

Cell Cycle Arrest by Colcemid Differs in Human Normal and Tumor Cells¹

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ABSTRACT

In the continuous presence of Colcemid, the mitotic index in cultures of nine human tumor cell lines began to increase immediately upon addition of the drug. For 12 human normal (nontumorigenic) cell lines, the mitotic index did not begin to increase for some 2 to 3 h after the addition of Colcemid. The effect was independent of whether the cells were of fibroblast or epithelial origin and occurred over a 1000-fold range of Colcemid concentrations. No such differential effect was seen with single concentrations of either Taxol or nocodazole, but a similar delayed effect was seen for two concentrations of vinblastine. These observations suggest a fundamental difference between human normal and human tumor cells involving a cell cycle checkpoint in G₂, about 1 to 2 h before mitosis.

INTRODUCTION

The medicinal uses of *Colchicum* have a recorded history over more than 35 centuries, and few, if any, drugs have been studied more extensively (1-3). Besides its use for treatment of gout and in (largely unsuccessful) trials as an anticancer chemotherapeutic agent, the active compound, colchicine, and some of its derivatives such as Colcemid have been widely used in cytogenetics. By binding to tubulin dimer, the drug inhibits polymerization of microtubules (4-6). Since depolymerization is unaffected, the mitotic spindle rapidly dissociates or is not formed, and cycling cells accumulate in a prometaphase-like state, in many cases for an extended period. The absence of a spindle and the increased frequency of mitotic-like cells facilitates cytogenetic analysis. During a study using Colcemid for this purpose, we made an interesting observation. In the continuous presence of Colcemid, the mitotic index of two low passage normal human cells in culture did not increase (no accumulation was observed) until about 2 to 3 h after addition of the drug. This was in sharp contrast to the immediate increase or accumulation of prometaphase-like cells seen for two human tumor cell lines and immediately raised several questions. Was it simply a random chance that the two lines responding one way happened to be of tumor origin while the two responding the other way happened to be normal? Most available tumor cell lines (including those we used) have been derived from carcinomas, while most available low passage normal human cells are of fibroblast origin. Was our observation more a reflection of a difference in properties of epithelial *versus* fibroblast than tumor *versus* normal cells? Do increases in the ability to exclude certain drugs (as, for example, in multidrug resistance) occur during G₂ and mitosis for some cells but not others? Does a similar difference in the accumulation of mitotic cells occur for other drugs interfering with tubulin polymerization-depolymerization by different mechanisms? Lastly, does Colcemid act in some cells nonspecifically by temporarily "freezing" cell cycle progression uniformly, or does it act specifically at some cell cycle checkpoint in G₂ about 1 to 2 h before mitosis? The results of experiments addressing these and certain other questions are reported below.

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MATERIALS AND METHODS

Cell Lines. The normal (nontumorigenic) human cells used in these studies were as follows. The cells designated GM2149, GM730, GM0077, GM3396, GM3397, GM3387, GM3389, and GM3395 are all skin fibroblasts obtained from the NIGMS Human Genetic Mutant Cell Repository. The GM3396, GM3397, GM3387, and GM3389 cells were from phenotypically normal ataxia-telangiectasia heterozygotes, while the GM3395 cells were from "normal" tissue (not tumor) of an ataxia-telangiectasia homozygote. The AG1522 cells were derived from normal human skin fibroblasts and obtained from the NIA Cell Repository. The HF-19 cells were derived from normal human skin fibroblasts and were kindly supplied by Dr. D. Goodhead of the MRC Radiobiology Unit (Chilton, Didcot, United Kingdom). The human lymphocytes were donated by one of us (M. N. J.). The SVHUC cells, while not entirely "normal," are still nontumorigenic and were derived from normal human urinary tract epithelial cells, subsequently immortalized by SV-40 virus treatment (7). Samples of these SVHUC nontumorigenic human epithelial cells were kindly supplied by Drs. E. J. Hall and S. Martin of Columbia University.

The human tumor cell lines were as follows: HeLa (cervical carcinoma) were kindly supplied to our laboratory in 1967 by Dr. L. J. Tolmarch, then of Washington University. The HT29 (colon adenocarcinoma) and HT1080 (human fibrosarcoma) were obtained from the American Type Culture Collection. The A549 (lung carcinoma), MCF7 (breast adenocarcinoma), OVGI (ovarian carcinoma) and SHAW (pancreatic carcinoma) were kindly supplied by Drs. J. B. Mitchell and J. A. Cook of the National Cancer Institute. MC-T11 and MCPP-T11 are two subclones of methylcholanthrene-transformed and tumorigenic human uroepithelial cells derived from the nontumorigenic SVHUC cells described above (7) and were also supplied by Drs. Hall and Martin of Columbia University.

