indicate that the mitotic arrest mediated by microtubule inhibitors such as paclitaxel may result in cytotoxicity due to alteration of normal mitotic signal transduction pathways, including prolonged CDC2 activity. Numerous studies suggest a link between CDC2 activation and apoptosis. In the current study, all of the HNSCC cells arrested with a 4N DNA content by 24 hours after paclitaxel treatment. The cell-cycle arrest was accompanied by elevated levels of cyclin B1 protein and cyclin B1–immunoprecipitable kinase activity (Figure 2A-D). The presence of a faster migrating or hypophosphorylated form of CDC2 after paclitaxel treatment was consistent with transition into mitosis and elevated levels of cyclin B1/CDC2 activity. We verified that paclitaxel induced mitotic arrest in all 4 HNSCC cell lines when we detected significant increases in MPM-2 epitope positivity on results of Western blotting (Figure 2A-D). A previous study by Davis et al has shown that the MPM-2 antibody recognizes phosphorylated protein epitopes found only in mitotic cells. Thus, the MPM-2 antibody can distinguish 4N DNA-containing mitotic cells from those in G2 phase. The length of the paclitaxel-mediated elevation in CDC2 activity varied among the different cell lines; however, a peak in activity preceded the accumulation of subdiploid cells that was observed at 24 hours after treatment.

Previously, sensitivity to anticancer agents has been shown to be influenced by alterations in posttranslational modifications of Bcl-2 family members, including the antiapoptotic protein Bcl-2. After paclitaxel treatment, Bcl-2 phosphorylation was evident in all the HNSCC cell lines by 12 hours, when cells first began to accumulate in mitosis and to acquire cyclin B1/CDC2 activity (Figure 2A-D). When peak levels of cyclin B1/CDC2 activity were apparent, there was a significant conversion of Bcl-2 to the phosphorylated forms in all the HNSCC cell lines. In fact, we observed a correlation between the relative levels of cyclin B1/CDC2 activity and Bcl-2 phosphorylation.

Significant differences in the regulation of select cell-cycle signaling pathways accompanied the differential cell-cycle arrest induced by carboplatin in all the HNSCC cell lines (Figure 2A-D). Carboplatin treatment resulted in an almost complete inhibition of cyclin B1/CDC2 activity, which was likely integral for the observed S-phase arrest. Between 24 and 36 hours, we saw a significant conversion of CDC2 from the hypophosphorylated, active form in the control cells to the hyperphosphorylated, inactive form of CDC2. Consistent with these changes in the phosphorylation status of CDC2 was an almost complete loss of cyclin B1–immunoprecipitable kinase activity and the absence of MPM-2 positivity on results of Western blotting. When carboplatin was given simultaneously with paclitaxel, attenuation of the paclitaxel-induced elevation in cyclin B1/CDC2 activity, mitotic entry, and Bcl-2 phosphorylation were seen in all 4 cell lines examined (Figure 2A-D).

**ALTERATION OF CELL-CYCLE SIGNALING AFTER DIFFERENTIAL ORDER OF DRUG ADDITION**

Because simultaneous treatment of the HNSCC cells with paclitaxel and carboplatin led to an attenuation of mitotic arrest, we explored whether pretreatment of the cells with one drug before addition of the second would provide clues to the molecular changes that may be consistent with apoptosis. To accomplish this, parallel experiments were designed in which HNSCC cell lines were subjected to one drug (paclitaxel or carboplatin) for 8 hours, followed by cotreatment with the second drug for 6, 12, 24, and 36 hours (Figure 3).

In the first set of experiments, cells were treated with paclitaxel for 8 hours, then treated with carboplatin. After this treatment regimen, an arrest of the G2/M phase occurred at 6 to 12 hours and the subdiploid population increased at 24 to 36 hours in the UNC-7, UNC-10, and UM-14C cells (Figure 3). The increase in S-phase fraction seen in the previous carboplatin treatments was reduced with this treatment regimen. As seen in the experiment, the UM-38 line maintained the arrest of the G2/M phase (Figure 3D). The same lines were exposed to carboplatin for 8 hours before paclitaxel treatment. The accumulation of a G2/M-phase population was delayed in all lines except the UM-38 cells (Figure 3). In the UM-38 cells, there was a transient elevation in S-phase cell levels at 6 to 12 hours, followed by an arrest of cells with a 4N DNA content by 24 hours (Figure 3). The remaining lines, UNC-7, UNC-10, and UM-14C, accumulated cells in the S phase without the appearance of a G2/M-phase arrest, similar to carboplatin treatment (Figure 3).