Cell Culture and Experimental Procedures. All cells were cultured in α -minimal essential medium containing 15% fetal bovine serum and were kept at 37°C in a humidified atmosphere of 5% CO₂ in air. For all experiments, actively growing cells from a partly confluent flask were subcultured, and typically for each drug treatment series, 20 to 30 identical T25 flasks were inoculated with 10⁵ cells each, approximately 40 h before the drug addition. This insured that the cultures would be in asynchronous log phase growth at the time of drug addition. Two replicate cultures were then taken for each sample time. In the case of lymphocytes, blood cultures were set up and stimulated with phytohemagglutinin 96 h before drug addition.

Just before the drug addition, the cultures were removed from the CO₂ incubator and placed in a 37°C warm room, where drug was added. The cultures were then sealed and remained in the warm room until the particular sample-pairs were removed. Then the cells were quantitatively harvested by trypsinization and fixed in methanol:acetic acid (3:1); slides were prepared and stained. During the incubation, cultures were kept in subdued light to minimize any cell cycle-perturbing photochemical reactions with the cells in the presence of drug as reported for Colcemid by Aronson and Inoué (8). Slides were coded so that the observer was unaware of the experimental conditions for any sample when the mitotic index was determined microscopically by scoring at least 1000 cells. The mitotic indices for the two replicate samples at each time point were then averaged. Each experiment was then repeated at least once and the log (1+MI) values (where MI is the fraction of mitotic cells) from the experiments were, in turn, averaged and plotted against the corresponding time the sample was taken. Uncertainties shown are estimates of 95% confidence limits of the plotted mean values. As first pointed out by Puck and Steffen (9, 10), since the fraction of cells of an age $t + \delta t$ after mitosis in a randomly dividing log phase cell population is an exponential function of time, t , any agent acting specifically to block cells at a certain point in the cell cycle will result in an exponential accumulation of cells at that point with time. If this point, or narrow window, is mitosis and if all cells are cycling with about the same cycle time, then log (1+MI) will be a linear function of time for

$0 \leq t \leq T_c$, with T_c being the total cell cycle transit time. For such plots, the slope of the linear portion of the curve is directly proportional to T_c .

In one experiment designed to follow the exit from and entry into mitosis of individual cells, Colcemid was added to a log phase culture of normal AG1522 cells, and the flask was sealed and immediately clamped onto the stage of an inverted phase contrast microscope located in a 37°C room. Minimizing the light intensity as much as possible, we then simply followed and periodically recorded the morphology of each cell in the field with respect to the rounded configuration of mitotic cells or flattened configuration of interphase.

RESULTS AND DISCUSSION

All the data shown in Figs. 1 and 2 are presented as “mitotic accumulation curves” as explained above (9, 10). The preliminary observations for normal and tumor cells referred to above are shown in Fig. 1, A and B. (normal) and Fig. 1, M and N (tumor), respectively. As already mentioned, there was a delay of about 2 h before normal cells began to accumulate in mitosis, but the accumulation began immediately for the tumor cells. To determine whether this difference

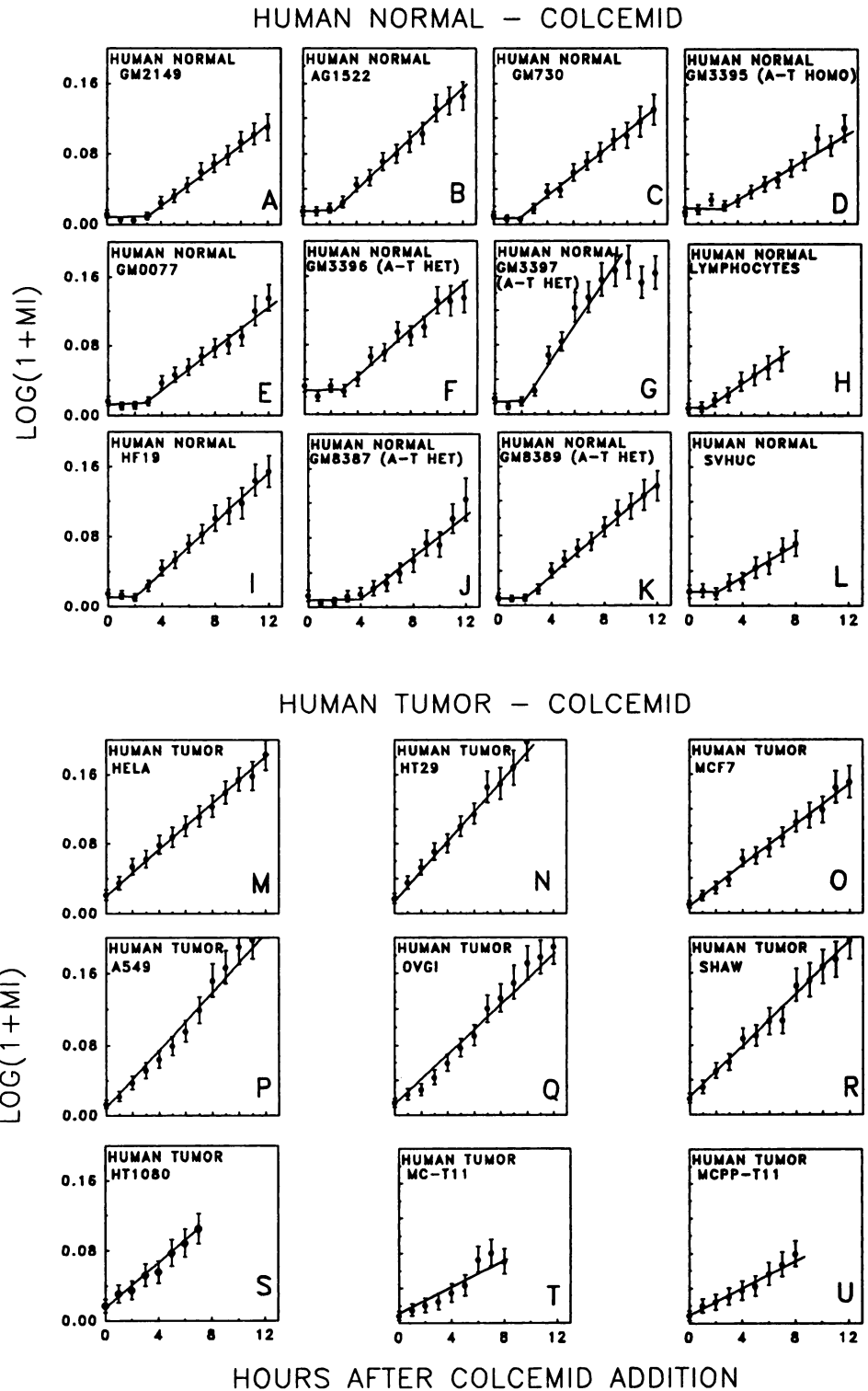
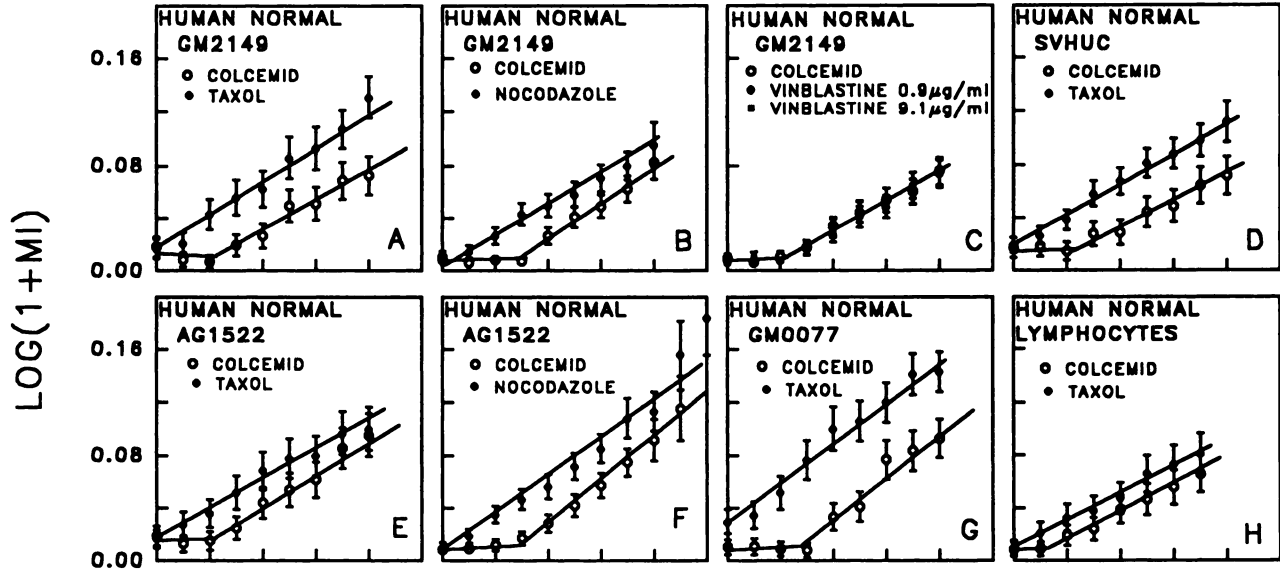
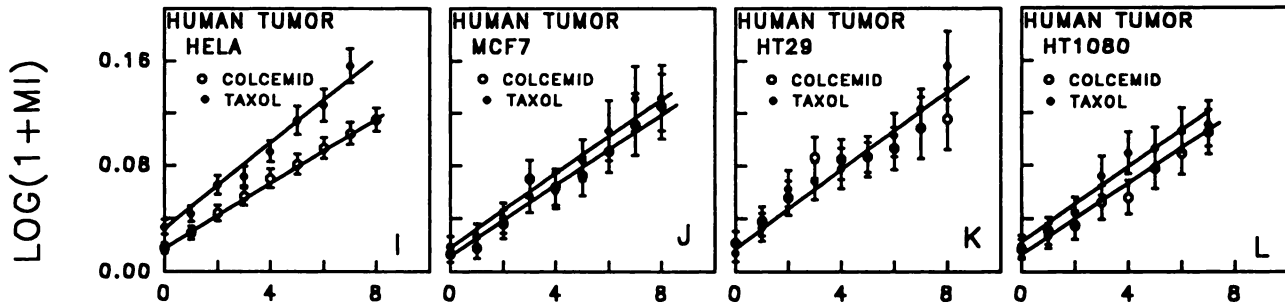