To determine what effect order of addition of paclitaxel and carboplatin had on molecular cell-cycle determinants, we examined the G1/S-phase checkpoint regulators, p53 and p21, and the mitotic markers examined in previous treatments (Figure 4). Modulation of p53 was not significantly different from that shown in the previous section. Levels of mutant p53 remained unchanged in the UM-14C, UM-38, and UNC-10 cells (Figure 4B-D). In the UNC-7 cells, p53 levels increased with the carboplatin pretreatment schedule, but not with paclitaxel pretreatment (Figure 4A). Again, p21 was regu-
lated in a p53-independent manner in the UM-14C and UM-38 cells (Figure 4C-D). In the UM-38 cells, a decrease in p21 protein was seen by 24 to 36 hours after pretreatment with paclitaxel or carboplatin. This latter change is likely the result of altered downstream p53 signaling. Again, as in the previous section, these differences did not significantly affect the cell-cycle kinetics.

Perhaps the most accurate molecular indicators of mitosis in paclitaxel-treated cells were the levels of MPM-2, Bcl-2, and cyclin B1 and the activity of CDC2.
In the UNC-7, UNC-10, and UM-38 cells pretreated with paclitaxel for 8 hours before the addition of carboplatin, the mitotic arrest was prolonged compared with the simultaneous treatment regimen (Figure 4A, B, D). Coadministration resulted in the mitotic markers reverting from the mitotic form to the premitotic form be-
tween 12 and 24 hours. In contrast, after paclitaxel pre-
treatment, the mitotic indices were still present at 24
hours, consistent with a prolonged mitotic arrest. In the
UM-14C cells, the patterns for simultaneous adminis-
tration were similar to those for the paclitaxel pretreat-
ment arm, which likely indicates cell-to-cell variability
(Figure 4C). In the carboplatin pretreatment schedule,
there was a conversion of CDC2 to the slower migrat-
ing, inactive form, a loss of cyclin B1 activity and MPM-2
positivity, and maintenance of Bcl-2 in the premitotic,
dephosphorylated form (Figure 4). These data correlate
with results of the flow cytometric analyses (Figure 3)
and suggest that these cells never entered mitosis.

EFFECTS OF PACLITAXEL
AND CARBOPLATIN ON
ANCHORAGE-INDEPENDENT GROWTH

To extend the studies performed in monolayer and to fur-
ther examine the chemosensitivity of the HNSCC cell
lines, the effect of paclitaxel and carboplatin on anchorage-
independent growth was assayed by means of growth in
soft agar (Figure 5). In 1 arm of the experiment, cells
were treated in soft agar with paclitaxel or carboplatin
alone or simultaneously. Alternatively, cells were pre-
treated with paclitaxel or carboplatin for 8 hours in mono-
layer culture, followed by anchorage-independent growth
in the presence of both agents. Colonies were grown for 14 days in soft agar before quantification. Results represent 8 independent determinations. Error bars represent SDs.

In an effort to increase effectiveness and decrease the tox-
icity of chemotherapeutic agents in the treatment of
HNSCC, combination therapy has evolved. Until re-
cently, these treatments have been reserved for inoper-
able or recurrent tumors. In the treatment of HNSCC,
chemoradiotherapy may have similar efficacy to surgery
in select cases. By avoiding surgery, organ preservation
and improved quality of life may be enjoyed by the pa-
tient. Several groups have demonstrated significant in-
creases in response rates of head and neck tumors when
treated with concomitant paclitaxel, carboplatin, and ra-
diation therapies, but improvement in survival has not yet been shown. The optimistic results of
these phase 2 trials were the impetus for determining
mechanism of action of these drugs in HNSCC cells.

We undertook the current study to delineate the re-
sponse of 4 HNSCC cell lines to treatment with paclitaxel
or carboplatin alone or in combination. Specifically, we

Figure 5. Effect of paclitaxel (Taxol) and carboplatin on the anchorage-independent growth of head and neck squamous cell carcinoma. UNC-7 (A), UNC-10 (B),
UM-14C (C), and UM-38 (D). Cells were treated with paclitaxel (100 nmol/L) and/or carboplatin (100 µmol/L). Cells were treated in soft agar with paclitaxel alone,
carboplatin alone, or simultaneously, or pretreated with carboplatin or paclitaxel for 8 hours in monolayer culture followed by soft-agar growth in the presence of
both agents. Colonies were grown for 14 days in soft agar before quantification. Results represent 8 independent determinations. Error bars represent SDs.
examined the cell-cycle progression of these lines and determined the effect of these treatments on molecular mechanisms of cell-cycle control. There was a defect in the G_1/S-phase checkpoint in all of the lines studied as determined by the lack of G_1-phase arrest after treatment with the genotoxic agent carboplatin. However, regardless of the genetic alteration(s) that led to the loss of G_1-phase checkpoint control, paclitaxel was more effective at inhibiting anchorage-independent cell growth compared with carboplatin, in all of the cell lines. The activity of paclitaxel was correlated with an elevation of cyclin B1/CDC2 activity, a prolonged mitotic arrest, and Bcl-2 phosphorylation. In contrast, carboplatin induced predominantly an S-phase arrest, except in the UM-38 cells. Combination treatment, simultaneously or sequentially, was more effective than the use of either agent alone in the inhibition of HNSCC growth. Furthermore, when used in combination, the order of drug administration differentially affected cell-cycle position. However, overall cell outcome was the same, regardless of which drug was used first.