Fig. 1. Mitotic accumulation for low passage normal human cells (A-L) and cultured human tumor cells (M-U) in the continuous presence of Colcemid (0.1 μ g/ml). Error bars are 95% confidence limits calculated for the plotted mean values. The curve slopes are directly proportional to the average cell cycle transit times.

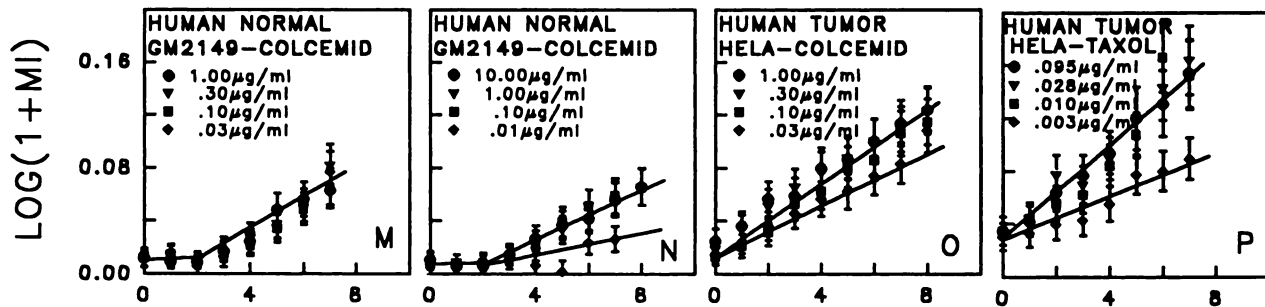
HUMAN NORMAL



HUMAN TUMOR



DRUG CONCENTRATION EFFECTS



HOURS AFTER DRUG ADDITION

Fig. 2. A comparison of mitotic accumulation of human normal (A-H) and tumor (I-L) cells in the continuous presence of either Colcemid (0.1 $\mu\text{g/ml}$; A-L), Taxol (0.028 $\mu\text{g/ml}$; A, D, E, and G-L), Nocodazole (0.044 $\mu\text{g/ml}$; B and F), or vinblastine (0.9 or 9.1 $\mu\text{g/ml}$; C). M-P, the mitotic collections for different concentrations of Colcemid for normal human GM2149 cells (M and N) and HeLa human tumor cells (O). P, the mitotic accumulation of HeLa cells with time in the presence of different concentrations of Taxol. The experimental conditions, replicate sample preparations, repetition of experiments, and uncertainties were as described for Fig. 1 except only one experiment was carried out for each result presented in M-P. For comparisons between Colcemid and Taxol for a given cell line or in two cases, Nocodazole (B and F), or in one case, vinblastine (C), both drugs were used in different series within the same experiment, *i.e.*, the same results for the Colcemid treatments were not simply replotted from Fig. 1. Bars, 95% confidence limits.

was perhaps random and unrelated to the tumor or normal tissue origin of the cells, we examined the response to Colcemid for 10 additional normal-derived low passage human cells and 7 additional tumor-derived cell lines. The additional results are also plotted in Fig.

1, C-L, for normal cells and Fig. 1, O-U, for tumor cells. The different modes of mitotic accumulation held in every case. Regarding a possible association with cells of fibroblast *versus* epithelial rather than normal *versus* tumor origin, we included two nonfibroblast

human normal cell samples and a fibroblast-derived human tumor cell line. For the normal cells, one was human lymphocytes (Fig. 1H) stimulated with phytohemagglutinin and the other (7) was derived from human urinary tract epithelial cells that were immortalized but nontumorigenic (Fig. 1L). Both showed a delay typical of normal cells before accumulation began, although for the human lymphocytes the delay was only about 1 h. Two tumorigenic cell lines derived as subclones from the parental human uroepithelial cells after treatment with methylcholanthrene (7) both displayed the immediate accumulation typical of the other tumor lines (Fig. 1, T and U). In addition, one fibroblast-derived tumor cell line, the fibrosarcoma HT1080, also showed the characteristic immediate accumulation of carcinoma-derived tumor lines (Fig. 1S). Thus, it appears that the different responses to Colcemid reflect an underlying difference between tumor and normal-derived cells.

Although it is possible that the differences observed between normal and tumor cells resulted from the ability of normal cells to exclude the drugs during a particular stage in the cell cycle (late G₂), this is considered most unlikely since it is tumor cells and not normal cells that are found to express elevated levels of the multidrug resistant P-glycoprotein (11). However, we did address the question by examining the Colcemid concentration dependence of the mitotic accumulation in both normal and tumor cells. As shown in Fig. 2, Colcemid concentrations ranging from 0.03 to 10 μg/ml (Fig. 2, M and N) all gave the delayed accumulation for normal GM2149 cells. Only for the lowest concentrations of the drug did we notice an alteration in the rate of mitotic accumulation. This occurred at 0.01 μg/ml in normal GM2149 cells and at 0.03 μg/ml in the human tumor-derived HeLa cell line (Fig. 2, N and O), suggesting that these tumor cells may indeed have higher levels of expression of the P-glycoprotein. Even at these low doses of Colcemid, the mitotic accumulation was continuous showing no preferential expression of drug resistance at any point of the cell cycle.

Taxol is another microtubule-active drug that has recently stimulated considerable interest as a chemotherapeutic anticancer agent. Its mechanism of action differs from that of Colcemid in that it binds to microtubules, preventing their depolymerization, but it also blocks mitosis (12, 13). Our experiments with Taxol showed that concentrations above about 0.01 μg/ml were as effective as Colcemid in blocking tumor cells in mitosis (Fig. 2, P and I-L), but as shown in Fig. 2, A, D, E, G, and H, at this single concentration of Taxol, there was no delay in mitotic accumulation for normal cells. From these and the drug concentration experiments, we conclude that if normal human G₂ and M cells can maintain an effective exclusion of Colcemid (M_r 371.4), they would have to be extremely efficient in doing so. As little as 0.03 μg/ml is fully effective at blocking cells in mitosis, and absolutely no increase or change was seen in this regard with concentrations up to 10 μg/ml. Furthermore, these same G₂ and M cells are unable to maintain an exclusion of the much larger Taxol molecule (M_r 853.9).

We also carried out several experiments on normal cells using either vinblastine (0.9 or 9 μg/ml; Fig. 2C) or nocodazole (0.044 μg/ml; Fig. 2, B and F). The action of vinblastine on normal human GM2149 cells at the two concentrations used was identical to that of Colcemid, but it was a little surprising that nocodazole, at this one concentration, was fully effective in immediately retaining mitotic and G₂ normal cells. Nocodazole is thought to act by a mechanism similar to that of Colcemid (14). However, populations of microtubules stable to these depolymerizing drugs have been detected in interphase cells (15, 16). The Colcemid stable microtubules have been shown to contain an acetylated lysine on the α-tubulin subunit (17), but this modification alone does not make the microtubules Colcemid-resistant. Presumably, this resistance arises from

the binding of one or more MAPs³ which recognize the modified tubulin (18, 19) or from exogenously acting factors such as Ca²⁺ or p34cdc2 kinase which have been recently shown to affect microtubule stability to nocodazole (20).