The mechanism of action of the agents under study is proposed to be cell-cycle dependent. Paclitaxel exerts its activity by polymerizing tubulin, causing its stabilization and the subsequent arrest of cells in mitosis. Carboplatin intercalates into DNA and interferes with DNA synthesis, arresting cells in the S phase. Since both drugs cause differential cell-cycle arrest with respect to one another, the activity of one drug may be affected by the second drug in the treatment of HNSCC. We hypothesized that the order of addition of these drugs would alter the effectiveness of the combination treatment against HNSCC. However, this was not the case. Although combination treatment was more effective than use of a single agent, the order of addition did not significantly alter outcome. Pretreatment or simultaneous cotreatment with carboplatin abrogated the mitotic response of paclitaxel as determined by molecular markers (eg, elevation of cyclin B1/CDC2 activity, MPM-2 positivity, and Bcl-2 phosphorylation). Other studies have shown that paclitaxel can act as a radiosensitizer by arresting cells in mitosis, the most radioresponsive phase of the cell cycle. If mitotic arrest is required for radiosensitization, then we hypothesize that the premitotic cell-cycle arrest we observed in HNSCC cells after simultaneous carboplatin and paclitaxel treatment would abrogate the radiosensitizing effects of paclitaxel. Additional preclinical studies will be required to test this hypothesis, the results of which may have significant clinical relevance.

Several studies have shown that tumor cells with defective checkpoint function are more vulnerable to anticancer agents. If one considers the frequency of alterations in p53 and pRB and their upstream and downstream regulatory pathways in HNSCC, most have defective checkpoint function. For example, more than 80% of primary HNSCCs examined by Reed et al contain inactivated p16INK4a, which would result in defective G_1/S-phase checkpoint function. Likewise, p53 and pRB mutations have been found in many HNSCCs examined. Approximately 30% of patients with HNSCC had a mutation within the p53 gene. Overexpression of MDM-2, which can inactivate p53, was found in 78% of oral squamous cell carcinomas and 52% of dysplastic, premalignant lesions, suggesting a role for MDM-2 overexpression as in the genesis of HNSCC. Elevated CDK6 levels, which can lead to hyperphosphorylation of pRB and deregulated S-phase entry, were found in all the HNSCC specimens examined in a recent study by Timmermann et al. However, it remains to be determined whether these genetic alterations have prognostic significance in HNSCC.

In patients with advanced inoperable HNSCC treated with platinum-based radiochemotherapy, mutant p53 was associated with improved local progression-free survival. One possible explanation for this observation is that defective DNA repair in tumor cells may lead to increased tumor cell death. Chomchai et al have shown improvement in overall survival and a trend toward improved disease-free survival in patients with p53 mutation, regardless of the treatment method. However, in a recent survey of patients undergoing primary radiochemotherapy with carboplatin, Haas et al showed no correlation of p53, p21, pRB, p16INK4A, or Bcl-2 status with remission rate, locoregional recurrence rate, or survival. Thus, the latter results refute the notion that these cell-cycle regulators may provide prognostic significance. However, Haas et al demonstrated that cyclin D1 overexpression was correlated with a significantly shortened overall survival. In other studies, the apoptotic family of proteins has been examined with regard to prognosis. In 1 study, ectopic expression of the antiapoptotic protein, Bax, in HNSCC cells was found to increase sensitivity of the cells to a variety of anticancer agents, including paclitaxel. Although an ever-increasing number of tumor specimens are undergoing molecular analyses, the prognostic significance of the findings is not well defined, and in some cases is controversial. Indeed, this area of research requires further investigation.

To our knowledge, this study is the first to explore how paclitaxel and carboplatin, used alone or in combination, differentially affect cell-cycle checkpoint response and HNSCC cell growth. Paclitaxel was a more effective single agent than carboplatin; however, combination therapy was the most effective at inhibiting tumor cell growth. Sequential combination of both drugs was equally effective at inhibiting cell growth as simultaneous co-treatment. Carboplatin pretreatment or cotreatment resulted in a prevention of the mitotic arrest seen with paclitaxel. This premitotic arrest may have implications for the radiosensitizing ability of this combination. These results provide molecular validation for the current clinical use of these 2 drugs in combination and set the stage for analyses of patient tumor specimens.

Many laboratories are now searching for compounds that interfere with cell-cycle checkpoints, in the hope that such agents will be more effective in anticancer therapy. As our knowledge of cell-cycle checkpoint regulation and the mechanism of action of currently used anticancer agents in the treatment of HNSCC increases, so will the number of signaling molecules and pathways that can be used as targets for rational drug design. We hope that a detailed understanding of these processes will lead to evolution of more incisive approaches to HNSCC.

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treatment that exploit the molecular defects in HNSCC cell-cycle control.

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