To determine whether Colcemid simply freezes all cell cycle progression temporarily in normal cells, we carried out the following experiment. Colcemid (0.1 μg/ml) was added to a log phase culture of normal AG1522 cells. The flask was sealed and immediately clamped onto the stage of an inverted phase contrast microscope located in a 37°C room. Minimizing the light intensity as much as possible, we identified a field containing about nine rounded mitotic cells. Every 5 min for the next 3 h the morphological status of each original rounded cell in the field was monitored and recorded, along with any flattened cells that assumed a rounded morphology during this period. After 3 h, observations were continued at 30-min intervals until a total of 4.5 h had elapsed. We found that all of the original nine rounded mitotic cells successfully completed division in the presence of the Colcemid and reassumed the flattened interphase configuration. For approximately the first 2 h, 20 additional flattened cells entered the rounded configuration at various times, and all but 2 divided normally within about an additional 1 to 1.5 h from the time they rounded. Cells that entered the rounded state more than about two h after Colcemid was added never succeeded in dividing during the remainder of the 4.5-h observation period. We conclude that cell cycle progression of normal cells is not temporarily frozen by the addition of Colcemid but that some event occurring approximately one to two h before mitosis renders the development and function of the mitotic apparatus refractory to interference by Colcemid.

Puck (10) made a similar observation on a difference in Colcemid collection for CHO cells compared to CHL cells. Accumulation was delayed for CHL cells but was immediate for CHO cells. CHO cells are known to possess a malignant phenotype (21), but we are unaware of any reports on similar properties for the CHL cells they used. It may also be worth noting that they found a small delay of about 45 min to 1 h for HeLa cells. The only obvious difference in experimental protocols that might account for slight differences in results is that the Puck and Steffen (9) experiments involved changing medium on cultures to fresh prewarmed medium one h before adding Colcemid, whereas our protocol did not involve a medium change at that time. Cell line-specific differences, particularly rodent *versus* human, in respect to the ability to undergo repeated cycles of DNA synthesis without cell division in the presence of Colcemid and other agents have also been reported previously by us (22) and by others (23). Kung *et al.* (23) found that in rodent (but not human) cells an increasingly transformed phenotype is correlated with an increasing propensity for undergoing repeated cycles of DNA synthesis without cell division. While this is clearly a different checkpoint than that concerning the cellular responses reported here, they are both generally consistent with the loss of growth control that is a hallmark of cancer cells.

How might these differences between human normal and tumor cells in microtubule stability to Colcemid during late G₂ arise? The tumor suppressor genes play a crucial role in cell cycle progression. Mutations in these genes are the most commonly found mutations in all human cancers (24–26). If, as seems likely, mutations in tumor suppressor genes are responsible for the differences we observe between normal and transformed cells, our results suggest that in normal cells the products of these genes regulate, through either synthesis or modification, proteins which alter microtubule stability to Colcemid during late G₂. How could such stability changes come about? While

³ The abbreviations used are: MAP, microtubule-associated protein; CHO, Chinese hamster ovary; CHL, Chinese hamster lung.

products of some tumor suppressor genes function as transcription factors (27), others may interact directly with cell cycle-regulated proteins (cyclins; Refs. 28, 29). Most, if not all, of these proteins are themselves subjected to regulation during the cell cycle, either through changes in steady-state concentration (24–26) or through phosphorylation, which may directly involve the tumor suppressor gene product (30), or via other proteins which bind and alter its activity (31). Cell cycle-dependent protein kinases and okadaic acid-sensitive protein phosphatases have been demonstrated to have a profound effect on microtubule dynamics in cells (20, 32, 33). Microtubule dynamics are regulated by the binding of specific MAPs, which can be spatially and temporally regulated by posttranslational modifications of both the tubulins and the MAPs (reviewed in Ref. 19). Given the complex but central role for mitogen-activated protein kinases in both cell cycle progression (34) and ability to phosphorylate MAPs (35), as well as the distribution of its different isoforms between the nucleus and cytoplasm (36), this enzyme appears to be a good candidate for future studies on the mechanism which gives rise to the observed differences in microtubule stability.

Much has been learned in recent years about cell cycle checkpoints and control mechanisms of cell cycle progression (37–43). We are currently investigating how some of these may be involved with regard to the observed difference in the effect of Colcemid on human normal and tumor cells.

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REFERENCES

- Eigsti, O. J., and Dustin, P., Jr. Colchicine in Agriculture, Medicine, Biology, and Chemistry. Ames, IA: The Iowa State College Press, 1955.
- Sluder, G. Role of spindle microtubule in the control of cell cycle timing. *J. Cell Biol.*, **80**: 674–691, 1979.
- Rieder, C. L., and Palazzo, R. E. Colcemid and the mitotic cycle. *J. Cell Sci.*, **102**: 387–392, 1992.
- Taylor, E. W. The mechanism of colchicine inhibition of mitosis. *J. Cell Biol.*, **25**: 145–160, 1965.
- Margulis, L. Colchicine-sensitive microtubules. *Int. Rev. Cytol.*, **34**: 333–361, 1973.
- Wilson, L., Anderson, K., and Chin, D. Nonstoichiometric poisoning of microtubule polymerization: a model for the mechanism of action of the Vinca alkaloids, podophyllotoxin and colchicine. In: R. Goldman, T. Pollard, and J. Roseblum (eds.), *Cell Motility*, pp. 1051–1064. New York: Cold Spring Harbor Laboratory, 1976.
- Reznikoff, C. A., Loretz, L. J., Christian, B. J., Wu, S. Q., and Meisner, L. F. Neoplastic transformation of SV40-immortalized human urinary tract epithelial cells by *in vitro* exposure to 3-methylcholanthrene. *Carcinogenesis (Lond.)*, **9**: 1427–1436, 1988.
- Aronson, J., and Inoué, S. Reversal by light of the action of *N*-methyl-*N*-desacetyl colchicine on mitosis. *J. Cell Biol.*, **45**: 470–477, 1970.
- Puck, T. T., and Steffen, J. Life cycle analysis of mammalian cells. I. A method for localizing metabolic events within the life cycle, and its application to the action of Colcemid and sublethal doses of X-irradiation. *Biophys. J.*, **3**: 379–397, 1963.
- Puck, T. T. Studies of the life cycle of mammalian cells. *Cold Spring Harbor Symp. Quant. Biol.*, **29**: 167–176, 1964.
- Gottesman, M. M., and Pastan, I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.*, **62**: 385–427, 1993.
- Schiff, P. P., and Horwitz, S. B. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA*, **77**: 1561–1565, 1980.
- Jordan, M. A., Toso, R. J., Thrower, D., and Wilson, L. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentration. *Proc. Natl. Acad. Sci. USA*, **90**: 9552–9556, 1993.
- DeBrabander, M. J., VandeVeire, R. M. L., Aerts, F. E. M., Borgers, M., and Janssen, P. A. J. The effects of methyl [5-(2-thienylcarbonyl)-1*H*-benzimidazol-2-yl]carbonate, (R 17934; NSC 238159), a new synthetic antitumor drug interfering with microtubule, on mammalian cells cultured *in vitro*. *Cancer Res.*, **36**: 905–916, 1976.
- Cassimeris, L. U., Wadsworth, P., and Salmon, E. D. Dynamics of microtubule depolymerization in monocytes. *J. Cell Biol.*, **102**: 2023–2032, 1986.
- Thompson, W. C., Asai, D. J., and Carney, D. H. Heterogeneity among microtubules of the cytoplasmic microtubule complex detected by a monoclonal antibody to α tubulin. *J. Cell Biol.*, **98**: 1017–1025, 1984.
- Schulze, E., Asai, D. J., Bulinski, J. C., and Kirschner, M. Posttranslational modification and microtubule stability. *J. Cell Biol.*, **105**: 2167–2177, 1987.
- Gelfand, V. I., and Bershadsky, A. D. Microtubule dynamics, mechanism, regulation, and function. *Annu. Rev. Cell Biol.*, **7**: 93–116, 1991.
- Masson, D., and Kreis, T. E. Identification and molecular characterization of E-MAP-115, a novel microtubule-associated protein predominantly expressed in epithelial cells. *J. Cell Biol.*, **123**: 357–371, 1993.
- Lieuvain, A., Labbé, J.-C., Dorée, M., and Job, D. Intrinsic microtubule stability in interphase cells. *J. Cell Biol.*, **124**: 985–996, 1994.
- Puck, T. T. Cyclic AMP, the microtubule-microfilament system, and cancer. *Proc. Natl. Acad. Sci. USA*, **74**: 4491–4495, 1977.
- Mitchell, J. B., Bedford, J. S., and Bailey, S. M., Dose rate effects on the cell cycle and survival of S3 HeLa and V79 cells. *Radiat. Res.*, **79**: 520–536, 1979.
- Kung, A. L., Sherwood, S. W., and Schimke, R. T. Cell line differences in the control of cell cycle progression in the absence of mitosis. *Proc. Natl. Acad. Sci. USA*, **87**: 9553–9557, 1990.
- Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. *p53* mutations in human cancers. *Science (Washington DC)*, **253**: 49–53, 1991.
- Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.*, **51**: 6304–6331, 1991.
- Weinberg, R. Tumor suppressor genes. *Neuron*, **11**: 191–196, 1993.
- Levine, A. J. The tumor suppressor gene. *Annu. Rev. Biochem.*, **62**: 623–651, 1993.
- Cobrinck, D., Dowdy, S. F., Hinds, P. W., Mittnacht, S., and Weinberg, R. A. The retinoblastoma protein and the regulation of cell cycling. *Trends Biochem. Sci.*, **17**: 312–315, 1992.
- Dowdy, S. F., Hinds, P. W., Lonic, K., Reed, S. I., Arnold, A., and Weinberg, R. A. Physical interaction of the retinoblastoma protein with human D cyclins. *Cell*, **73**: 499–511, 1993.
- Ewen, M. E., Sluss, H. K., Sherr, C. J., Matsushime, H., Kato, J., and Livingston, D. M. Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell*, **73**: 487–497, 1993.
- Momand, J., Zambetti, G. P., Olson, D. C., George, D., and Levine, A. J. The *mdm-2* oncogene product forms a complex with the p53 proteins and inhibits p53 mediated transactivation. *Cell*, **69**: 1237–1245, 1992.
- Verde, F., Labbé, J. C., Dorée, M., and Karsenti, E. Regulation of microtubule dynamics by cdc2 protein kinase in cell free extracts of *Xenopus* eggs. *Nature (Lond.)*, **343**: 233–237, 1990.
- Gliksman, N. R., Parsons, S. F., and Salmon, E. D. Okadaic acid induces interphase to mitotic-like microtubule dynamic instability by inactivating rescue. *J. Cell Biol.*, **119**: 1271–1276, 1992.
- Blenis, J. Signal transduction via the MAP kinase: proceed at your own risk. *Proc. Natl. Acad. Sci. USA*, **90**: 5889–5892, 1993.
- Ray, L. B., and Sturgill, T. W. Rapid stimulation by insulin of a serine/threonine kinase in 3T3-L1 adipocytes that phosphorylates microtubule-associated protein 2 *in vitro*. *Proc. Natl. Acad. Sci. USA*, **84**: 1502–1506, 1987.
- Gonzales, F. A., Seth, A., Raden, D. L., Bowman, D. S., Fay, F. S., and Davis, R. J. Serum induced translocation of mitogen-activated protein kinase to the cell surface ruffling membrane and the nucleus. *J. Cell Biol.*, **122**: 1089–1101, 1993.
- Hartwell, L. H., and Weinert, T. A. Checkpoints: controls that ensure the order of cell cycle events. *Science (Washington DC)*, **246**: 629–634, 1989.
- Murray, A. W., and Kirschner, M. W. Dominoes and clocks: the union of two views of the cell cycle. *Science (Washington DC)*, **246**: 614–621, 1989.
- Murray, A. W. Creative blocks: cell-cycle checkpoints and feedback controls. *Nature (Lond.)*, **359**: 599–604, 1992.
- Solomon, M. J., Glotzer, M., Lee, T., Philippe, M., and Kirchner, M. Cyclin activation of p34^{cdc2}. *Cell*, **63**: 1013–1014, 1990.
- Whitfield, W. G. F., Gonzalez, C., Maldonado-Codina, G., and Glover, D. M. The A- and B-type cyclins of *Drosophila* are accumulated and destroyed in temporally distinct events that define separable phases of the G₂-M transition. *EMBO J.*, **9**: 2563–2572, 1990.
- Pardee, A. B., and Keyomarsi, K. Modification of cell proliferation with inhibitors. *Curr. Opin. Cell Biol.*, **4**: 186–191, 1992.
- Nishimoto, T., Uzawa, S., and Schlegel, R. Mitotic checkpoints. *Curr. Opin. Cell Biol.*, **4**: 174–179, 1992.